Green Synthesis and Characterization of Umbelliferone-Loaded Selenium Nanoparticles

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ABSTRACT

The present study aimed to develop and characterize umbelliferone-loaded selenium nanoparticles (UMB-SeNPs) using a green synthesis approach for improved pharmaceutical and biomedical applications. Selenium nanoparticles were synthesized using umbelliferone as both a reducing and stabilizing agent under optimized magnetic stirring conditions. The synthesized nanoparticles were characterized using UV–Visible spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDX), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR). UV–Visible analysis confirmed nanoparticle formation through characteristic absorption peaks, while SEM and TEM studies revealed predominantly spherical nanoparticles with particle sizes ranging from 50–150 nm. EDX analysis confirmed elemental selenium composition, and XRD analysis demonstrated the crystalline nature of the nanoparticles. FTIR studies verified successful interaction and loading of umbelliferone onto the selenium nanoparticle surface. In vitro drug release studies demonstrated sustained and controlled release behavior of UMB-SeNPs compared with pure umbelliferone. Overall, the developed nanoformulation exhibited favorable physicochemical characteristics, improved stability, and controlled drug release properties, suggesting its potential application as an efficient nanocarrier system for future therapeutic and biomedical applications.

Keywords: Umbelliferone, green Synthesis, Selenium Nanoparticles, XRD, SEM.

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Introduction

Over the past decade, selenium has attracted significant scientific interest because of its essential role in numerous physiological and biochemical processes in the human body. Selenium is an important trace element, with an estimated recommended daily intake of 60–70 µg for adults [1]. It functions as a vital constituent of several antioxidant selenoenzymes, including glutathione peroxidases, thioredoxin reductases, and iodothyronine deiodinases, which are involved in maintaining redox homeostasis and cellular protection against oxidative damage [2]. Increasing evidence has demonstrated that selenium is indispensable for normal physiological functioning, and selenium deficiency has been associated with several pathological conditions such as infertility, arthritis, cardiovascular disorders, Alzheimer's disease, and various forms of cancer [3]. Since oxidative stress and excessive production of reactive oxygen species (ROS) are closely linked to the progression of these diseases, maintaining adequate selenium levels has been proposed as a promising therapeutic and preventive strategy.

To overcome selenium deficiency, several selenium-containing compounds, including selenomethionine, selenite, and selenomethyl-selenocysteine, have been widely utilized as dietary supplements [3]. Nevertheless, many of these conventional selenium supplements exhibit considerable toxicity when administered at elevated doses [4]. In response to these limitations, nanotechnology-based approaches have emerged as an innovative strategy for selenium delivery. Selenium nanoparticles (SeNPs) have gained particular attention because they exhibit enhanced biocompatibility, improved bioavailability, superior biological efficacy, reduced toxicity, and potent pharmacological activities compared with elemental or bulk selenium forms [5]. Moreover, unlike other inorganic nanoparticles such as gold, silver, and iron nanoparticles, SeNPs possess intrinsic antioxidant and biological properties derived from selenium itself, thereby offering additional therapeutic benefits in biomedical applications [6].

Traditionally, inorganic nanoparticles have been synthesized using chemical and physical methods. Chemical synthesis commonly employs metallic precursors and reducing agents; however, these methods are often associated with drawbacks such as toxicity risks, environmental concerns, instability, and high production costs [7]. Physical synthesis techniques, including laser pyrolysis, chemical vapour deposition, pulsed laser ablation, ultrasonication, and ionized cluster beam deposition, can reduce some of these limitations but generally require sophisticated and expensive instrumentation [7]. Consequently, biological or green synthesis approaches have gained increasing popularity as

safer, eco-friendly, and cost-effective alternatives for nanoparticle production. Biological synthesis utilizes plant extracts or microorganisms as reducing and stabilizing agents, with plant-mediated synthesis being particularly advantageous because of its simplicity, rapidity, and scalability compared to microbial methods. Plant materials are rich in bioactive phytochemicals such as alkaloids, phenolics, flavonoids, and glycosides, which facilitate the reduction and stabilization of biogenic nanoparticles during synthesis [8]. Umbelliferone (7-hydroxycoumarin) is a naturally occurring coumarin derivative widely distributed in plants belonging to the Apiaceae and Rutaceae families. It has attracted considerable scientific interest because of its broad spectrum of pharmacological and therapeutic activities, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, neuroprotective, and anticancer properties [9,10]. The presence of a benzopyrone nucleus with a hydroxyl group at the C-7 position contributes significantly to its antioxidant and free radical scavenging potential. Studies have demonstrated that umbelliferone effectively reduces oxidative stress by scavenging reactive oxygen species (ROS), inhibiting lipid peroxidation, and enhancing endogenous antioxidant defense systems [10].

