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Clove Essential Oil–Hydroxypropyl-β-Cyclodextrin Inclusion Complexes: Preparation, Characterization and Incorporation in Biodegradable Chitosan Films

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Abstract: The encapsulation of clove essential oil (CEO) in hydroxypropyl-β-cyclodextrin (HP-β-CD) and the subsequent incorporation of the inclusion complex in an elastic chitosan film in order to achieve a controlled release profile of the volatile CEO are herein presented. Freshly distilled CEO was found to contain eugenol in concentrations higher than 70%. The kneading method was implemented for the preparation of a CEO-HP-β-CD inclusion complex, resulting in a 50% inclusion efficiency of the essential oil in particles sized 214.40 nm with ζ-potential −27.5 mV. Free CEO and CEO-HP-β-CD inclusion complex were tested for their ability to scavenge the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical, and it was found that the CEO-HP-β-CD complex presented enhanced antioxidant activity (88%) compared to the free CEO (71%). Choline chloride-containing chitosan (CS) films were prepared, incorporating either the pure CEO or the CEO-HP-β-CD inclusion complex, and their mechanical properties were determined. The study of the release profile in different pH values demonstrated the capacity of the CS-HP-β-CD system to provide sustained release of CEO, noting its potential use in food processing as smart packaging.

Keywords: hydroxypropyl-β-cyclodextrin; clove essential oil; inclusion complexes; antioxidant activity; chitosan elastic films

Citation: Adjali, A.; Pontillo, A.R.N.; Kavetsou, E.; Katopodi, A.; Tzani, A.; Grigorakis, S.; Loupassaki, S.; Detsi, A. Clove Essential Oil–Hydroxypropyl-β-Cyclodextrin Inclusion Complexes: Preparation, Characterization and Incorporation in Biodegradable Chitosan Films. *Micro* **2022**, *2*, 212–224. https://doi.org/10.3390/micro2010014

Academic Editor: Hiroshi Furuta

Received: 11 January 2022
Accepted: 11 March 2022
Published: 16 March 2022

1. Introduction

Clove essential oil (*Eugenia caryophyllata* (*Syzigium aromaticum* L. Myrtaceae)) has been known since ancient times and is currently used in a wide variety of applications [1,2]. The therapeutic properties of clove essential oil (CEO) are constantly being explored [3,4]. CEO exhibits remarkable activity against foodborne pathogens such as *Listeria monocytogenes* [5], *Campylobacter Jejuni* [6], *E. coli, S. aureus, B. cereus* and *Y. enterocolitica* [7], *Salmonella typhimurium* (FICI: 0.41), and *Aspergillus niger* [8]; thus, it is suitable as a food preservative. Recently, a mixture of clove and lemongrass essential oil was found to effectively control the growth of the red flour beetle (*Tribolium castaneum*), acting as antifeedants [9].

CEO is an effective 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenger [8]. The antioxidant activity of CEO is mainly owed to the presence of high levels of eugenol, a phenylpropanoid, and caryophyllene. The anti-inflammatory as well as wound healing and anticancer activities shown by CEO further justify their extensive applications in the pharmaceutical and cosmetics industries [10,11].

However, the volatility of the essential oils, along with their sensitivity when exposed to air and light, severely limit their use. Encapsulation of bioactive molecules, extracts

or essential oils, in appropriately selected carriers, is a well-studied technique, offering protection towards oxidation, evaporation and degradation [12–14]. Cyclodextrins (CDs) are often the selected carriers of hydrophobic substances because of their amphiphilic character and the orientation of glycosidic linkages inside the cavity of the carrier [15,16]. Their biocompatibility, biodegradability and edible character allows the use of cyclodextrins in numerous applications. The effect of encapsulation of EOs in β-cyclodextrin has been demonstrated through several scientific studies.

Chitosan is a biopolymer, a cationic polysaccharide derived from the partial deacetylation of chitin. Chitosan is considered as Generally Recognized as Safe (GRAS) by the Food and Drug Administration (FDA) and possesses antioxidant, lipid-lowering and antimicrobial activities. In addition, this biopolymer has film-forming and gelling properties, while it has been widely used as an encapsulation matrix for the preparation of nanosystems containing natural products, active pharmaceutical agents as well as essential oils and plant extracts. Owing to the above properties, chitosan has found a vast number of applications in the pharmaceutic, food and cosmetic sectors [14,17].

