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FABRICATION AND CHARACTERIZATION OF CHITOSAN NANOCARRIERS FOR ERUCIN DELIVERY USING IONIC GELATION TECHNIQUES

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Abstract
Background: The incorporation of nanotechnology and bioactive molecules represents a boundary in modern pharmacology, aiming to improve the bioavailability and stability of plant-derived chemicals. This study focuses on the evolution of a novel drug delivery system using chitosan nanocarriers to encapsulate erucin, a potent isothiocyanate derived from Eruca sativa known for its significant anticancer, anti-inflammatory, and antimicrobial properties.
Methods: Fresh chopped Eruca sativa leaves were macerated in water to get erucin phytochemical by enzymatic conversion with myrosinase, followed by an organic solvent extraction using dichloromethane. In the production of nano erucin, sodium tripolyphosphate -a cross-linker - was used in a process called ionic gelation. Three definite solutions were prepared with chitosan-to-erucin ratios of 0.5:1, 1:1, and 1.5:1. Then, centrifugation performed to purify the resulting nanoparticles which were characterized using Scanning Electron Microscopy (SEM), Energy Dispersive X-ray (EDX), and Zeta potential analysis to evaluate morphology, elemental composition, and surface charge, respectively.

1. Introduction

Nanotechnology is a continuous development in the field of drug delivery, offering innovative solutions to improve therapeutic efficacy and reduce side effects of pharmaceutical compounds (Koynova, et al. ,2025). Chitosan nanoparticles are among the most prominent natural drug carriers that have received significant attention in recent years due to their outstanding properties, such as biocompatibility, biodegradability, and chemical modification (Akdaşçi, et al. ,2025). At the same time, interest has grown in bioactive compounds extracted from natural plants, particularly those belonging to the isothiocyanate family, such as erucin from Jirjeer (Eruca sativa) which exhibits anti-cancer and anti-inflammatory activities (Rizwan, & Masoodi, 2023).

The combination of nanotechnology and natural active compounds represents a new trend in the development of advanced drug delivery systems. Nanoparticles can improve the chemical stability and bioavailability of natural compounds while reducing their systemic toxicity through targeted delivery (Çiftçi, et al.,2025).

1.1 Chitosan Nanoparticles: Properties and Applications

1.1.1. Physical and Chemical Properties

Chitosan is a natural polymer derived from chitin and possesses unique properties that make it suitable as a nano pharmaceutical carrier. Their particles are characterized by their positive charge resulting from amino groups, enabling them to interact with negatively charged cell membranes and enhance absorption across mucosal membranes (Omidian, et al., 2024). These particles also exhibit high chemical modifiability, allowing the attachment of various functional groups to improve loading properties and controlled release (Herdiana, et al., 2024).

Chitosan nanoparticle sizes typically range from tens to hundreds of nanometers, placing them in the ideal range for biomedical applications. This nanoscale size enables the particles to bypass

biological barriers and achieve effective distribution in target tissues. In addition, these particles demonstrate good stability in physiological environments and the ability to protect the loaded compounds from degradation (Jha, , et al. ,2024).

1.1.2. Medical and Pharmaceutical Applications

Chitosan nanoparticles are used in a variety of medical applications, ranging from topical skin delivery to complex applications such as delivery across the blood-brain barrier (Gomes, et al. ,2024). In oncology, these particles have proven effective in improving the delivery of anticancer drugs while reducing their side effects through precise targeting of tumor cells (Yadav, et al. ,2024).

Chitosan nanoparticles are also used in antimicrobial applications, where they exhibit natural activity against bacteria and fungi, in addition to their ability to carry antibiotics and enhance their effect (Shi, S., et al.,2024). These particles are also used to improve drug delivery to the lungs with increased stability and controlled release (Mercan, & Selçuk, 2024).

1.2. Eruca sativa

Eruca sativa is a rich source of bioactive compounds, containing a wide variety of glucosinolates, with quantitative and qualitative variations depending on geographical origin and growing conditions. In addition to glucosinolates and their isothiocyanate derivatives, it contains phenolic compounds and flavonoids such as isoramantin and rhamantin (Kumar , et al.,2022)

The plant also contains other sulfur compounds such as dimethyl succinate (DMTS), as well as chlorophyll and pigment compounds that contribute to antioxidant activity This chemical diversity makes watercress a valuable source of natural compounds with multiple therapeutic potentials (Kumar , et al.,2022) .

