MICROENCAPSULATION OF *ORIGANUM MAJORANA* L. ESSENTIAL OIL FOR ANTIBACTERIAL APPLICATIONS IN DRUG DELIVERY SYSTEMS

Elif Can Aydogdumu[1], Deniz Akin Sahbaz[1,2]*, Nazan Karapinar[1]

[1]Department of Chemical Engineering, Faculty of Engineering, Pamukkale University, Denizli, 20070, Turkey

[2]Department of Environmental Engineering, Faculty of Çorlu Engineering, Tekirdağ Namık Kemal University, Çorlu, Tekirdağ, 59860, Turkey

Abstract

Microencapsulation is an advanced technique used to protect active ingredients by encapsulating them within a protective shell, enabling controlled release in various fields, including pharmaceuticals, food, agriculture, cosmetics, and chemistry. This study aims to synthesize and characterize the microencapsulation of Origanum majorana L. essential oil to impart antibacterial properties for potential applications in drug delivery systems. Origanum majorana L., a plant native to the Mediterranean region and widely cultivated in Turkey, is recognized for its antimicrobial activity against various microorganisms. The microencapsulation process was carried out using the coacervation method, and the effect of ultrasonication time on the structure of the nano/microcapsules was evaluated. The resulting nano/microcapsules were characterized using scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), and differential scanning calorimetry (DSC). Additionally, the surface oil and total oil content of the nano/microcapsules were determined, and their encapsulation efficiency were calculated. The results revealed that ultrasonication time significantly influenced the morphology, structural interactions, and thermal behavior of the microcapsules. Shorter sonication durations yielded more compact and thermally resistant polymeric structures, while intermediate durations provided a favorable balance between stability and porosity. These findings highlight the potential of Origanum majorana L. essential oil-loaded microcapsules as promising candidates for antimicrobial delivery systems with enhanced structural integrity and thermal performance.

Keywords: *Microencapsulation, Origanum Majorana L., drug delivery systems, complex coacervation, ultrasonication*

1. INTRODUCTION

The demand for natural, bio-based compounds with therapeutic potential has significantly increased in recent years due to rising concerns over synthetic additives, antibiotic resistance, and environmental sustainability. Essential oils, in particular, have attracted considerable attention as alternatives to synthetic antimicrobials owing to their broad-spectrum biological activities, including antibacterial, antifungal, antiviral, antioxidant, and anti-inflammatory properties [1-2]. Among these, *Origanum majorana* L. (commonly known as sweet marjoram), a medicinal and aromatic plant widely cultivated in the Mediterranean region and especially in Turkey, has been recognized for its strong antimicrobial potential [3]. Its essential oil contains a complex mixture of bioactive components such as carvacrol, thymol, γ-terpinene, and terpinen-4-ol, which are known to disrupt bacterial membranes and inhibit microbial growth [4].

Despite its promising biological activity, the direct application of *Origanum majorana* L. essential oil in pharmaceutical formulations faces significant limitations due to its high volatility, poor water solubility, and sensitivity to heat, light, and oxygen [5]. These characteristics lead to rapid degradation and loss of efficacy over time. To overcome these challenges, microencapsulation has emerged as a powerful and versatile technique for improving the stability, bioavailability, and targeted delivery of essential oils [6]. By entrapping the oil within a protective matrix or shell, microencapsulation not only

protects the core material from environmental degradation but also enables controlled and sustained release, which is particularly important in drug delivery applications [7-8].

Among various encapsulation strategies, complex coacervation is a widely employed method that involves the phase separation of two oppositely charged polymers under controlled pH and temperature conditions [9]. This technique is especially suitable for encapsulating hydrophobic or volatile compounds such as essential oils, as it offers high encapsulation efficiency and tunable capsule morphology [10]. Moreover, recent advancements have introduced the use of ultrasonication as an auxiliary method to enhance dispersion, reduce droplet size, and improve capsule uniformity during the coacervation process [11-13].