Chitosan-based films are gaining ground as alternative, biodegradable and environmentally friendly packaging materials, the properties of which can be further modified by the incorporation of essential oils, plant extracts or pure natural products in the film. The food industry is the main receptor of such edible films and coatings, applied mostly in meat, seafood, fruit, vegetable and dairy products [18]. Thus, several food products, including fresh pistachio fruit, figs (*Ficus carica*), deepwater pink shrimp, etc., have been incorporated in edible chitosan or alignate–chitosan films for food packaging applications. Their coating with chitosan seems to reduce the growth of bacteria and fungi and most importantly increase their shelf lives, therefore improving the quality of the final product [19–21].

In this context, clove essential oil has been incorporated into chitosan films that presented enhanced antioxidant and antimicrobial properties and were tested for their ability to act as protective coatings for fresh-cut apples. The results showed that the films decreased the deterioration rate as well as the microbial counts of the tested food samples [22]. In an analogous work, Shukla et al. proved that chitosan edible films containing CEO significantly improved the quality and shelf-life of chicken products [23]. Moreover, essential oils have also been incorporated in chitosan films for pharmaceutical and biomedical applications. CEO has been incorporated in chitosan films for wound healing applications and was found to improve the mechanical properties of the films [24], whereas Lee et al. prepared chitosan films containing clove essential oil and halloysite nanotubes and showed that the nanocomposites possessed high antioxidant activity [25].

Although CEO-β-cyclodextrin inclusion complexes have been reported since 2008 [26], only a few research works describing the preparation and complete characterization of the inclusion complexes of CEO in hydroxypropyl-β-cyclodextrin (HP-β-CD) appear in the literature [27,28], while to our knowledge, this is the first time that CEO-β-HP-β-CD inclusion complexes have been incorporated in chitosan films in order to combine the benefits of both carriers in one advantageous system.

In the present study, freshly distilled clove essential oil (CEO) was encapsulated into hydroxypropyl-β-cyclodextrin (HP-β-CD), and the formed inclusion complexes (CEO-HP-β-CD) were evaluated for their DPPH radical scavenging ability and were subsequently entrapped into chitosan films, which to our knowledge is being reported for the first time in the literature. To increase the elasticity of the films, choline chloride was incorporated, as it has been previously found that its addition has a plasticising effect [29]. The physical and mechanical properties of the films were studied as well as the release profile of the CEO from the inclusion complexes and the films.

2. Materials and Methods

2.1. Materials

Hydroxypropyl-β-Cyclodextrin with 99% purity was from Aldrich, Germany, Chitosan (5–20 mPa·s, 0.5% in 0.5% Acetic Acid at 20 °C) and 2,2-Diphenyl-1-picrylhydrazyl

(DPPH) 97 +% were from TCI (Oxford, UK), DL-lactic acid 80–85% aq. soln. and choline chloride 98 +% were purchased from Alfa Aesar, and ethyl acetate GC grade was from ChemLab. All other reagents and solvents were of laboratory grade and were used without further process.

2.2. Extraction of Clove Essential Oil (CEO)

A total 150 g of dry matter of commercially available clove was mortared until becoming fine grains, which was then incorporated in a Clevenger apparatus and left for more than 3 h. The extracted CEO was collected in a dark glass vial and stored in a deep freezer until further use.

2.3. Preparation of HP-β-CD-CEO Inclusion Complexes

A total 125 mg of CEO was dissolved in 4 mL acetonitrile:water 1:2 *v/v*. Then, 500 mg of Hydroxypropyl-β-Cyclodextrin was poured in a mortar followed by the gradual addition of the CEO solution. The mixture was kneaded for 50 min until a homogenous paste was formed. Thereafter, the sample was dried for 24 h in a desiccator at room temperature under vacuum, and finally mixed well and stored in the refrigerator for further study.

2.4. Preparation of Chitosan Films

Firstly, a solution of 0.4% chitosan was prepared by adding 400 mg of chitosan in 100 mL of water. A total of 9 mL lactic acid was added dropwise, until pH 2.5, for the complete dissolution of chitosan. The solution was left under stirring overnight, after which the acidic chitosan solution was filtered and choline chloride was added to the solution at a ratio of 1:15 (*w*:*w*) choline chloride:lactic acid. A total of 20 mL of the solution was poured in a Petri dish of 5.6 cm diameter and was casted to dry in an air flow oven under 45 °C for 24 h. Finally, the film was peeled off and stored in a deep freezer for further study and characterization. This film is designated as CS film.