1.3. Erucin Properties and Biological Activities (Chemical Structure and Properties)

Erucin belongs to the isothiocyanate family, which is a group of sulfur compounds derived from glucosinolates found in plants of the Brassicaceae family These compounds are characterized by their chemical structures containing the isothiocyanate group (-N=C=S), which is responsible for their biological activity (Hoch, et al. ,2024).

Erucin exhibits variable physicochemical properties in terms of solubility and stability, making the development of improved delivery systems essential to maximize its therapeutic potential (Wang, et al. , 2024) . Isothiocyanates undergo biochemical changes in the body, which may affect their efficacy and bioavailability (Narra, et al., 2025).

Isothiocyanates, including erucin, exhibit multiple anticancer mechanisms. These mechanisms include 1) inducing oxidative stress within cancer cells (Kyriakou, et al. 2024). 2) activating the intrinsic pathway of apoptosis by affecting mitochondria and causing loss of membrane polarity, which leads to the activation of caspases and the initiation of the apoptotic process (Tang & Zhang,2005.) 3) The ability to disrupt the cell cycle and halt cancer cell division at specific stages (Zhang, et al.,2023).

Isothiocyanates exhibit anti-inflammatory properties by modulating various inflammatory signaling pathways. These compounds inhibit the transcription factor NF-κB, a key regulator of the inflammatory response, and activate the Nrf2/Keap1 pathway responsible for the antioxidant response. These mechanisms contribute to reducing the production of inflammatory mediators such as inflammatory cytokines and prostaglandins, making these compounds promising candidates for the treatment of chronic inflammatory diseases. Some studies also show that these compounds may help alleviate inflammation associated with tumors and metabolic diseases(Habtemariam,2024).

Researches indicate that erucin may act by inhibiting quorum sensing and virulence in pathogenic bacteria like Escherichia coli and Pseudomonas aeruginosa in a concentration-dependent manner. This suggests it could disrupt bacterial communication and reduce their pathogenicity (Romeo, et al.,2018).

1.4. Nanotechnology in Drug Delivery

1.4.1. Basic Principles and Mechanisms

Nanotechnology in drug delivery relies on the use of various Nano carriers such as liposomes, dendrimers, and polymeric molecules to enhance the delivery of therapeutic compounds to target sites in the body (Cheng, et al., 2023). This technology relies on two main targeting mechanisms: passive targeting through the effect of increased vascular permeability (EPR), and active targeting using ligands or antibodies immobilized on the surface of the Nano carrier (Cheng, et al., 2023).

Nano systems enable controlled release induced by environmental conditions such as changes in pH or temperature, ensuring drug release at the appropriate site and time .This technology also allows for the integration of diagnostic and therapeutic functions into a single platform, paving the way for the development of advanced therapeutic systems (Kim, et al. ,2024) .

1.4.2. Advantages and Benefits

Nanotechnology in drug delivery offers multiple advantages that make it a promising technology in the medical field. These advantages include improving drug bioavailability by protecting active compounds from degradation and enhancing their absorption and distribution in the body. They also contribute to reducing systemic toxicity by precisely targeting drugs to diseased tissues, thus reducing exposure of healthy tissues to the active substance (Zubair, et al. ,2024).

Nanotechnology enables controlled and prolonged drug release, reducing dosing frequency and improving patient compliance with treatment . In addition, the nanoscale properties of carriers help them bypass challenging biological barriers such as the blood-brain barrier, opening up new possibilities for treating diseases of the nervous system (Koynova, et al. ,2025).

1.4.3. Challenges and Limitations

Despite the promising potential of nanotechnology, this technology faces numerous challenges that limit its clinical deployment. These challenges include the potential toxicity risks of nanomaterials, which may include oxidative stress and inflammatory responses .It also faces challenges in large-scale manufacturing, as the production of homogeneous and stable particles requires complex and expensive processes (Çiftçi, et al. ,2025).