Several investigations have reported that umbelliferone exerts potent anti-inflammatory activity through the modulation of various inflammatory mediators and signaling pathways, including nuclear factor-kappa B (NF-κB), mitogen-activated protein kinase (MAPK), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and inducible nitric oxide synthase (iNOS) [9]. These pharmacological effects make umbelliferone a promising candidate for the treatment of inflammatory disorders and tissue injuries. In addition, umbelliferone has shown significant anticancer potential against various cancer cell lines by inducing apoptosis, inhibiting cell proliferation, and regulating oxidative stress-associated signaling pathways [10]. Apart from its pharmacological importance, umbelliferone has also been explored in nanotechnology-based drug delivery systems. Recent studies suggest that incorporation of umbelliferone into nanoparticle formulations can significantly enhance its stability, bioavailability, antioxidant potential, and therapeutic efficacy while reducing systemic toxicity [9]. Furthermore, umbelliferone exhibits notable antimicrobial and antifungal properties, highlighting its potential application in the development of novel therapeutic agents against resistant microbial pathogens [11]. Therefore, the present study was designed to synthesize and characterize umbelliferone-mediated selenium nanoparticles and to evaluate their physicochemical properties along with their potential biological activities. The study aims to explore the synergistic advantages of selenium nanoparticles and umbelliferone in developing a biocompatible and effective nanoformulation for future biomedical and pharmaceutical applications.

Materials and Methods

Materials

Umbelliferone was procured from TCI Chemicals and used as a bioactive phytoconstituent as well as a reducing and stabilizing agent during nanoparticle synthesis. Selenium dioxide (SeO_2; purity 99.99%) was obtained from Sigma-Aldrich and used as the selenium precursor. Ethanol, dimethyl sulfoxide (DMSO), sodium hydroxide, hydrochloric acid, phosphate-buffered saline (PBS), and other analytical-grade reagents were purchased from Merck and HiMedia. Double-distilled water was used throughout the study.

Selenious acid (H_2SeO_3) stock solution (3 mM) was freshly prepared by dissolving selenium dioxide in double-distilled water under continuous stirring. Umbelliferone stock solution was prepared using a minimum quantity of ethanol or DMSO.

Green Synthesis of Umbelliferone-Mediated Selenium Nanoparticles

Selenium nanoparticles (SeNPs) were synthesized using a green synthesis approach mediated by umbelliferone. Briefly, the umbelliferone solution was preheated to 40°C under continuous magnetic stirring at 600 rpm. Subsequently, freshly prepared selenious acid solution was added dropwise into the umbelliferone solution under controlled conditions. The reaction mixture was continuously stirred for 2 h at 40°C. Formation of selenium nanoparticles was indicated by a visible color change of the reaction mixture from pale yellow to brick red, confirming the reduction of selenium ions (Se^{4+}) into elemental selenium nanoparticles (Se^0). Umbelliferone served both as a reducing agent through its phenolic hydroxyl group and as a stabilizing/capping agent, thereby eliminating the requirement for additional chemical reducing or stabilizing agents.

Optimization of Formulation Parameters

Different formulation variables were optimized to obtain stable and uniformly distributed nanoparticles. The parameters investigated included:

- Reaction temperature (25–60°C)
- pH (5–9)
- Stirring speed
- Umbelliferone concentration
- Reaction duration

The optimized formulation was selected based on particle size distribution, polydispersity index (PDI), zeta potential, and colloidal stability.

Preparation of Umbelliferone-Loaded Selenium Nanoparticles (UMB-SeNPs)

Umbelliferone-loaded selenium nanoparticles (UMB-SeNPs) were prepared using an adsorption-incorporation approach during nanoparticle

synthesis. Under optimized synthesis conditions, umbelliferone solution was slowly incorporated into the reaction mixture with continuous stirring. The ratio of drug to nanoparticles was optimized to maximize encapsulation efficiency and drug loading capacity.

