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MOLECULAR INFLUENCE OF TERPENE COMPOSITION ON ENCAPSULATION EFFICIENCY AND CYTOCOMPATIBILITY OF PCL/PF68 NANOPARTICLES LOADED WITH ESSENTIAL OILS

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Abstract: Essential oils present promising biological activities; however, their volatility, instability, and low aqueous solubility limit direct application in health-related formulations. This study aimed to develop and characterize poly(ε-caprolactone) (PCL)/Pluronic F-68 (PF68) nanoparticles loaded with different essential oils (10% w/w) and to investigate the influence of terpene molecular characteristics on encapsulation efficiency and cytocompatibility. Nanoparticles were prepared by the nanoprecipitation method and evaluated by dynamic light scattering, zeta potential analysis, encapsulation efficiency determination, and in vitro cytotoxicity using L929 fibroblasts after 24 h exposure. All formulations exhibited nanometric diameters (approximately 205–213 nm), low polydispersity indices (0.11–0.13), and moderately negative zeta potentials (≈ −14 mV), indicating homogeneous and colloidally stable systems. Encapsulation efficiency ranged from 71% to 87% and was strongly influenced by terpene composition. Oils rich in highly hydrophobic monoterpene hydrocarbons, such as limonene, showed higher retention compared to formulations containing oxygenated or phenolic terpenes. Cytotoxicity assays demonstrated that all 10% formulations maintained cell viability above 79%, satisfying ISO 10993-5 criteria for non-cytotoxic materials. The results demonstrate that terpene hydrophobicity plays a central role in nanoparticle loading performance and that PCL/PF68 nanosystems represent a promising platform for essential oil stabilization in health-related applications.

Keywords: Polymeric nanoparticles; Essential oils; Encapsulation efficiency; Terpene hydrophobicity; Cytocompatibility; PCL

Introduction

Essential oils (EOs) are volatile natural compounds obtained from different plant parts, such as leaves, roots, flowers, fruits, and bark. Due to their complex chemical composition, mainly consisting of terpenes, phenolic derivatives, aldehydes, and alcohols, essential oils exhibit a broad spectrum of biological activities (Khalid & Ahmed, 2020; Oğuz, Oğuz & Güler, 2023). Their antimicrobial, antioxidant, anti-inflammatory, and antiviral properties have stimulated growing interest for applications in food, agricultural, and pharmaceutical contexts (Haro-González et al., 2021; Diass et al., 2021; Pedreiro et al., 2023; Sousa et al., 2023).

Despite their promising biological potential, the direct application of essential oils in health-related formulations remains limited. Their high volatility, susceptibility to oxidation, photosensitivity, and low solubility in aqueous environments may reduce stability and bioavailability, compromising their effectiveness (Diass et al., 2021; Kant & Kumar, 2022; Chakravarty, Parmar & Mandavgane, 2023). In addition, the variability in chemical composition depending on cultivation conditions and geographic origin can influence performance and reproducibility (Khalid & Ahmed, 2020; Oğuz, Oğuz & Güler, 2023).

To overcome these limitations, encapsulation strategies have been explored as a technological approach to improve stability, protect volatile compounds, and modulate release profiles. Polymeric nanosystems represent a particularly promising alternative, as they combine biocompatibility with structural versatility, enabling the incorporation of hydrophobic compounds

and contributing to enhanced dispersion in aqueous media.

Poly(ε-caprolactone) (PCL) is a biodegradable polyester widely employed in biomedical and pharmaceutical applications due to its favorable mechanical behavior and controlled degradation rate. When associated with nonionic surfactants such as Pluronic F-68 (PF68), the resulting nanosystems may present improved colloidal stability and steric stabilization in aqueous environments. Previous investigations from our research group have demonstrated the feasibility of PCL/PF68 nanostructures for bioactive compound incorporation (Ferreira; Menezes; Tavares, 2022; Ferreira; Menezes; Tavares, 2024).

Different essential oils present distinct chemical profiles that can directly influence their interaction with the polymeric matrix. Oils rich in phenolic constituents, such as eugenol, may exhibit stronger hydrophobic interactions with PCL, while oils predominantly composed of monoterpenes, such as limonene or terpinen-4-ol, may present different affinity behaviors. These interactions can impact nanoparticle size, surface charge, and encapsulation efficiency, ultimately affecting the physicochemical performance of the carrier system.