Regulatory requirements for the approval of nanoproducts are complex, as the behavior of the nanocarrier, the drug payload, and biological interactions must be evaluated, increasing development time and cost . In addition, biological variability between patients and tissues is a challenge that requires the development of customized strategies to achieve optimal efficacy (Csóka, et al., 2021).

1.4.4. Therapeutic and Application Potential

The development of chitosan nanoparticles loaded with aerucin holds promising therapeutic potential in several areas. In oncology, this system could combine the precise targeting provided by chitosan nanoparticles with the anticancer activity of aerucin, potentially leading to the development of more effective and less toxic treatments (Herdiana, et al. ,2024).

In preventive medicine, this system could be used as an advanced nutritional supplement or chemo preventive agent, especially for individuals at risk of cancer. Further applications could also be explored in regenerative medicine and wound healing, where the anti-inflammatory and antimicrobial properties could contribute to accelerating the healing process (Zubair, et al. ,2024).

Aim and Future Directions

This study aims to explore the potential of manufacturing chitosan nanoparticles as a carrier for erucin extracted from Eruca sativa as it represents an important step in the development of advanced natural drug delivery systems. This approach combines the advantages of nanotechnology with the potential of active natural compounds which may open new horizons in the field of nanotechnology-assisted physical therapy.

2. Method

2.1 Erucin Extract

200 g of fresh Eruca sativa leaves were washed, then chopped into small pieces and mixed with distilled water at a 1:3 w/v ratio. The mixture was left for 20–30 minutes at room temperature to

allow the natural enzyme myrosinase to convert glucoerucin to erucin (Hanschen, et al., 2014), then it was transferred to a separating funnel, and an organic solvent dichloromethane (DCM) was added. The funnel was shaken gently to allow the separation of the aqueous layer from the organic layer which contained erucin and other organic compounds, the process is repeated two or three times to increase the extracted amount. The organic layers was collected in a clean beaker, washed with saline to remove polar impurities (Bell, et al., 2020), then anhydrous sodium sulfate (Na_2SO_4) was added and mixed for a few minutes to absorb the remaining water (Moldoveanu & David, 2021). The extract is filtered to obtain a dry organic solvent. The solvent is removed using a rotary evaporator under reduced pressure and a temperature below 30 °C to avoid the decomposition of erucin (Poulev, et al., 2003).

2.1.1. Erucin identification by HPLC (HPLC analysis)

HPLC model SYKAM (Germany) was used to analyze and detect anthocyanin. The mobile phase was an isocratic flow of a 95 / 5 (v/v) mixture of water (pH 7.0) and (2 %) formic acid flow rate at 0.8 mL/min , column was C18 – ODS (25 cm * 4.6 mm) and the detector UV-Vis at = 520 nm .

2.1.2. Preparation of erucin solution

To prepare 2% of Erucin solution; 2 g of the extract was dissolved in 100 ml of 85% ethanol using magnetic stirrer for 1 hour. Adjust the pH of the solution to 5.0.

2.2. Chitosan Solution Preparation

100 ml of a 1% v/v acetic acid solution was first prepared by adding 1 ml of concentrated acetic acid to 99 ml of distilled water. 2 grams of chitosan were then dissolved in this acid solution under continuous stirring using a magnetic stirrer for 12–18 hours until the chitosan completely dissolved. After ensuring dissolution, the pH of the solution was adjusted to 5.0 using 0.1M NaOH solution. The final result was a 2% chitosan solution ready for use in laboratory applications (Sikorski, et al., 2021).

2.3. Chitosan encapsulated erucin

Three clean flasks were prepared with 5 ml , 10ml and 15 ml of chitosan solution respectively, the flasks were placed on magnetic stirrers, and erucin solution was slowly added dropwise at a constant volume of 10 ml to each flask with continuous stirring for 60 minutes which were resulted in chitosan-to-erucin ratios of 0.5:1, 1:1, and 1.5:1 .

The three solutions were placed in an ultrasonic bath for 10 minutes to ensure the homogeneity of the solution and the deposition of erucin within the chitosan particles. At the same time, the pH was monitored and maintained within the range of 4.5–5.0 to ensure the stability of phenolic compounds and to protect erucin from degradation (Bhardwaj, et al.,2025; Singh, et, al. ,2023).