For the preparation of the CEO-containing films (CS-CEO), 15 mg of pure CEO was added directly to the film-forming solution before oven casting at 40 °C, while for the preparation of the CS-HP-β-CD and CS-CEO-HP-β-CD inclusion complex-containing films, 30 mg of the HP-β-CD or HP-β-CD-CEO complex was incorporated prior to film casting.

2.5. GC-MS Analysis of Clove Oil

Gas chromatography–mass spectroscopy was implemented to determine the components of extracted essential oil, after being diluted 1/1000 in ethyl acetate. The instrument used was Shimadzu QP 2010, associated with a mass spectrophotometer (Zebron, ZB-5) and capillary column (type Phenomenex 0.25 um × 0.25 mm × 30 m) and a selective mass detector of QP2010. Retention time (RT) and the Kovats Retention Index (KI) were compared with authentic standards, NIST and Wiley libraries Literatures and Spectra.

2.6. CEO Inclusion Efficiency and Drug Loading

The method followed for determining the encapsulation efficiency (EE) of the CEO in the inclusion complex is similar to the method presented in [16] with few modifications; in 50 mg of the CEO-HP-β-CD inclusion complex, 4 mL of deionized water and 2 mL hexane were added, and the mixture was inserted in an ultrasonic bath at 70 °C for 20 min. The solvent extraction method with hexane was implemented in order to obtain the encapsulated CEO followed by two consecutive washes of the aqueous phase with 5 mL of the organic solvent. Hexane was evaporated under reduced pressure. A total of 80 mL of ethyl acetate was added and the concentration of the CEO in the sample was determined via UV-Vis analysis (UviLine 9400 UV-Visible spectrophotometer −280 nm SCHOTT instruments) using a calibration curve. The inclusion efficiency (IE%) of the CEO was calculated with the following Equation (1):

$$IE\ (\%) = \frac{mass\ of\ the\ CEO\ in\ the\ inclusion\ complexes}{initial\ mass\ of\ CEO} \times 100 \quad (1)$$

Finally, for the calculation of the drug loading percentage, Equation (2) was used.

$$Drug\ loading\ (\%) = \frac{mass\ of\ the\ encapsulated\ CEO}{mass\ of\ the\ dried\ inclusion\ complexes} \times 100 \quad (2)$$

Experiments were repeated three times and the results are expressed as mean ± SD.

2.7. Characterization

2.7.1. FT-IR Spectroscopy

The FT-IR spectra were obtained (on a JASCO FT/IR-4200 apparatus; Company of Japan Spectroscopic, Tokyo, Japan) as KBr pellets. The FTIR measurements (32 scans per spectrum) were carried out in the 4000–400 cm^{-1} scanning range with a resolution of 4 cm^{-1}.

2.7.2. Dynamic Light Scattering (DLS)

The size, polydispersity index (PDI) and ζ-potential of the dried HP-β-CD-CEO complex were determined using a Zetasizer Nano ZS of the Malvern Instruments Ltd. The samples for DLS analysis were prepared by diluting 1 mg of nanocomplex powder in double deionized water and vortexing for 2 min. Folded capillary cells (type DTS1070) were used for the measurements of size and ζ-potential carried out at room temperature 25 ± 1 °C, reporting the results as mean ± SD.

2.7.3. Thermogravimetric Analysis (TGA)

TGA analysis was performed on a Mettler Toledo Company reference TGA/DSC 1 STARe System thermobalance. The samples were heated from 25 °C to 600 °C, with gas flow of nitrogen (10 mL/min) and rate of heating of 10 °C/min.

2.7.4. Mechanical Properties of Films

A digital micrometre (Hogetex 0–25 mm) has been used to measure the thickness of the films. Measurements were taken from 5 different parts of each film. The force required to rupture the film, referred to as burst strength (BS), and total elongation of the film before breakage, referred to as distance at burst (DB), of the films were measured with a TA.XT2i Texture Analyzer; Stable Micro System, equipped with a stainless-steel penetration probe and analysed with the software Texture Exponent 32. The film was secured in a film supporting rig and the probe moved vertically towards the centre of the film at a speed of 1 mm/sec until the film was ruptured. Experiments were repeated three times and the results are expressed as mean ± SD.