In this context, the present study aimed to develop and characterize PCL/PF68 polymeric nanoparticles loaded with different essential oils, including rosemary, clove, grapefruit, sweet orange, basil, and tea tree oils, using the nanoprecipitation method. The influence of oil type and concentration on the physicochemical characteristics of the nanosystems was evaluated, considering their potential application in health-related formulations.

Materials and Methods

Materials

Poly(ε-caprolactone) (PCL, Mn ≈ 80,000 g mol^{-1}) and Pluronic® F-68 (PF68, Mn ≈ 8,400 g mol^{-1}) were purchased from Sigma-Aldrich. Acetone (analytical grade) was obtained from Isofar. Essential oils of rosemary (*Rosmarinus officinalis*), clove (*Syzygium aromaticum*), grapefruit (*Citrus paradisi*), sweet orange (*Citrus sinensis*), basil (*Ocimum basilicum*), and tea tree (*Melaleuca alternifolia*) were supplied by Bioessências. All reagents were used as received, without further purification.

Preparation of Polymeric Nanoparticles

Polymeric nanoparticles were prepared by the nanoprecipitation method, adapted from previously reported procedures. Briefly, an aqueous phase was prepared by dissolving PF68 (0.2% w/v) in distilled water under magnetic stirring at room temperature for 1 h. The organic phase consisted of PCL (0.4% w/v) dissolved in acetone under magnetic stirring at 40 °C in a closed system to prevent solvent loss.

The organic phase was slowly added to the aqueous phase using a laminar flow funnel under continuous stirring. For the preparation of oil-loaded nanoparticles, the essential oils were previously incorporated into the organic phase at concentration of 10% (w/w) relative to the mass of PCL. After mixing, the colloidal systems were maintained under magnetic stirring in a fume hood for 72 h to ensure complete evaporation of acetone.

The resulting nanoparticle suspensions were filtered by gravity using a 14 μm pore-size filter to remove residual polymer aggregates. When necessary, distilled water was added to restore the initial aqueous volume. Part of each suspension was used for colloidal characterization, while the remaining samples were freeze-dried for further analyses.

Nanoparticle suspensions were frozen in liquid nitrogen for approximately 5 min and subsequently lyophilized for 72 h under the following conditions: pressure of 487 μHg, voltage of 217 V, and temperature of −47 °C. The obtained powders were stored in a desiccator until further characterization.

Dynamic Light Scattering (DLS)

The hydrodynamic diameter and polydispersity index (PDI) of the nanoparticles were determined by dynamic light scattering (DLS) using a Nano S90 Zetasizer. Samples were diluted in distilled water and vortexed for 1 min prior to analysis. All measurements were performed at 25 °C.

Zeta Potential Analysis

Zeta potential measurements were carried out using a NanoBrook ZetaPALS (Brookhaven Instruments). Each sample was analyzed at room temperature (25 °C), and results were reported as the average of ten measurements.

Encapsulation Efficiency

Encapsulation efficiency (EE%) was determined by ultraviolet–visible (UV–Vis) spectrophotometry through indirect quantification of non-encapsulated essential oil in the supernatant.

Freshly prepared nanoparticle suspensions were centrifuged at 10,000 rpm for 10 minutes to separate the nanoparticulate fraction from the unencapsulated oil. The supernatant containing the free essential oil was carefully collected and appropriately diluted for spectrophotometric analysis.

Calibration curves were constructed for each essential oil using standard solutions prepared in acetone at known concentrations. The wavelength of maximum absorbance (λmax) for each oil was determined by spectral scanning between 200 and 400 nm. Quantification was performed at the following wavelengths:

- Melaleuca oil: 222 nm
- Grapefruit oil: 212 nm
- Sweet orange oil: 212 nm
- Clove oil: 281 nm
- Basil oil: 259 nm
- Rosemary oil: 215 nm

The concentration of non-encapsulated oil in the supernatant was determined from the respective calibration curve. Encapsulation efficiency was calculated as the percentage of oil retained within the nanoparticles relative to the total amount initially added to the formulation. All analyses were performed in triplicate, and results were expressed as mean ± standard deviation.