In the present study, nano/microcapsules containing *Origanum majorana* L. essential oil were synthesized and characterized via the complex coacervation method, with particular emphasis on their antibacterial potential for application in drug delivery systems. The influence of ultrasonication duration on capsule formation and morphology was systematically evaluated to optimize encapsulation conditions. The resulting capsules were analyzed using field emission scanning electron microscopy (FESEM) for morphological evaluation, Fourier-transform infrared spectroscopy (FTIR) for chemical structure confirmation, and thermal analyses including thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). This research contributes to the growing body of work on natural, plant-derived antimicrobial delivery systems by demonstrating the effectiveness of *Origanum majorana* L. essential oil microcapsules as promising alternatives to conventional antimicrobial agents. The findings offer valuable insights for the formulation and optimization of essential oil-based carriers with enhanced stability, controlled release, and improved efficacy in pharmaceutical applications.

2. MATERIALS AND METHODS

2.1. Materials

Origanum majorana L. essential oil, used as the core material in this study, was obtained from HEAL&FLORA (Istanbul, Turkey) and HLY Aromaterapi (Istanbul, Turkey). Gelatin (type B, gel strength 220 Bloom) and sodium alginate, used as wall-forming biopolymers, were supplied by Alfasol (Istanbul, Turkey). Glutaraldehyde (50 wt.% solution in ethanol; Acros Organics) was used as a cross-linking agent in the complex coacervation process. Hydrochloric acid (HCl, 37%) and sodium hydroxide (NaOH, reagent grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Methods

2.2.1. Synthesis of Origanum majorana L. essential oil microcapsules

The microcapsules were synthesized via the complex coacervation method [14-15]. During the synthesis, 100 mL of gelatin solution (6%, w/w) and 25 mL of sodium alginate solution (2%, w/w) were first prepared separately. The gelatin solution was continuously stirred using a magnetic stirrer (Isolab Laborgerate GmbH, Germany) at 1000 rpm and maintained at 40 °C. Then, 2.5 mL of *Origanum majorana* L. essential oil was gradually added to the gelatin solution under constant stirring. After 5 minutes of stirring, the emulsion was formed by applying ultrasonic treatment using an ultrasonicator device (Bandelin, HD4100, Germany) at varying durations (5, 10, 15, and 20 minutes). The vibration induced by the ultrasonic waves facilitated the dispersion of oil droplets into the aqueous phase. Successful emulsification was visually confirmed by the formation of a milky-white emulsion, indicating uniform droplet dispersion. Following the emulsification, the sodium alginate solution was added to the mixture at 40 °C. The pH of the solution was then adjusted to 4.0 using 0.5 M hydrochloric acid (HCl) to initiate the complex coacervation process. Once the desired pH was achieved, the emulsion was transferred to an ice bath and the temperature was gradually lowered to 10 °C. At this stage, 5 mL of glutaraldehyde solution (50 wt. % solution in ethanol) was introduced into the cold emulsion maintained at 10 °C, and the mixture was stirred for 1 hour at 1000 rpm using a magnetic stirrer to ensure uniform crosslinking. Upon completion of the reaction, the formed microcapsules were separated from the suspension by centrifugation (Nuve, NF 400, Turkey) at 4000 rpm for 60 minutes. The collected

microcapsules were then dried in a laboratory oven (Natural Convection Oven, JSON-100, Republic of Korea) at 30 °C.

The microcapsules synthesized with different ultrasonication durations were denoted as OMEO-MC- *x*, where *x* refers to the ultrasonication time in minutes (i.e., OMEO-MC-5, OMEO-MC-10, OMEO-MC-15, and OMEO-MC-20 for 5, 10, 15, and 20 minutes, respectively).