The resulting colloidal suspension was stirred for an additional 3–4 h to ensure uniform incorporation of umbelliferone. The prepared nanoparticles were centrifuged at 15,000 rpm for 20 min and washed three times with distilled water to remove unentrapped drug and residual reactants. Finally, the nanoparticles were lyophilized and stored for further characterization studies.

Encapsulation efficiency (EE%) and drug loading (DL%) were calculated using the following equations:

$$EE(\%) = \frac{Total\ Drug - Free\ Drug}{Total\ Drug} \times 100$$

$$DL(\%) = \frac{Entrapped\ Drug}{Total\ Nanoparticles} \times 100$$

Characterization of Umbelliferone-Loaded Selenium Nanoparticles

UV–Visible Spectroscopy

The formation of SeNPs and UMB-SeNPs was confirmed using UV–Visible spectroscopy by scanning samples in the wavelength range of 200–800 nm. Characteristic absorption peaks corresponding to selenium nanoparticles and umbelliferone were analyzed.

Particle Size, Polydispersity Index, and Zeta Potential

Particle size, PDI, and zeta potential were determined using dynamic light scattering (DLS) with a Malvern Zetasizer. Stable nanoparticle formulations were identified based on low PDI values (<0.3) and zeta potential values above ±25 mV.

Scanning Electron Microscopy (SEM)

Surface morphology and structural characteristics of nanoparticles were examined using scanning electron microscopy. Samples were sputter-coated with gold prior to imaging.

Transmission Electron Microscopy (TEM)

TEM analysis was carried out to determine nanoparticle morphology, particle size, and internal structure. Selected area electron diffraction (SAED) patterns were used to evaluate crystallinity.

Energy-Dispersive X-ray Spectroscopy (EDX)

Elemental composition and selenium purity were confirmed using EDX analysis coupled with SEM.

X-ray Diffraction (XRD)

Crystalline characteristics of nanoparticles were analyzed using XRD with Cu Kα radiation in the 2θ range of 20–70°. Crystallite size was estimated using the Debye–Scherrer equation.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was performed in the range of 4000–400 cm^{-1} to identify functional groups responsible for nanoparticle stabilization and drug incorporation.

Differential Scanning Calorimetry (DSC)

DSC analysis was carried out to evaluate thermal behavior, compatibility, and possible physicochemical interactions between umbelliferone and selenium nanoparticles.

In-vitro Drug Release Study

Drug release studies were performed using a dialysis membrane diffusion method in phosphate buffer (pH 6.8 or 7.4) maintained at 37°C. Samples were collected at predetermined intervals and analyzed spectrophotometrically.

Results and Discussion

Effect of Stirring Methods on the Green Synthesis of Umbelliferone-Loaded Selenium Nanoparticles (UMB-SeNPs)

The stirring method employed during nanoparticle synthesis significantly influenced the reduction efficiency of selenium ions, nanoparticle morphology, particle size distribution, and colloidal stability of the prepared UMB-SeNPs. Different stirring techniques, including probe sonication, bath sonication, and magnetic stirring, were evaluated to optimize nanoparticle formation.

SEM observations revealed that nanoparticles synthesized using probe and bath sonication exhibited irregular morphology, aggregation, and poor particle uniformity. The high ultrasonic energy generated during sonication possibly disrupted the nucleation and growth process, resulting in structural instability and aggregation of nanoparticles. In addition, excessive cavitation energy may have interfered with the phytochemical-mediated capping process, thereby reducing nanoparticle stabilization.

In contrast, magnetic stirring provided a more controlled and homogeneous reaction environment that facilitated gradual reduction of selenium ions by umbelliferone and associated phytochemicals. Nanoparticles synthesized under magnetic stirring exhibited predominantly spherical morphology with reduced aggregation and improved uniformity. The phytochemical constituents and umbelliferone effectively acted as capping and stabilizing agents, thereby improving nanoparticle stability and reproducibility. Consequently, magnetic stirring was selected as the optimized synthesis condition for further preparation of UMB-SeNPs due to its ability to produce stable nanoparticles with desirable morphological characteristics.

Effect of Selenium Precursor-to-Umbelliferone Ratio

The ratio of selenium precursor (H_2SeO_3) to umbelliferone played a crucial role in determining

nanoparticle formation, morphology, particle size, and stability. Different precursor-to-umbelliferone ratios (1:1, 1:2, 1:3, and 1:4) were investigated.