2.4 TPP Solution and Nano particles preparation

Sodium tripolyphosphate (TPP) was dissolved in distilled water at a concentration of approximately 1 mg/ml, stored at 0-2C for 4 hours (Esquivel, et al., 2015).

The TPP solution was added dropwise to erucin -loaded chitosan solution in ice bath on magnetic stirrer at 600–1000 rpm (Gomes, et al. ,2024) . A Nano suspension was observed, with cloudiness or slight turbidity which was gradually transformed to a uniform white or light yellow turbidity, indicating ionic crosslinking between the positively charged chitosan and the negatively charged TPP (Jiang, et al., 2024). At this point, the addition of TPP was discontinued to avoid the formation of large particles or agglomerates. The nanoparticles were separated from the free extract by centrifugation, and washed with (phosphate buffer solution) PBS solution to ensure particle purification (Gutiérrez-Ruíz ,et al., 2024). The process was repeated two to three times to remove any residual unloaded extract or salts. For characterization, the three samples were sent for particle size and shape examination by SEM, Zeta potential and EDX examination.

3. Results

Since chitosan is what creates nano- or micro-particles, it is the most important variable in the study. The effects of increasing the volume or concentration of chitosan on stability, particle size, and encapsulation efficiency are compared, that's why, modification aids in figuring out the ideal ratio for the active ingredient's encapsulation.

Particle size, encapsulation effectiveness, and physical stability are frequently improved by increasing chitosan.

3.1. Erucin extract HPLC

The curve shows property values which include: 5.85 minutes retention time , 1419.74 mAU·s area under the curve, 710.00 mAU peak height, 100% area percentage (only one compound), 0.15 minutes peak width at half height (W 0.5). The quantity of the is reflected as "the area under the peak " while, the concentration is accurately determined by a comparison with the calibration curve. At the same time, the peak width represent the quality of the separation; the wider the peak, the less precise the separation. Since no other compounds are visible in the chromatogram and the sample contains only one compound (Erucin), this is mean that the sample was separated and analyzed accurately with a clear and defined peak, figure (1).