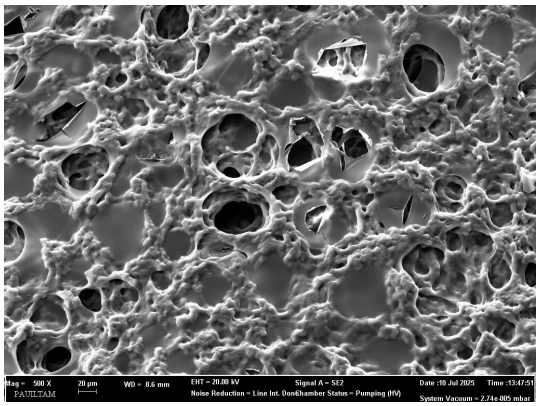
2.2.2. Characterization

The chemical structure of the microcapsules was analyzed using a Fourier Transform Infrared Spectrometer (Thermo Scientific Nicolet iS50 FT-IR). Spectral data were collected in the wavenumber range of 400–4000 cm^{-1}, with 50 scans at a resolution of 4 cm^{-1}. The thermal behavior of the microcapsules was examined using TGA and DSC. For this purpose, a known quantity of each sample was placed in a sealed aluminum pan and analyzed using a Simultaneous Thermal Analyzer (STA, Netzsch STA 449 F3, USA). The measurements were carried out under a dynamic nitrogen atmosphere (50 mL/min) at a constant heating rate of 10 °C/min. TGA thermograms were recorded and evaluated using NETZSCH Proteus Thermal Analysis software. The surface morphology of the microcapsules was observed using FESEM (Gemini Supra 40 VP, Zeiss, Germany) at an accelerating voltage of 20 kV. Prior to imaging, the samples were coated with a thin layer of gold using a sputter coater (Quorum Q150R ES, UK) to enhance conductivity. SEM images were acquired at magnifications of 500×.

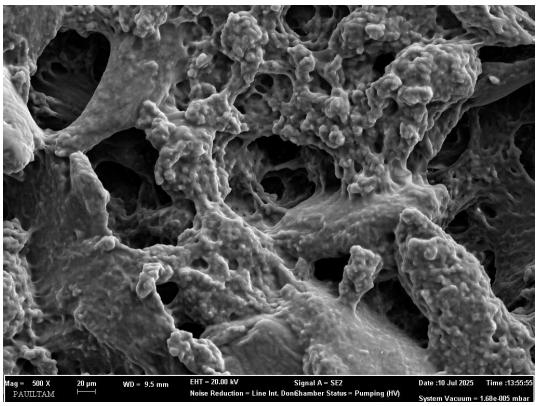
3. RESULTS AND DISCUSSION

3.1. Morphological evaluation of microcapsules

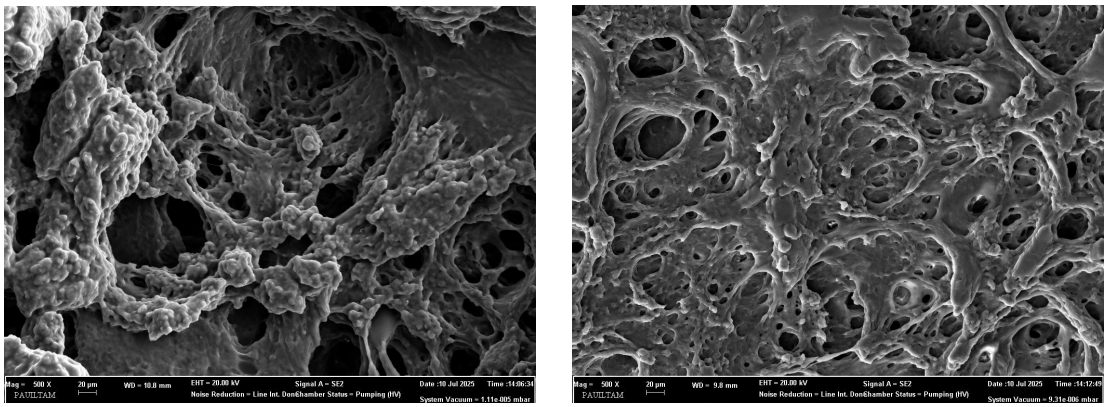
The surface morphology of *Origanum majorana* L. essential oil microcapsules synthesized at different ultrasonication durations was investigated using FESEM analysis. As shown in Figure 1, significant differences in the microcapsule structures were observed depending on the sonication time. In OMEO-MC-5 (Fig. 1a), the surface appears relatively smooth with fewer and smaller pores, indicating limited dispersion of the oil phase. OMEO-MC-10 (Fig. 1b) exhibits a more irregular and porous surface morphology, suggesting improved emulsification and more homogeneous microcapsule formation. For OMEO-MC-15 (Fig. 1c), a highly porous and interconnected network structure is evident, which may be attributed to optimal ultrasonic energy facilitating better droplet dispersion. However, in OMEO-MC-20 (Fig. 1d), the surface shows excessive porosity and partial wall disruption, possibly due to prolonged sonication causing structural degradation or over-fragmentation of the capsules. These results indicate that ultrasonication time plays a critical role in shaping the microcapsule morphology, affecting wall integrity, porosity, and uniformity.