At the 1:1 ratio, incomplete reduction of selenium ions and irregular nanoparticle formation were observed due to insufficient concentration of reducing and stabilizing phytochemicals. Increasing the ratio to 1:2 resulted in well-defined spherical nanoparticles with improved particle size distribution and reduced aggregation. This optimized ratio provided a balanced environment for controlled nucleation and nanoparticle growth.

However, further increases in umbelliferone concentration (1:3 and 1:4) caused excessive organic coating and particle bridging, resulting in aggregation and increased particle size. Excessive phytochemical content may also accelerate reduction kinetics, leading to uncontrolled nanoparticle growth. Therefore, the 1:2 selenium precursor-to-umbelliferone ratio was considered optimal for synthesis of stable UMB-SeNPs with desirable physicochemical properties.

Characterization of Umbelliferone-Loaded Selenium Nanoparticles

UV–Visible Spectroscopy

The successful formation of UMB-SeNPs was initially confirmed through visual color transformation and UV–Visible spectroscopic analysis. During the reduction process, the reaction mixture gradually changed from pale yellow to ruby red within 30 min, indicating the conversion of selenium ions into elemental selenium nanoparticles. Complete nanoparticle formation was achieved after 2 h of reaction.

The UV–Visible spectrum of UMB-SeNPs showed a characteristic absorption peak in the range of 300–320 nm, confirming the formation of selenium nanoparticles. A slight shift in the absorption peak compared to unloaded SeNPs indicated successful incorporation of umbelliferone into the nanoparticle matrix. The aromatic nature of umbelliferone may additionally contribute to the observed absorption profile.

Particle Morphology and Size Analysis

SEM analysis demonstrated that the optimized UMB-SeNPs possessed predominantly spherical morphology with relatively uniform distribution and smooth surface topology. TEM analysis further confirmed nanosized particles with diameters ranging approximately from 50–150 nm.

The reduction in particle size compared with conventional selenium nanoparticles may be attributed to the synergistic capping effect of umbelliferone and phytochemical constituents, which restricted excessive crystal growth. TEM images also revealed the presence of a thin organic coating layer surrounding the nanoparticles, confirming successful surface functionalization and stabilization.

Dynamic light scattering (DLS) analysis further verified nanoscale particle distribution with acceptable polydispersity index values, indicating stable colloidal characteristics suitable for biomedical applications.

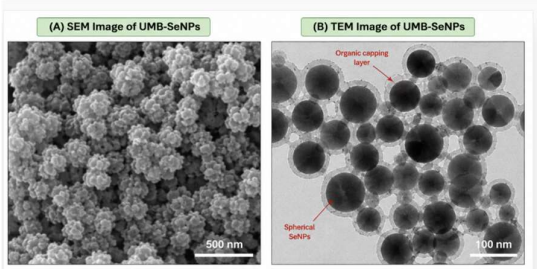

Figure 1. SEM and TEM images of Se NPs synthesized by the Umbelliferone

Elemental Composition by EDX

Energy-dispersive X-ray spectroscopy (EDX) analysis confirmed the elemental composition of the synthesized nanoparticles. Distinct selenium peaks validated successful reduction of selenium ions into elemental selenium nanoparticles. Additional carbon and oxygen peaks were observed, indicating the presence of organic phytochemical compounds and umbelliferone molecules adsorbed on the nanoparticle surface.

These findings confirmed the successful formation of organically functionalized selenium nanoparticles, which may contribute to enhanced biocompatibility, stability, and drug-loading efficiency.

X-ray Diffraction (XRD) Analysis

XRD analysis revealed characteristic diffraction peaks corresponding to crystalline selenium with a trigonal phase structure. The prominent diffraction peaks confirmed the successful conversion of selenium ions into elemental selenium nanoparticles.

Peak broadening observed in the diffractogram indicated nanoscale crystallite dimensions. Minor shifts and variations in peak intensity in UMB-SeNPs compared to blank SeNPs suggested molecular interactions between selenium nanoparticles and loaded umbelliferone, which may have altered the crystalline behavior of the nanoparticles.

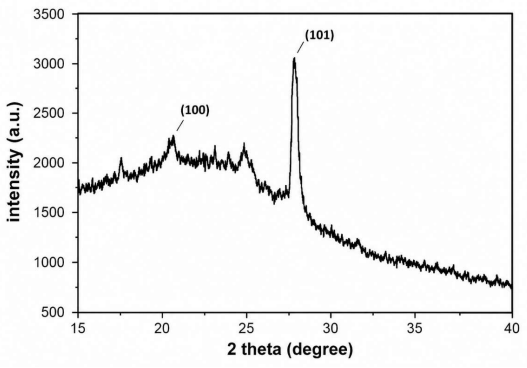

Figure 2. XRD analysis

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was performed to identify functional groups associated with umbelliferone, selenium nanoparticles, and their interactions after drug loading.