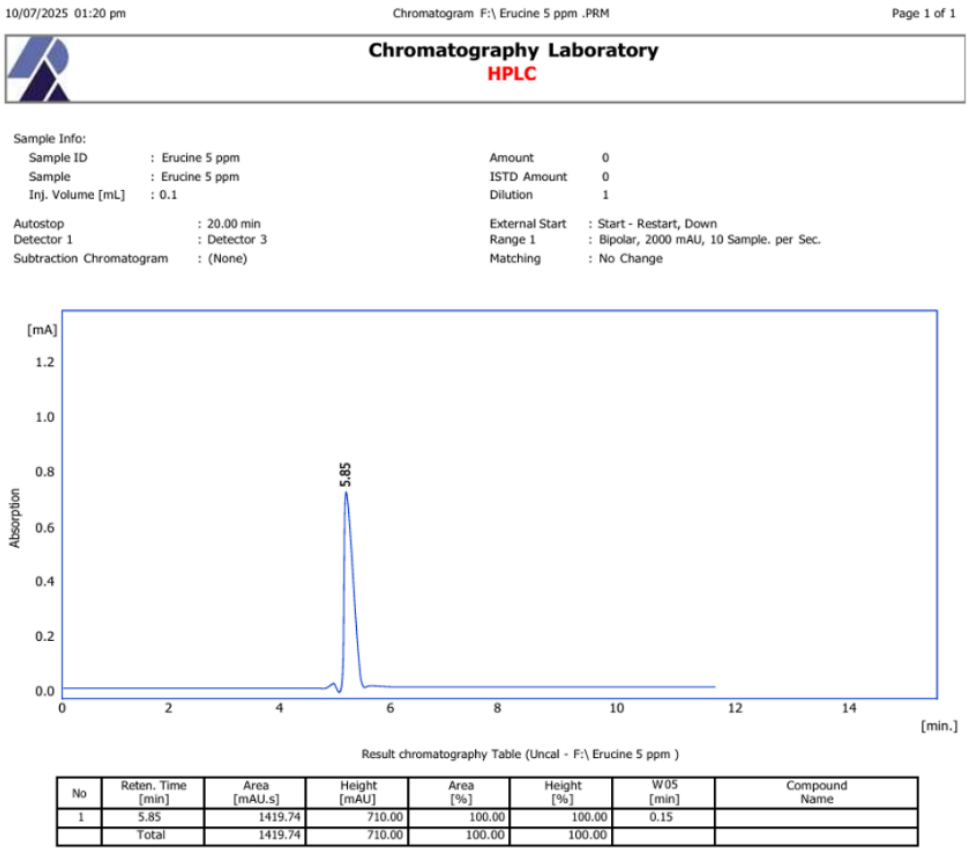


Figure 2. Erucin extract HPLC.

3.2. Scanning Electron Chromatography (SEM)

SEM images show a very porous sponge-like structure. Chitosan-based matrices created by special methods ionic gelation, frequently have these connected pores.

Pores appear uneven but uniformly distributed, with diameters in the micron range. Even though the entire matrix is porous at the micro-scale, the encapsulated erucin nanoparticles might reside inside these networks or in the smaller nano-domains that are not individually resolved .

The increment in surface area through the porous morphology suggests facilitates drug loading, in addition, Erucin encapsulated in chitosan is likely protected from degradation by the holes that function as diffusion channels for controlled release.

Also these open porosity network suggests that the technology may allow erucin to be released gradually, which is appropriate for prolonged delivery.

Rough, uneven texture supports strong trapping of the bioactive chemical.

The muco adhesive and biodegradable nature of chitosan makes this system potentially useful for oral, topical, or injection drug administration.