(a)



(b)



(c) (d)

Fig. 1. FESEM images of OMEO-MC-5 (a), OMEO-MC-10 (b), OMEO-MC-15 (c), and OMEO-MC-20 (d) microcapsules at 500× magnification.

3.2. FTIR analysis of the microcapsules

FTIR spectroscopy was performed to investigate the chemical structure and possible interactions between the core (*Origanum majorana* L. essential oil) and the wall-forming biopolymers (gelatin and sodium alginate) in the synthesized microcapsules. The FTIR spectra of microcapsules prepared at different ultrasonication durations (OMEO-MC-5, OMEO-MC-10, OMEO-MC-15, and OMEO-MC-20) exhibited characteristic bands corresponding to both the essential oil and the encapsulating polymers.

All microcapsule samples showed a broad absorption band around 3200–3400 cm^{-1}, which can be attributed to O–H and N–H stretching vibrations, indicating the presence of hydroxyl and amine groups from gelatin and alginate [16]. The bands observed near 2920 and 2850 cm^{-1} correspond to the asymmetric and symmetric stretching vibrations of aliphatic $–CH_2$ groups [17], mainly originating from the essential oil components. A prominent peak around 1640–1650 cm^{-1} is assigned to the amide I band (C=O stretching) of gelatin, while the peak near 1540 cm^{-1} is attributed to amide II (N–H bending and C–N stretching) [18]. Additionally, absorption bands near 1400–1450 cm^{-1} and around 1030–1080 cm^{-1} can be associated with the symmetric stretching of carboxylate groups (COO^-) and C–O–C stretching vibrations from the alginate structure [19].

Comparing the FTIR spectra of OMEO-MC-5 to OMEO-MC-20, minor shifts and changes in peak intensity were observed, especially in the regions around 1640 cm^{-1} and 2920 cm^{-1}, which may indicate varying degrees of interaction and encapsulation efficiency influenced by the ultrasonication duration. The more defined bands observed in OMEO-MC-15 suggest improved emulsification and encapsulation, consistent with morphological observations. These results confirm the successful encapsulation of *Origanum majorana* L. essential oil within the gelatin–alginate matrix and demonstrate that ultrasonication time affects not only the morphology but also the structural interactions within the microcapsules.