In Vitro Cytotoxicity Assay

The cytotoxicity of unloaded and essential oil-loaded PCL/PF68 nanoparticles was evaluated *in vitro* using the murine fibroblast cell line L929, commonly employed for biocompatibility screening of biomaterials.

L929 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) antibiotic solution, and maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were seeded in 96-well plates at an appropriate density and allowed to adhere for 24 h prior to treatment.

Cell viability was assessed using the MTT assay. Briefly, following the 24 h exposure period, the treatment medium was removed and MTT solution (at a final concentration of 0.5 mg mL^{-1}) was added to each well. The plates were incubated for 3–4 h to allow the formation of formazan crystals by metabolically active cells. Subsequently, the supernatant was carefully discarded, and the formed crystals were solubilized using dimethyl sulfoxide (DMSO). Absorbance was measured at 570 nm using a microplate reader.

Cell viability was expressed as a percentage relative to the untreated control group, which was considered as 100% viability. All experiments were performed in triplicate, and results were presented as mean ± standard deviation.

Results and Discussion

Dynamic Light Scattering (DLS)

The hydrodynamic diameter of unloaded and essential oil-loaded nanoparticles (10% w/w) was evaluated by dynamic light scattering (Figure 1). Unloaded nanoparticles (NNP) presented a mean diameter of 184.3 ± 7.6 nm, confirming the formation of nanometric systems through nanoprecipitation.

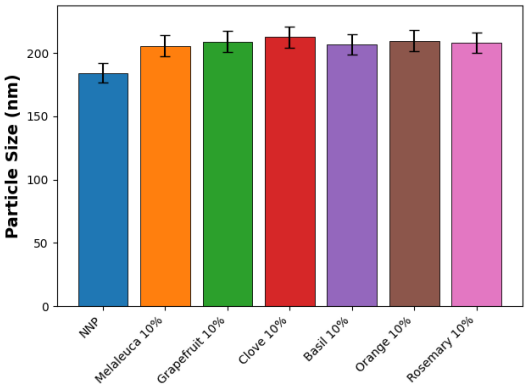

Figure 1. Hydrodynamic diameter of unloaded nanoparticles (NNP) and nanoparticles loaded with essential oils (10% w/w), determined by dynamic light scattering (DLS). Results are expressed as mean ± standard deviation (n = 3).

All formulations containing 10% essential oil exhibited increased particle sizes compared to the unloaded nanoparticles, with values ranging from approximately 205 to 213 nm. This increase indicates successful incorporation of the essential oils into the polymeric matrix.

Among the oil-loaded systems, nanoparticles containing clove oil (212.5 ± 8.6 nm) showed the largest mean diameter. This behavior is consistent with the predominance of eugenol in clove oil, a hydrophobic phenolic compound with high affinity for the PCL matrix. The strong hydrophobic interactions between eugenol and PCL likely favor greater incorporation within the polymer core, contributing to the slight enlargement of particle size.

Conversely, melaleuca-loaded nanoparticles showed slightly lower diameters (205.6 ± 8.4 nm) compared to other oil-loaded systems. The presence of oxygenated monoterpenes such as terpinen-4-ol may alter the interaction profile with PCL, in-

fluencing nanoparticle formation and resulting in comparatively smaller structures.

The polydispersity index (PDI) values remained low for all formulations, ranging from 0.11 to 0.13 (Figure 2). These results indicate narrow size distributions and high homogeneity among the nanosystems, suggesting good reproducibility of the preparation method and adequate colloidal stability — important characteristics for health-related applications.

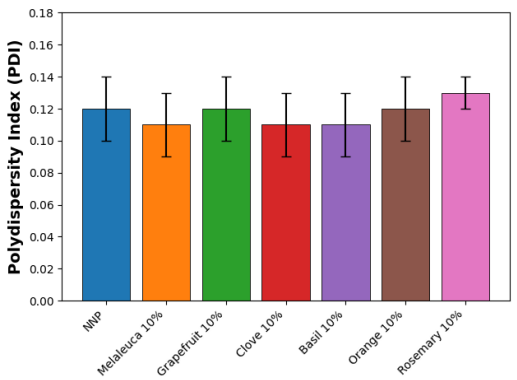

Figure 2. Polydispersity index (PDI) of unloaded and oil-loaded nanoparticles (10% w/w) obtained by dynamic light scattering. Values represent mean ± standard deviation (n = 3).