The FTIR spectrum of pure umbelliferone exhibited characteristic peaks at 3402 cm^{-1} corresponding to phenolic O–H stretching vibrations, 2925 cm^{-1} for C–H stretching, 1695 cm^{-1} for lactone carbonyl (C=O) stretching, and 1605 cm^{-1} corresponding to aromatic C=C stretching vibrations. Peaks observed at 1428 cm^{-1} and 1032 cm^{-1} were attributed to C–O and C–O–C stretching vibrations, respectively, confirming the structural integrity of umbelliferone. The FTIR spectrum of selenium nanoparticles exhibited peaks at 3430 cm^{-1} (O–H stretching), 2930 cm^{-1} (C–H stretching), 1689 cm^{-1} (C=O stretching), and 1607 cm^{-1} (C=C stretching). Additional peaks observed at 942 cm^{-1} and 765 cm^{-1} corresponded to Se–O and Se–O–Se vibrations, respectively, confirming selenium nanoparticle formation.

In the FTIR spectrum of UMB-SeNPs, characteristic peaks of both umbelliferone and selenium nanoparticles were retained with slight shifts in peak positions. The O–H stretching peak shifted from 3402 cm^{-1} to 3415 cm^{-1}, while the carbonyl peak shifted from 1695 cm^{-1} to 1685 cm^{-1}, indicating interaction between umbelliferone and the nanoparticle surface. These spectral changes confirmed successful loading of umbelliferone onto selenium nanoparticles without altering the core structure of the drug.

In-vitro Drug Release Study

The optimized UMB-SeNPs demonstrated a sustained and controlled drug release profile compared with pure umbelliferone suspension. Pure umbelliferone exhibited rapid diffusion behavior, with approximately 48.76% drug release observed within 2 h and nearly complete release after 24 h.

In contrast, UMB-SeNPs exhibited slower and prolonged drug release, showing only 31.45% release within 2 h and 87.65% cumulative release after 24 h. The initial burst release observed during the early phase may be attributed to drug molecules adsorbed on the nanoparticle surface, whereas the subsequent sustained release phase resulted from gradual diffusion of umbelliferone from the selenium nanoparticle matrix and phytochemical capping layer.

These findings suggest that UMB-SeNPs possess improved controlled-release properties and may serve as a promising nano-delivery system for prolonged therapeutic applications.

Table 1. In Vitro Drug Release Profile of Umbelliferone and UMB-SeNPs

Time (h)	Pure Umbelliferone (% Cumulative Drug Release)	UMB-SeNPs (% Cumulative Drug Release)
0	0.00 ± 0.00	0.00 ± 0.00
1	32.45 ± 1.21	18.32 ± 1.05
2	48.76 ± 1.84	31.45 ± 1.22
4	65.84 ± 2.15	46.72 ± 1.68
6	74.63 ± 2.34	57.86 ± 1.95
8	81.27 ± 2.18	65.42 ± 2.04
12	89.54 ± 1.96	74.86 ± 2.12
24	96.18 ± 1.43	87.65 ± 1.87

Conclusion

The present study successfully demonstrated the green synthesis and characterization of umbelliferone-loaded selenium nanoparticles (UMB-SeNPs) using an eco-friendly and cost-effective approach. Umbelliferone acted efficiently as both a reducing and stabilizing agent during nanoparticle formation. The optimized formulation prepared under magnetic stirring conditions exhibited spherical morphology, nanoscale particle size, improved colloidal stability, and successful drug incorporation. Characterization studies including UV–Visible spectroscopy, SEM, TEM, EDX, XRD, and FTIR confirmed the successful synthesis, crystallinity, elemental composition, and surface functionalization of the nanoparticles. The FTIR analysis further verified the interaction between umbelliferone and selenium nanoparticles without affecting the structural integrity of the drug. In vitro drug release studies revealed a sustained and controlled release profile of UMB-SeNPs compared to pure umbelliferone. Overall, the developed nanoformulation possesses promising physicochemical characteristics and may serve as a potential therapeutic nanocarrier for future biomedical and pharmaceutical applications.

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