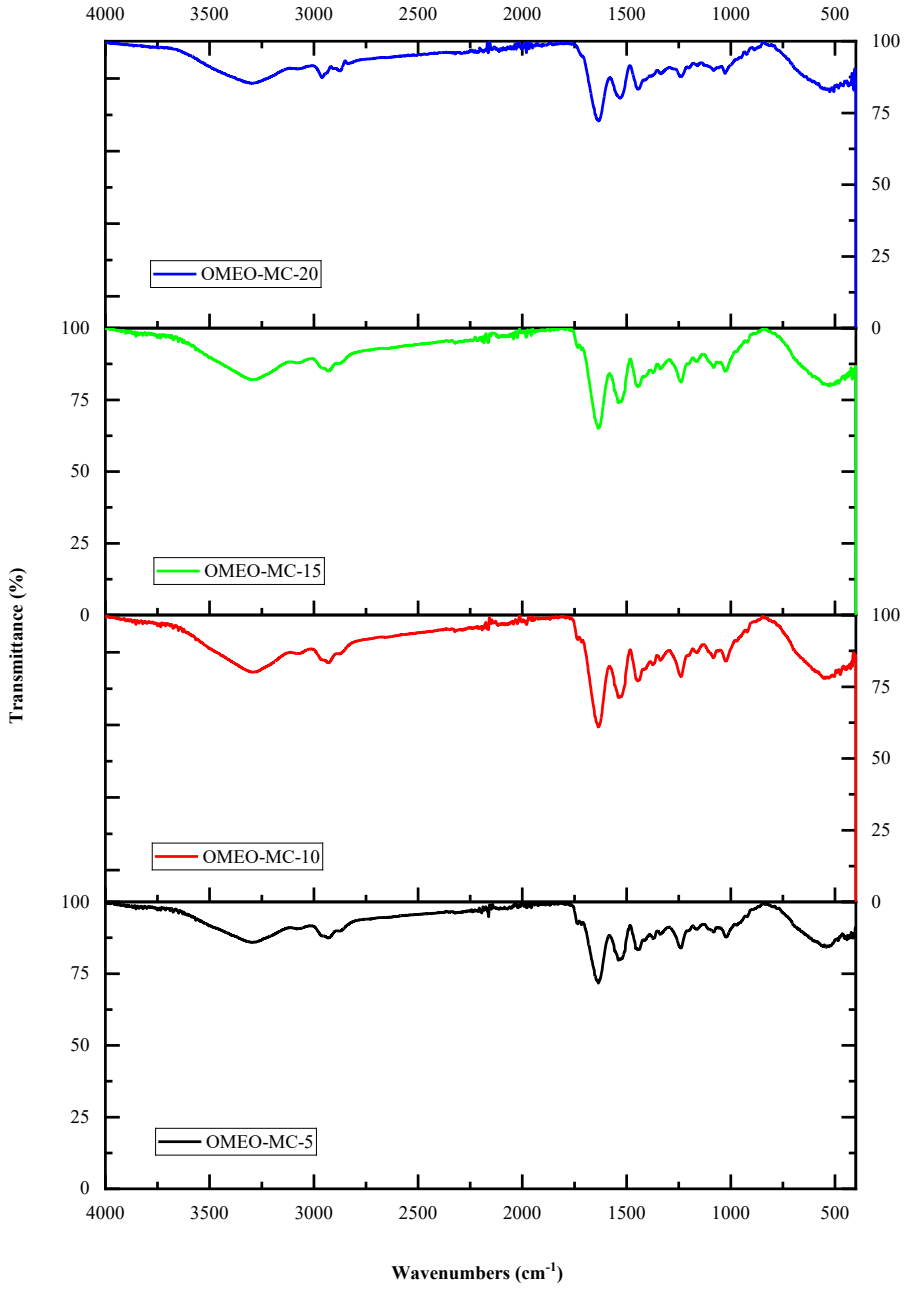


Fig. 2. FTIR spectra of OMEO-MC-5, OMEO-MC-10, OMEO-MC-15, and OMEO-MC-20 microcapsules prepared with different ultrasonication durations.

3.3. Thermal stability of microcapsules

The thermal stability of the microcapsules prepared at different ultrasonication durations was evaluated using TGA and DSC, as presented in Figures 3 and 4. The TGA thermograms (Figure 3) revealed multi-stage degradation profiles for all samples. The initial minor weight loss observed below 100 °C corresponds to the evaporation of physically adsorbed moisture [20]. The major degradation occurred

between approximately 200 °C and 400 °C and is attributed to the thermal decomposition of the gelatin–alginate wall matrix and the encapsulated *Origanum majorana* L. essential oil components [21-22].

Among all samples, OMEO-MC-15 exhibited the highest thermal stability, as indicated by a delayed onset of major degradation and a smoother, more gradual weight loss curve. This suggests that a 15-minute ultrasonication period may promote optimal droplet dispersion and effective crosslinking, resulting in enhanced thermal resistance. Although OMEO-MC-5 showed slightly higher residual mass at elevated temperatures, its earlier degradation onset suggests a less thermally robust structure. This behavior may result from insufficient emulsification and weaker matrix formation due to shorter sonication.

The DSC thermograms (Figure 4) provide further insight into the thermal transitions of the microcapsules. OMEO-MC-5 and OMEO-MC-10 demonstrated more pronounced and higher-temperature thermal transitions compared to OMEO-MC-15 and OMEO-MC-20. These characteristics indicate more compact and thermally resistant polymeric networks in the former two samples. In contrast, OMEO-MC-15 exhibited a broader and lower-temperature thermal transition, which may be associated with improved oil–polymer interactions and a more porous structure, consistent with its FESEM morphology and FTIR spectral characteristics. OMEO-MC-20 displayed the least defined transition, likely due to structural damage from over-sonication.

Overall, these results suggest that while OMEO-MC-5 and OMEO-MC-10 exhibit compact and thermally resistant wall structures, OMEO-MC-15 offers a favorable balance between thermal stability, encapsulation efficiency, and structural integration. Therefore, the 15-minute ultrasonication duration appears to be the most effective condition for achieving thermally stable and functionally optimized microcapsules.