2.8. Release Studies

The release study of the CEO from the inclusion complex and the two chitosan films was performed at different pH values and temperatures. To that end, 100 mg of CEO-HP-β-CD complex was dispersed into a 25 mL glass vial for 12 h. At predetermined times, 1 mL of the solution was taken, centrifuged in order to separate the HP-β-CD, and the supernatant was measured with a UV-Vis spectrophotometer. After each sampling, 1 mL of buffer solution was added to the solution. In this experiment, three different conditions were studied: pH 3 and 37 °C, resembling the stomach conditions, pH 5.5 and 34 °C, imitating the skin, and pH 8 and 37 °C for intestines, all using phosphate buffers. Experiments were repeated three times and the results are expressed as mean ± SD.

2.9. DPPH Radical Scavenging Ability

The antioxidant activity of CEO, HP-β-CD and the CEO-HP-β-CD inclusion complex was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. In brief, 195 μL of DPPH (0.05 mg/mL) and 5 μL of different concentrations of the samples were added in each well of a 96-well plate and the absorbance was measured at 515 nm after 30 min and

60 min. Control wells contained solvent (DMSO or water) instead of the test samples. The scavenging activity of the samples was calculated using the following Equation (3):

$$Inhibition\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (3)$$

where $A_{control}$ is the absorbance of control samples and A_{sample} is the absorbance of the test samples.

Experiments were repeated three times and the results are expressed as mean ± SD.

3. Results and Discussion

3.1. Composition of the CEO

The major component of the CEO isolated and used in this study was eugenol, constituting 71.3% of the essential oil. This value is analogous with the 68.95% reported in the literature [22]. Caryophyllene constituted 22.6% of the obtained CEO. Seven more components were found in traces. The chemical composition of the CEO used in this study is presented in Figure 1 and Table 1.

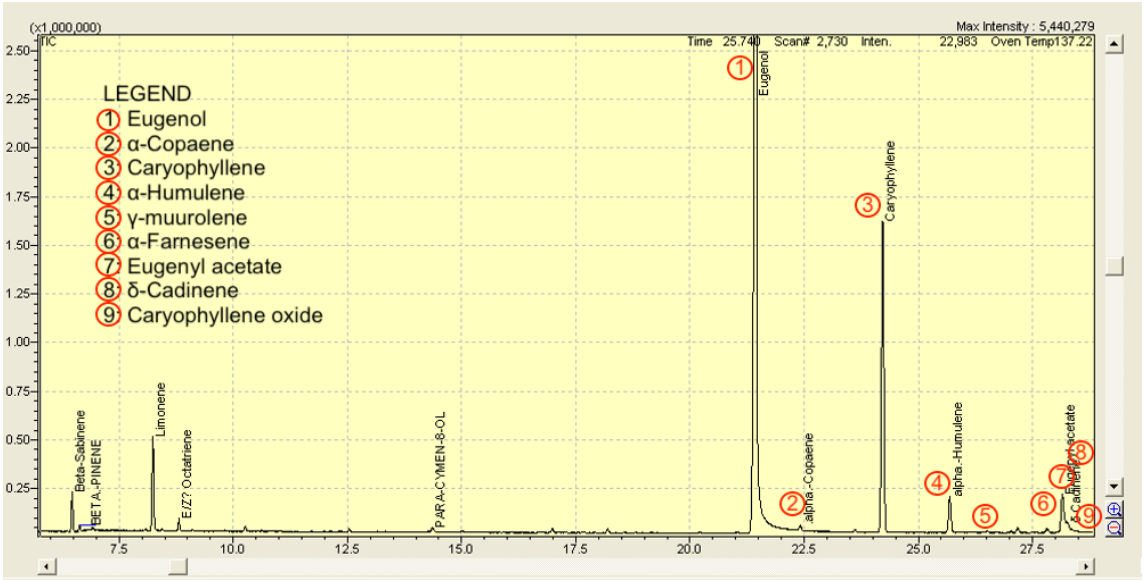

Figure 1. Clove essential oil gas chromatogram.

Table 1. The chemical composition of CEO used in the present study.