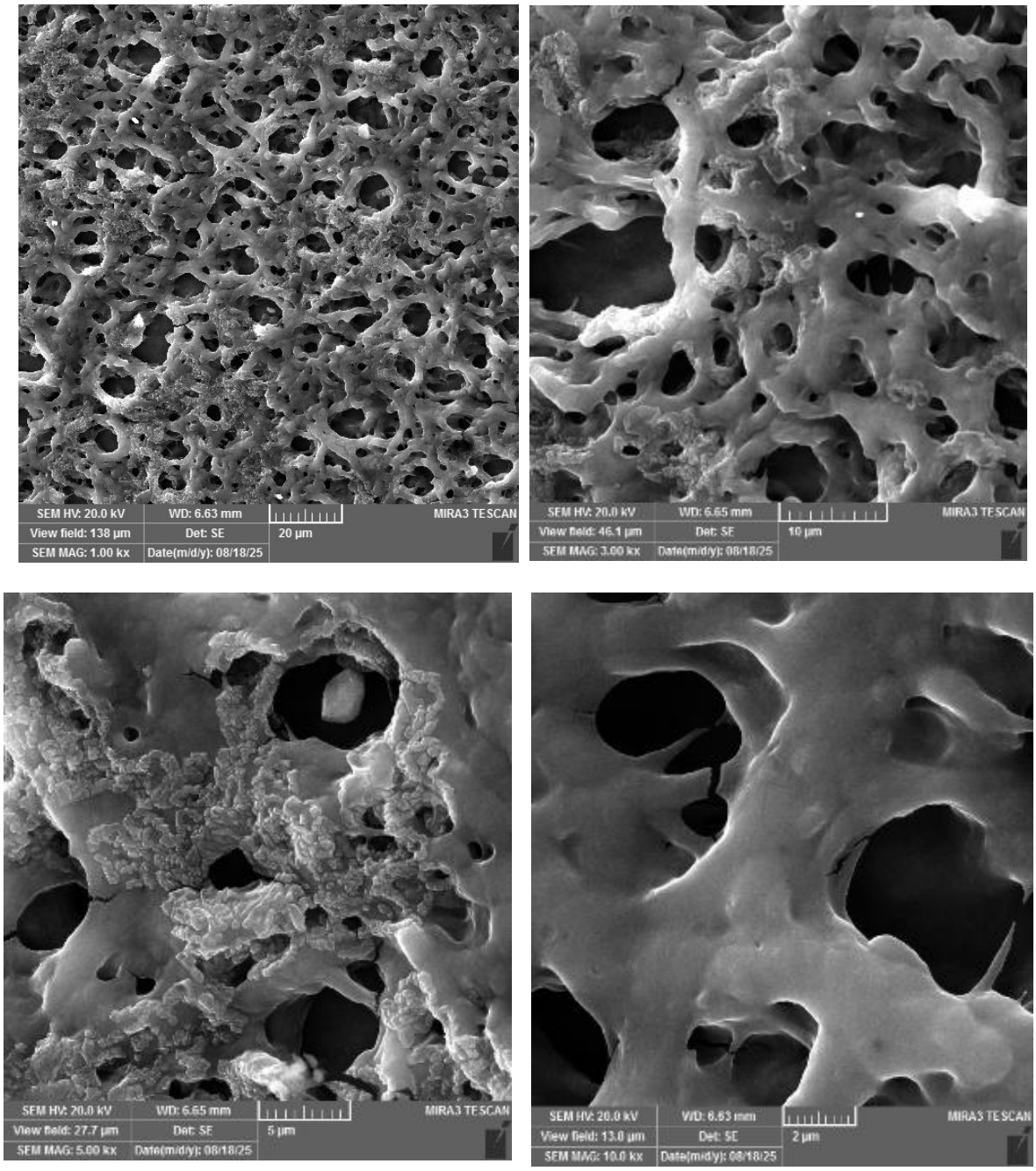


Figure 2. Erucin extract HPLC

3.3. EDS (Energy Dispersive X- ray Spectrometer)

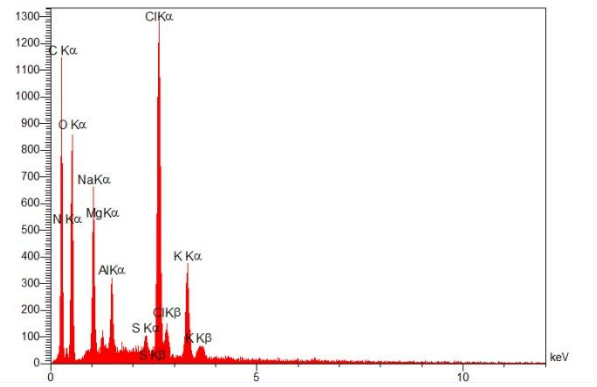
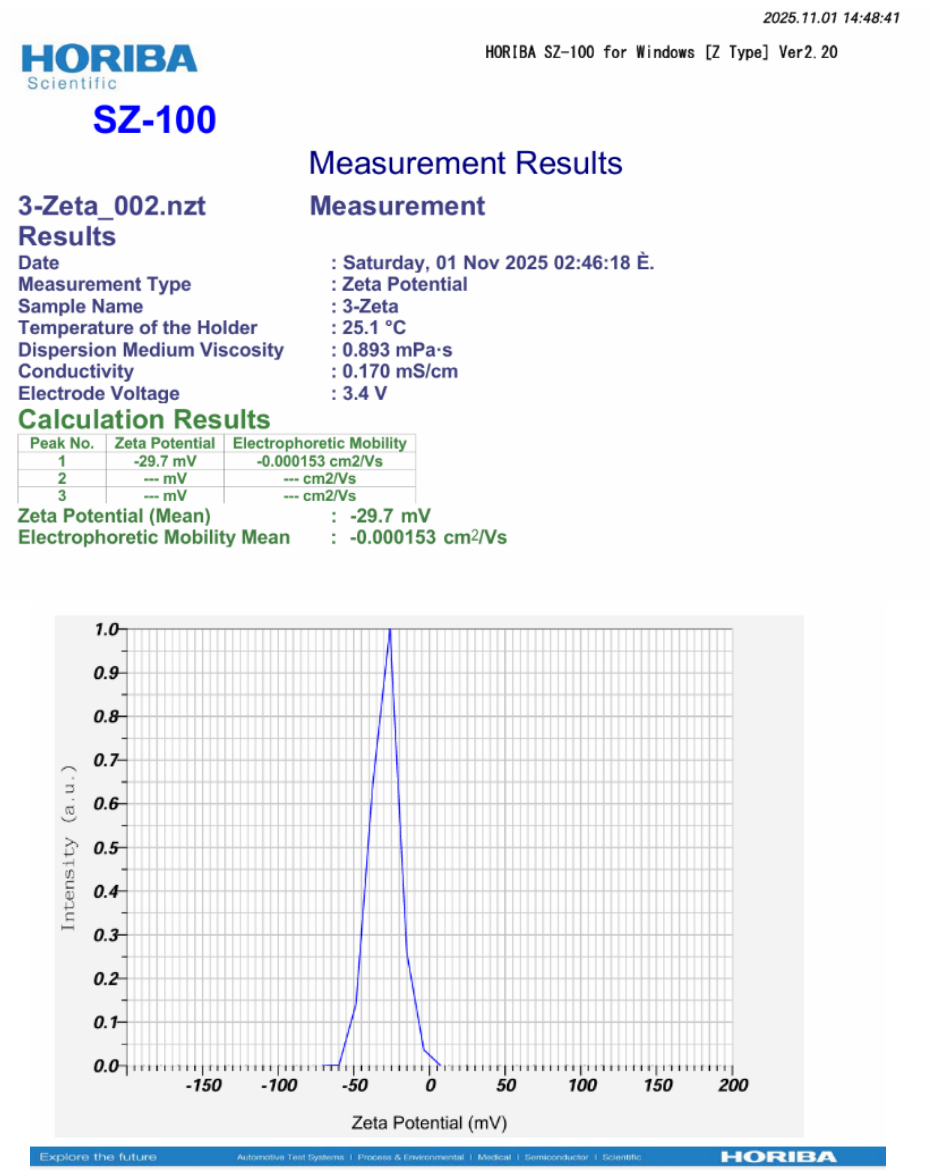