Zeta Potential (ZP)

Zeta potential results are shown in Figure 3. Unloaded nanoparticles exhibited a negative surface charge (–15.3 ± 1.2 mV), attributed to terminal carboxyl groups of PCL. Since Pluronic F-68 is a nonionic surfactant, its contribution to electrostatic charge is limited, although it provides steric stabilization through its hydrophilic chains.

All oil-loaded nanoparticles presented slightly less negative surface charge values, varying between –14.5 and –14.0 mV. This shift suggests that essential oil constituents may partially localize at or near the nanoparticle surface, modulating surface characteristics.

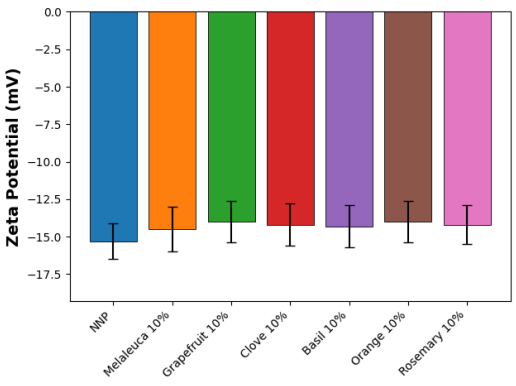

Figure 3. Zeta potential of unloaded nanoparticles (NNP) and nanoparticles loaded with essential oils (10% w/w). Measurements were performed in aqueous dispersion at 25 °C. Results are expressed as mean ± standard deviation (n = 3).

Nanoparticles containing melaleuca oil exhibited one of the least negative values (–14.5 ± 1.5 mV), which may be associated with the presence of oxygenated compounds capable of influencing surface electron distribution. In contrast, citrus-based oils (orange and grapefruit), rich in limonene, maintained zeta potential values closer to those of unloaded nanoparticles, likely due to the nonpolar nature of limonene, which does not significantly alter surface charge.

Despite these variations, all formulations maintained negative zeta potentials combined with steric stabilization from PF68, indicating suitable colloidal stability in aqueous dispersion.

Encapsulation efficiency

Encapsulation efficiency was determined exclusively for the oil-loaded nanoparticles (10% w/w), and values ranged from 71.0% to 87.1%, demonstrating satisfac-

tory incorporation of essential oils into the PCL matrix (Figure 4).

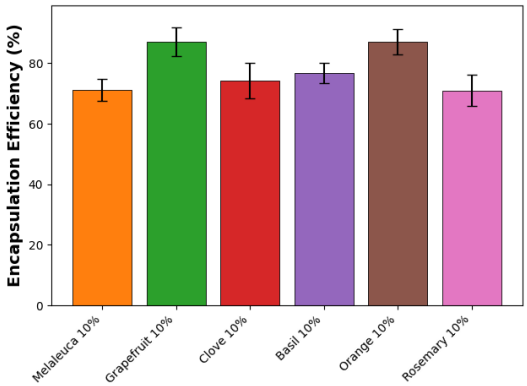

Figure 4. Encapsulation efficiency (%) of essential oils (10% w/w) incorporated into PCL/PF68 nanoparticles, determined by indirect UV–Vis spectrophotometry through quantification of non-encapsulated oil in the supernatant. Results are expressed as mean ± standard deviation (n = 3).

The highest retention values were observed for grapefruit and sweet orange formulations (87.1%), both predominantly composed of limonene (≈85–95%; 136.24 g/mol; logP ≈ 4.5). The strong hydrophobic character of limonene enhances its affinity for the apolar PCL core, favoring partitioning into the polymeric phase during nanoprecipitation. Its relatively small molecular size may also facilitate diffusion into the forming matrix before solvent evaporation is completed, contributing to enhanced retention.

Basil- and clove-loaded nanoparticles showed intermediate efficiencies (76.7% and 74.2%, respectively). Estragole, the major compound in basil oil, possesses moderate hydrophobicity (logP ≈ 3.4) but contains an ether functionality, slightly increasing polarity relative to limonene. Clove oil, rich in eugenol (logP ≈ 2.6), contains a phenolic hydroxyl group capable of hydrogen bonding, which may reduce full compatibility with the hydrophobic polymeric core despite the presence of an aromatic ring.