No.	Compound	RT (min)	Area (%)
1	Eugenol	21.450	71.3
2	α-Copaene	22.383	0.4
3	Caryophyllene	24.200	22.6
4	α-Humulene	25.650	2.1
5	γ-muurolene	26.483	0.1
6	α -Farnesene	27.783	0.3
7	Eugenyl acetate	28.117	2.5
8	δ-Cadinene	28.233	0.4
9	Caryophyllene oxide	30.667	0.2

3.2. Size, Size Distribution and Zeta-Potential (ζ-Potential)

The mean hydrodynamic diameter of the CEO-HP-β-CD inclusion complexes was found to be 214.40 ± 15.81 nm, in accordance with the values reported for analogous systems in the recent literature [16,30] and slightly higher than the value 165.87 ± 61.65 nm reported by Cetin Babaoglu et al. for the inclusion complexes of CEO in HP-β-CD [28]. The

polydispersity index (PDI) was 0.498 ± 0.11, which proved a moderate uniformity of size dispersion. The ζ-potential was found to be −27.5 ± 3.25 mV, demonstrating the stability of the aqueous dispersion of the inclusion complex towards the aggregation tendency. The negative charge at the surface of the CEO-HP-β-CD inclusion complexes is indicative of the aligning of the –OH groups of the HP-β-CD towards the aqueous surrounding [31].

3.3. Inclusion Efficiency and Drug Loading of CEO in the CEO-HP-β-CD Complexes

The inclusion efficiency of CEO in the CEO-HP-β-CD complexes was found to be around 50 ± 2% and the drug loading was calculated as 12 ± 0.2%, which tended to be reproducible based on the kneading method used for encapsulation.

3.4. FT-IR Analysis

The FT-IR spectra of CEO, HP-β-CD and the CEO-HP-β-CD inclusion complex were obtained in order to observe probable shifts in the wavenumbers of the characteristic absorption bands, which can be indicative of efficient intermolecular interactions between the CEO components and HP-β-CD in the inclusion complexes. The spectra are shown in Figure 2 and the characteristic absorption bands of each spectrum are presented in Table 2.

Table 2. Characteristic FT-IR absorption bands of CEO, HP-β-CD and the CEO-HP-β-CD inclusion complex.

	Characteristic Absorption Bands (cm^{-1})						
	OH Stretching	**C-H Stretching**	**C-H Asymmetric Stretching of CH_2**	**C=C Stretching (Aromatic)**	**O-H Bending (Alcohol)**	**C-O Stretching (Aryl-Alkyl Ether)**	**C-O Stretching (Secondary Alcohols)**
CEO	3512	2935	1637	1514	1464	1269	1036
HP-β-CD	3403	2929	1637	-	1462	-	1032
HP-β-CD-CEO *inclusion complex*	3406	2929	1639	1516	1460	1273	1034

The FT-IR spectrum of the clove essential oil shows characteristic absorption bands that can be attributed to eugenol, which is the main constituent of this essential oil. The bands at 3512 cm^{-1} and at 1464 cm^{-1} are owed to the OH stretching and bending vibrations, respectively. The absorption at 1637 cm^{-1} is attributed to the → C-H asymmetric stretching of CH_2, whereas the band at 2935 cm^{-1} is owed to the C-H stretching vibration. Finally, the band at 1269 cm^{-1} is due to the C-O stretching vibration of the aryl-alkyl ether group, present at the eugenol structure and other components.

The spectrum of HP-β-CD is characterized by the bands presented at 3403 cm^{-1} and 1462 cm^{-1}, owed to the alcohol OH stretching and bending vibrations, respectively. The absorption bands at 2930 cm^{-1} and 1637 cm^{-1} are attributed to the C-H symmetric and asymmetric stretching, respectively. Moreover, the absorption band at 1032 cm^{-1} is attributed to the C-O stretching vibration of the secondary alcohol groups, presented in the HP-β-CD molecule.