Figure 3. Chitosan loaded erucin nanoparticles EDS.

3.4. Zeta potential

In zeta potential teqnique; measured temperature is 25.1°C, medium viscosity is 0.893 mPa·s, with 0.170 mS/cm conductivity ,and 3.4V electrode voltage. Zeta potential is −29.7 mV with a distinct peak around the same value. The particle dispersion state in the medium is thus logically depicted.



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Measurement Results

3-Zeta_002.nzt

Measurement Results

Date	: Saturday, 01 Nov 2025 02:46:18 È.
Measurement Type	: Zeta Potential
Sample Name	: 3-Zeta
Temperature of the Holder	: 25.1 °C
Dispersion Medium Viscosity	: 0.893 mPa·s
Conductivity	: 0.170 mS/cm
Electrode Voltage	: 3.4 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-29.7 mV	-0.000153 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean) : -29.7 mV

Electrophoretic Mobility Mean : -0.000153 cm²/Vs

Figure 4. Zeta potential report.

4. Discussion

Ionic gelation, as a method for nano preparation, can be carried out at lower temperatures, like 4°C, which can slow down the reaction's kinetics and produce smaller, more uniformly distributed nanoparticles. By improving the dispersion of the reactants, high agitation rates during the synthetic process can aid in a particle size reduction. Chitosan solution's pH should ideally fall between 4.5 and 5.5 otherwise, Particle sizes and quality may be reduced as a result of uncontrolled aggregation phenomena caused by departures from this designated range (Sreekumar, et al., 2018).

The employment of scanning electron microscopy is to scan the surface morphology and structural quality or characteristics of these nanoparticles. High-resolution images produced by SEM demonstrate the smoothness and homogeneity of the coated nanoparticles, which is essential for ensuring reliable drug release profiles (Leonard, et al, 2012). By using SEM to characterize these nanoparticles, scientists can assess important factors that affect how they behave in biological systems (Mardani,et al.,2017).

SEM results for erucin-loaded chitosan nanoparticles would probably show a distinct surface morphology, indicating stable and efficient drug encapsulation within the nanoparticle matrix, also an understanding of the nanoparticles' interactions with biological tissues and possible biodistribution, which can only be achieved through this characterization (El-Saadony,et al., 2025).