Melaleuca and rosemary formulations exhibited lower encapsulation efficiencies (~71%). Their major constituents, terpinen-4-ol and 1,8-cineole (logP ≈ 2.7), are oxygenated monoterpenes with increased polarity compared to hydrocarbon terpenes. The presence of hydroxyl and ether groups likely reduces hydrophobic driving force toward the PCL matrix, limiting retention during nanoparticle formation.

Collectively, these findings indicate that encapsulation efficiency is primarily influenced by the balance between molecular hydrophobicity and functional group polarity. Oils dominated by nonpolar hydrocarbon terpenes demonstrated enhanced compatibility with the PCL core, whereas oxygenated or partially polar terpenes exhibited comparatively reduced incorporation. This behavior highlights the relevance of molecular affinity in the rational design of polymeric carriers for essential oils in health-related formulations.

In Vitro Cytotoxicity (L929)

The cytocompatibility of unloaded and 10% oil-loaded nanoparticles was evaluated in L929 fibroblasts after 24 h exposure (Figure 5). The unloaded nanoparticles (NNP) exhibited high cell viability (96.0 ± 3.0%), indicating that the PCL/PF68 system itself does not induce significant cytotoxic effects.

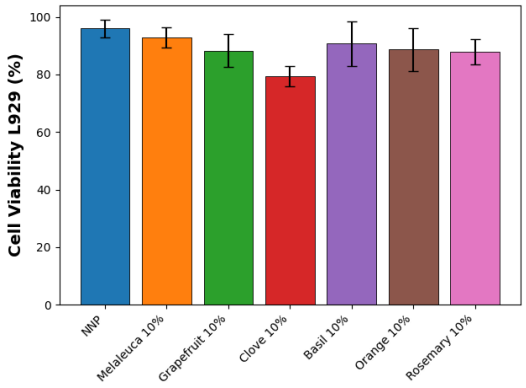

Figure 5. In vitro cytocompatibility of unloaded and oil-loaded nanoparticles (10% w/w) evaluated in L929 fibroblasts after 24 h exposure using the MTT assay. Cell viability is expressed as percentage relative to untreated control (100%). Results are presented as mean ± standard deviation (n = 3).

All oil-loaded formulations maintained cell viability above 79%, demonstrating acceptable cytocompatibility within the evaluated conditions. According to ISO 10993-5 guidelines, materials are considered non-cytotoxic when cell viability remains above 70%, which was satisfied by all 10% formulations.

Melaleuca-loaded nanoparticles showed viability of 92.8 ± 3.5%, closely approaching the unloaded control, suggesting minimal biological impact upon incorporation of terpinen-4-ol–rich oil. Basil (90.7 ± 7.8%), grapefruit (88.2 ± 5.7%), orange (88.7 ± 7.4%), and rosemary (87.9 ± 4.5%) also demonstrated high viability values, indicating that incorporation of these essential oils at 10% does not significantly compromise cellular metabolic activity.

Clove-loaded nanoparticles presented the lowest cell viability among the evaluated samples (79.4 ± 3.6%). Although still above the cytotoxicity threshold, this reduction may be attributed to the high concentration of eugenol in clove oil. Eugenol contains a phenolic hydroxyl group, which is known to interact with cellular membranes and may exert stronger biological effects compared to hydrocarbon terpenes such as limonene. The partial decrease in viability observed for this formulation is therefore consistent with its chemical composition.

Overall, the results indicate that at 10% loading, the PCL/PF68 nanoparticles preserve adequate cytocompatibility regardless of oil type. The slight differences observed among formulations appear to be associated with the intrinsic bioactivity and polarity of the major terpene constituents rather than with nanoparticle structure itself.

These findings support the potential applicability of the developed nanosystems in health-related formulations, particularly at the 10% concentration level, which combines satisfactory encapsulation efficiency with acceptable cellular compatibility.

Conclusion

This study demonstrated that PCL/PF68 nanoparticles effectively incorporate different essential oils at 10% loading through the nanoprecipitation method, yielding stable nanosystems with controlled physicochemical characteristics.