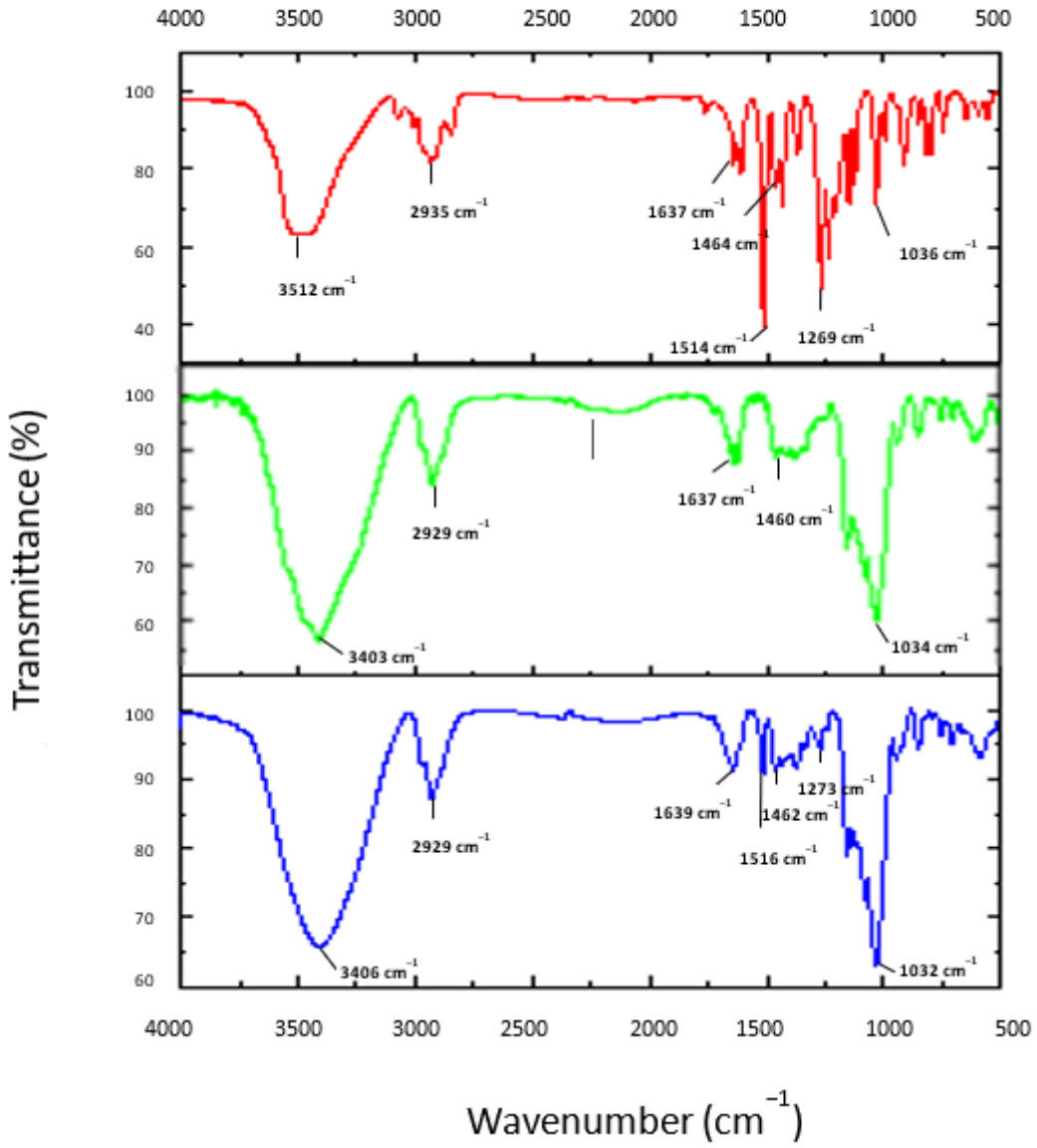

Figure 2. FT-IR (KBr) spectra of pure CEO (red), HP-β-CD (green) and CEO-HP-β-CD inclusion complex (blue).

As far as the FT-IR spectrum of the CEO-HP-β-CD inclusion complex is concerned, the shifts of the characteristic absorption bands of CEO and HP-β-CD compared to their spectrum in free form indicate the presence of CEO in the inclusion complex and its interaction with HP-β-CD. Moreover, the absence of several characteristic bands of CEO in the FT-IR spectrum of the inclusion complex proves the successful encapsulation of CEO in the HP-β-CD cavity and the formation of inclusion complexes [32].

3.5. Thermogravimetric Analysis

From the TGA graph of the clove essential oil (Figure 3), it can be clearly seen that the decomposition occurs in the temperature range between 47 °C to 251 °C with the highest rate observed at 197 °C.