Increased polymer concentrations lead to more contacts and the formation of larger and more compact particles or aggregates through the electrostatic attraction between negatively charged polyanions, such as tripolyphosphate (TPP), and positively charged chitosan amino groups (Abd El Hady, et al., 2024). More complex and extensive cross-linked structures can be formed by more polymer chains which can explain agglomerates formation (Gutiérrez-Ruíz,et al., 2024). For instance, depending on the chitosan ratio and the technique used, chitosan nanoparticle formulations have shown size variations which are consistent with other articles providing chitosan nanoparticles ranging from 40 nm to 299.8 nm (Sreekumar, et al., 2018).

A research has demonstrated that encapsulation efficiency (EE), which varies from 26% to 87% based on formulation ratios, rises with chitosan content (Cheng, et al., 2023). The optimal chitosan-to-active ingredient ratio is very important to determine the maximum capacity for encapsulation process before stability issues arise.(Cheng, et al., 2023)

EDS analysis was used to verify the presence of oxygen, a key component of erucin and chitosan. Nevertheless, carbon and nitrogen were not found in this analysis; this is typical in X-ray microanalysis since light elements' low-energy X-rays are readily absorbed by the sample or the detector's window, frequently falling below the trustworthy detection threshold (Newbury, et al., 2015).

Sodium, magnesium, and chlorine were considered as contaminants which are most likely came from sources like improper cleaning glassware with chlorine or reagents like TPP as a cross-linking compound or NaOH as a buffer.

A zeta potential of -29.7 mV indicates that the produced nanoparticles have a moderate net negative surface charge when dispersed in the measurement medium (likely water or a buffer).

-29.7 mV places the suspension on the cusp of being stable. It is very close to the -30 mV threshold but not quite over it.

Finding a negative Zeta potential for chitosan nanoparticles which is naturally positive; in many cases, it comes down to "over-shielding" the particles by an excess of TPP which carries a strong negative charge (Gonçalves, et al., 2023) .During the ionic gelation process, the negative phosphate groups of TPP neutralize the positive amino groups (NH_3^+) of chitosan. Excess anionic cross-linker carries significant implications for their colloidal stability and overall physicochemical behavior which refers to the resistance of a nanoparticle dispersion to aggregation and sedimentation over a defined period which ensure paramount storage of nanoparticle formulations and their performance in various applications. (Gonçalves, et al., 2023).

pH of the liquid used for the measurement is another big factor, Since chitosan only keeps its positive "personality" in acidic conditions, any shift toward a neutral or basic pH causes it to lose its charge. Essentially, the negative reading tells that the outer layer of your particles is currently dominated by negative ions rather than the chitosan core itself.

5. Conclusion

In conclusion, we have successfully developed a novel nanocarrier-based drug delivery system through the use of ecologically benign ionic gelation techniques that employ the bioactive isothiocyanate, erucin, derived from the edible plant Eruca sativa for use as a target-specific, long-term release (sustained) formulation for the treatment/prevention of cancer and other diseases.

The successfully isolated, pure form of erucin was accomplished using high-performance liquid chromatography (HPLC) and resulted in a distinct peak (5.85 minutes' retention time) with remarkable clarity and accuracy due to precision in the extraction of the bioactive compound.

The loading ratio between chitosan and erucin was found to be a critical factor in the matrix architecture of the drug delivery system and its physical and morphological properties. The scanning electron microscope (SEM) provided evidence of a highly porous sponge-like microstructure which

functions to provide the most efficient matrix trap for the bioactive and to provide the necessary pathways for subsequent sustained-controlled release by diffusion.

Energy dispersive X-ray spectroscopy (EDS) confirmed the identity of the elemental structural components of the nanocarrier and the presence of the major element—oxygen—and minimal trace impurities from the mineral processing of the cross-linked sodium tripolyphosphate (TPP) in the overall structure.

Zeta potential analysis revealed a net negative charge of -29.7 mV, thereby confirming the electrostatic "over-shielding" effect of the abundant polyanionic sodium TPP cross-linkers over the chitosan (cationic) core, thus bringing the colloidal dispersion very close to the physical stable range of zeta potentials.

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