All formulations presented nanometric particle sizes (≈205–213 nm), low polydispersity indices (0.11–0.13), and moderately negative zeta potentials (approximately –14 mV), indicating homogeneous and colloidally stable dispersions. The incorporation of essential oils slightly increased particle diameter compared to unloaded nanoparti-

cles, confirming successful encapsulation without compromising structural uniformity.

Encapsulation efficiency ranged from 71% to 87%, being strongly influenced by the molecular characteristics of the major terpene constituents. Oils rich in highly hydrophobic monoterpene hydrocarbons, such as limonene, exhibited higher retention, whereas formulations containing oxygenated or partially polar terpenes showed comparatively lower incorporation. These results highlight that terpene hydrophobicity and functional group composition play a key role in determining affinity for the PCL matrix.

In vitro cytotoxicity evaluation using L929 fibroblasts demonstrated that all 10% formulations maintained cell viability above 79% after 24 h exposure, satisfying the ISO 10993-5 criterion for non-cytotoxic materials. The slight reduction observed for clove-loaded nanoparticles may be associated with the biological activity of eugenol, rather than structural instability of the carrier system.

Overall, the developed nanosystems combine satisfactory encapsulation performance, controlled physicochemical properties, and acceptable cytocompatibility, supporting their potential as carriers for essential oils in health-related formulations. The findings reinforce the importance of considering molecular compatibility between terpene structure and polymer matrix in the rational design of polymeric nanocarriers.

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Declaration of AI Use

The authors declare that artificial intelligence (AI)-assisted tools were used exclusively for data visualization purposes, specifically for generating graphical representations of experimental results. All experimental procedures, data collection, analysis, interpretation, and scientific conclusions were performed entirely by the authors. The use of AI did not influence the study design, data integrity, or interpretation of the results.

References

Alexis, F., Pridgen, E., Molnar, L. K., & Farokhzad, O. C. (2008). Factors affecting the clearance and biodistribution of polymeric nanoparticles. *Molecular Pharmaceutics, 5*(4), 505–515.

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils. *Food and Chemical Toxicology, 46*, 446–475.

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods. *International Journal of Food Microbiology, 94*, 223–253.

Diass, K., Merzouki, M., & Elfazazi, K., et al. (2021). Effect of essential oils on microbial growth and oxidative stability of meat products: A review. *Food Research International, 148*, 110–125.

Danhier, F., Ansorena, E., Silva, J., et al. (2012). PLGA-based nanoparticles: An overview of biomedical applications. *Journal of Controlled Release, 161*, 505–522.

Ferreira, I. J., Menezes, L. R., & Tavares, M. I. B. (2022). Morphological and structural evaluation of nanoparticles loaded with tea tree oil for the therapeutic treatment of HPV. *Polymer Bulletin, 79*, 5457–5479.

Ferreira, I. J., Menezes, L. R., & Tavares, M. I. B. (2024). Obtaining PCL/tea tree oil particles with antimicrobial capacity and high cytocompatibility. *Polymer Bulletin, 81*, 15995–16020.

Haro-González, J., Mateo, A., Manso, T., et al. (2021). Essential oils as natural agents for food preservation: A review. *Foods, 10*(301), 1–25.

Hyldgaard, M., Mygind, T., & Meyer, R. (2012). Essential oils in food preservation. *Frontiers in Microbiology, 3*, 12.

Khalid, A. A., & Ahmed, A. M. A. (2020). Agriculture locations induced variations in the essential oil of Valencia orange. *Journal of Essential Oil Bearing Plants, 23*(6), 1438–1449.

Oğuz, M. Ç., Oğuz, E., & Güler, M. (2023). Seed priming with essential oils for sustainable wheat agriculture in semi-arid region. *PeerJ, 11*, e15023.

Tavares, M. R., Menezes, L. R., Dutra Filho, J. C., Cabral, L. M., & Tavares, M. I. B. (2017). Surface-coated polycaprolactone nanoparticles with pharmaceutical application: Structural and molecular mobility evaluation by TD-NMR. *Polymer Testing, 60*, 39–48.

Turek, C., & Stintzing, F. C. (2013). Stability of essential oils. *Comprehensive Reviews in Food Science and Food Safety, 12*, 40–53.

Woodruff, M. A., & Hutmacher, D. W. (2010). The return of a forgotten polymer: Polycaprolactone in the 21st century. *Progress in Polymer Science, 35*, 1217–1256.