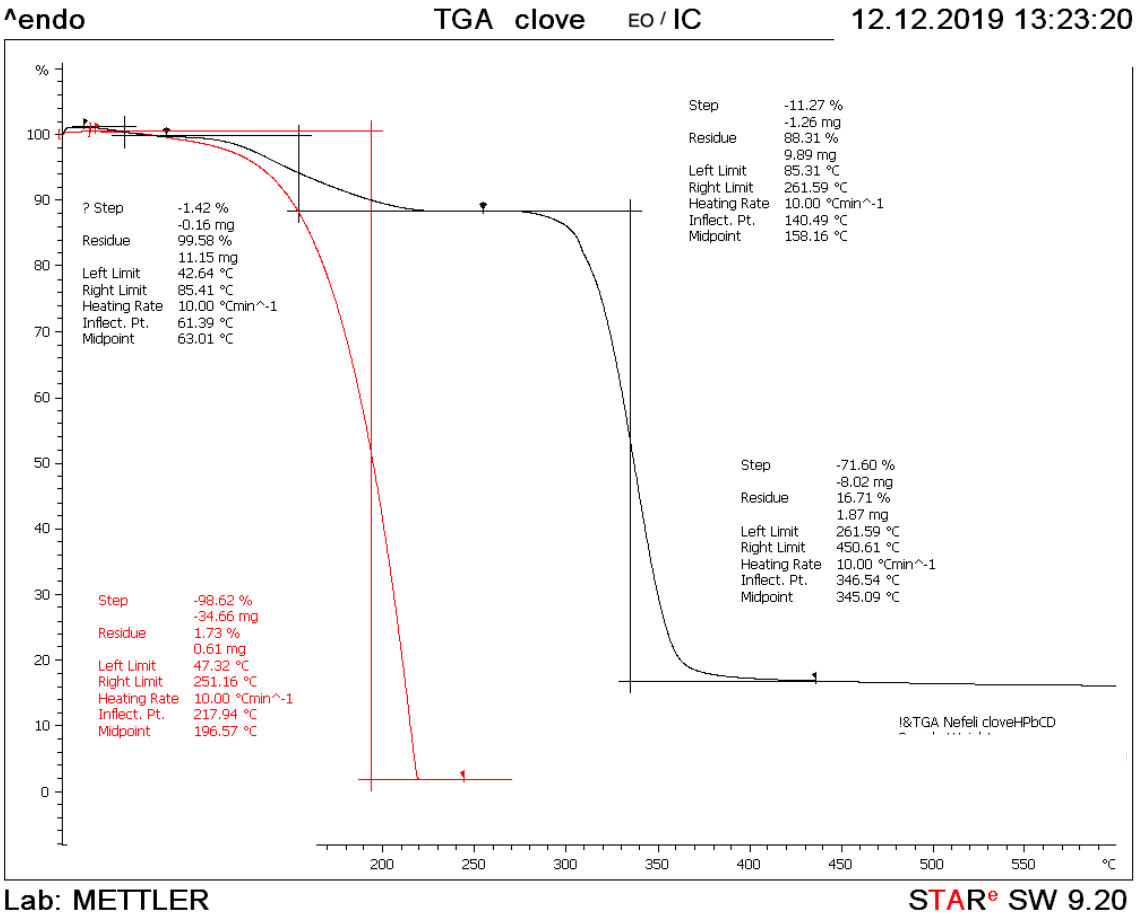

Figure 3. Comparative TGA graphs of CEO (red) and CEO-HP-β-CD (black) heated from 25 °C to 600 °C, at a heating rate of 10 °C/min under nitrogen gas flow (10 mL/min).

At the TGA curve of the HP-β-CD-CEO complex, three phases can be observed: In the range of 42–85 °C, a 1.42% mass loss is observed, which is attributed to the evaporation of water from the complex [16,33]. The second mass loss (11.27%) represents the evaporation of the clove essential oil probably retained at the surface of HP-β-CD in the temperature range of 85–261 °C, reaching a maximum rate at 218 °C. The most significant mass loss (71.60%) is observed at the temperature range 262–451 and is attributed to the decomposition of the inclusion complex (T_d 345 °C). This high T_d of the inclusion complex shows that the strong host–guest interactions provide efficient thermal stability to the CEO constituents.

3.6. Film Characterization

Table 3 represents the film burst strength (BS) and distance at the burst (DB) of the different films prepared.

Table 3. Properties of the prepared films.