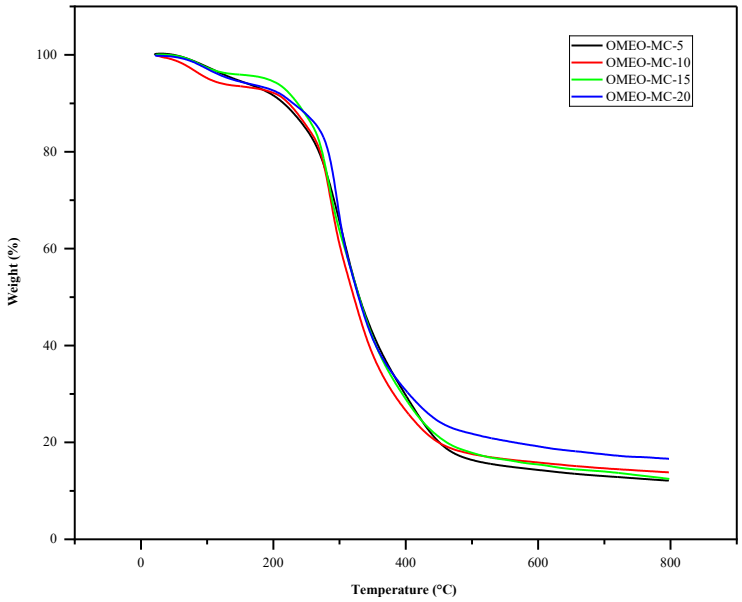


Fig. 3. TGA thermograms of OMEO-MC-5, OMEO-MC-10, OMEO-MC-15, and OMEO-MC-20 microcapsules, illustrating thermal degradation behavior and weight loss profiles.

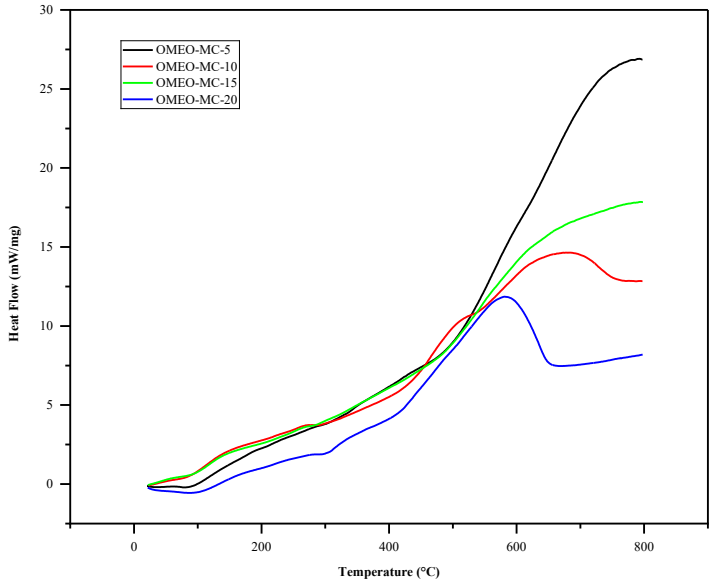


Fig. 4. DSC thermograms of OMEO-MC-5, OMEO-MC-10, OMEO-MC-15, and OMEO-MC-20 microcapsules illustrating thermal transitions and phase behavior.

4. CONCLUSIONS

This study successfully demonstrated the microencapsulation of *Origanum majorana* L. essential oil using a complex coacervation method with gelatin and sodium alginate as wall-forming biopolymers. Ultrasonication was systematically investigated as a key parameter affecting the formation, morphology, and thermal characteristics of the resulting microcapsules. The findings revealed that ultrasonication duration plays a critical role in determining the physical and chemical properties of the microcapsules.

Shorter ultrasonication durations, particularly 5 minutes, led to more compact and thermally resistant microstructures, as confirmed by FESEM, FTIR, TGA, and DSC analyses. Notably, a 15-minute ultrasonication treatment resulted in a more homogeneous and porous capsule network, offering a favorable balance between encapsulation efficiency and structural integrity. In contrast, prolonged ultrasonication (20 minutes) caused excessive wall disruption and diminished thermal stability.

These results demonstrate the significance of optimizing process parameters in essential oil microencapsulation for drug delivery applications. The *Origanum majorana* L. essential oil-loaded microcapsules developed in this study hold strong potential for antimicrobial delivery systems, offering enhanced protection, stability, and performance. Future work may explore the in vitro release behavior and antimicrobial efficacy of the optimized capsules to further support their application in pharmaceutical formulations.

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