Sample	Thickness (mm)	Burst Strength, BS (N)	Distance at Burst, DB (mm)
Chitosan film (CS)	0.0584 ± 0.018	10.66 ± 0.09	25.09 ± 0.06
Chitosan film incorporating HP-β-CD (CS-HP-β-CD)	0.0660 ± 0.018	8.24 ± 0.05	27.17 ± 0.04
Chitosan film incorporating CEO (CS-CEO)	0.0618 ± 0.008	5.64 ± 0.03	23.40 ± 0.03
Chitosan film incorporating CEO-HP-β-CD inclusion complex (CS-CEO-HP-β-CD)	0.0648 ± 0.012	8.41 ± 0.03	27.55 ± 0.05

The study of the mechanical properties of the films revealed some interesting differences. Firstly, the resistance at the burst of the film prepared using only chitosan dissolved in lactic acid and choline chloride (CS) is the highest, followed by the films containing HP-β-CD (CS-HP-β-CD) or the inclusion complex (CS-CEO-HP-β-CD), while the film containing free essential oil (CS-CEO) was the most easily ruptured. The incorporation of HP-β-CD or of the inclusion complex slightly increased the distance of the probe before the burst of the film, while the addition of the free essential oil had the opposite outcome.

The incorporation of the hydrophobic CEO in the chitosan film leads to a less flexible material with lower resistance to fracture. This is in accordance with the observations of [25,34], who prepared chitosan films containing CEO and α-tocopherol, respectively. The plausible explanation of the phenomenon is that hydrophobic compounds tend to influence the film structure by restricting the mobility of the hydrophilic polymeric chains of chitosan as well as by reducing the moisture content, thus inducing lower flexibility and resistance at burst [25,34].

The chitosan film prepared by the incorporation of HP-β-CD (2.7%) (CS-HP-β-CD) showed lower burst strength but higher distance at burst than the CS film, in accordance with the study of Sun et al. [35]. The incorporation of CEO in the chitosan films in the form of the inclusion complex (CS-CEO-HP-β-CD) results in a film with analogous mechanical properties with the CS-HP-β-CD film and not the one containing pure CEO (CS-CEO). This indicates that the CEO is protected inside the cyclodextrin cavity and does not interact with the chitosan polymeric chains in the same mode as in the case of the CS-CEO film.

3.7. Release Studies

The release profile of CEO from the three systems prepared, namely the CEO-HP-β-CD inclusion complex, the chitosan film incorporating CEO (CS-CEO) and the chitosan film incorporating the inclusion complex (CS-CEO-HP-β-CD), was studied over a 12 h period at three different pH values for each system (Figure 4).

For all systems, an initial burst release of the CEO is observed and lasts for approximately 60 min. Moreover, in all pH values investigated, the release of CEO was the highest from the chitosan film incorporating CEO (CS-CEO), thus providing further evidence that the CEO is efficiently protected inside the cyclodextrin cavity.

The higher release rate of the CEO in the case of the CS-CEO system can be attributed to the rapid discharge of the clove essential oil from the surface of the film, achieving almost 50% release of the CEO in the first few minutes at pH 5.5 and 8.0 and 70% release at pH 3.0. The remaining CEO was further released at a lower rate until a plateau was reached due to the migration of the essential oil from the deep to the shallow matrix of the film [36]. Another factor favouring the higher release rate of the EO from the film is the preparation process of the film, which includes the drying of the film in an air flow oven under 45 °C for 24 h, which could lead to the migration of the EO constituents to the film's surface.

However, in the case of CS-CEO-HP-β-CD film, this burst effect is reduced due to the entrapment of the EO's constituents into the HP-β-CD cavity, which leads to the protection of the constituents and thereafter their slower release from the film matrix.

The release rate of the CEO from the CEO-HP-β-CD complex was significantly lower (30–50% plateau reached in the first 12 h), as has also been reported in the literature, which indicated that only 30% of the EO was released during the first 10 h [28]. This limitation could therefore be overcome by the incorporation of the complex in the chitosan film as was also observed in this study, which, due to the preparation process, enhances the release rate of the essential oil.

Another important observation is that, when the CEO is entrapped into chitosan films, whether it is in its free form or encapsulated in the cyclodextrin, its release rate is higher at a lower pH. More specifically, after 12 h, the release of 92% of the essential oil has occurred from the CS-CEO at pH 3, while at pH 5.5 and 8, the respective amounts are 81 and 80%. Similarly, the cumulative release of CEO from the chitosan film incorporating the inclusion complex (CS-CEO-HP-β-CD) is 54, 52 and 56% at pH 3, 5.5 and 8, respectively.

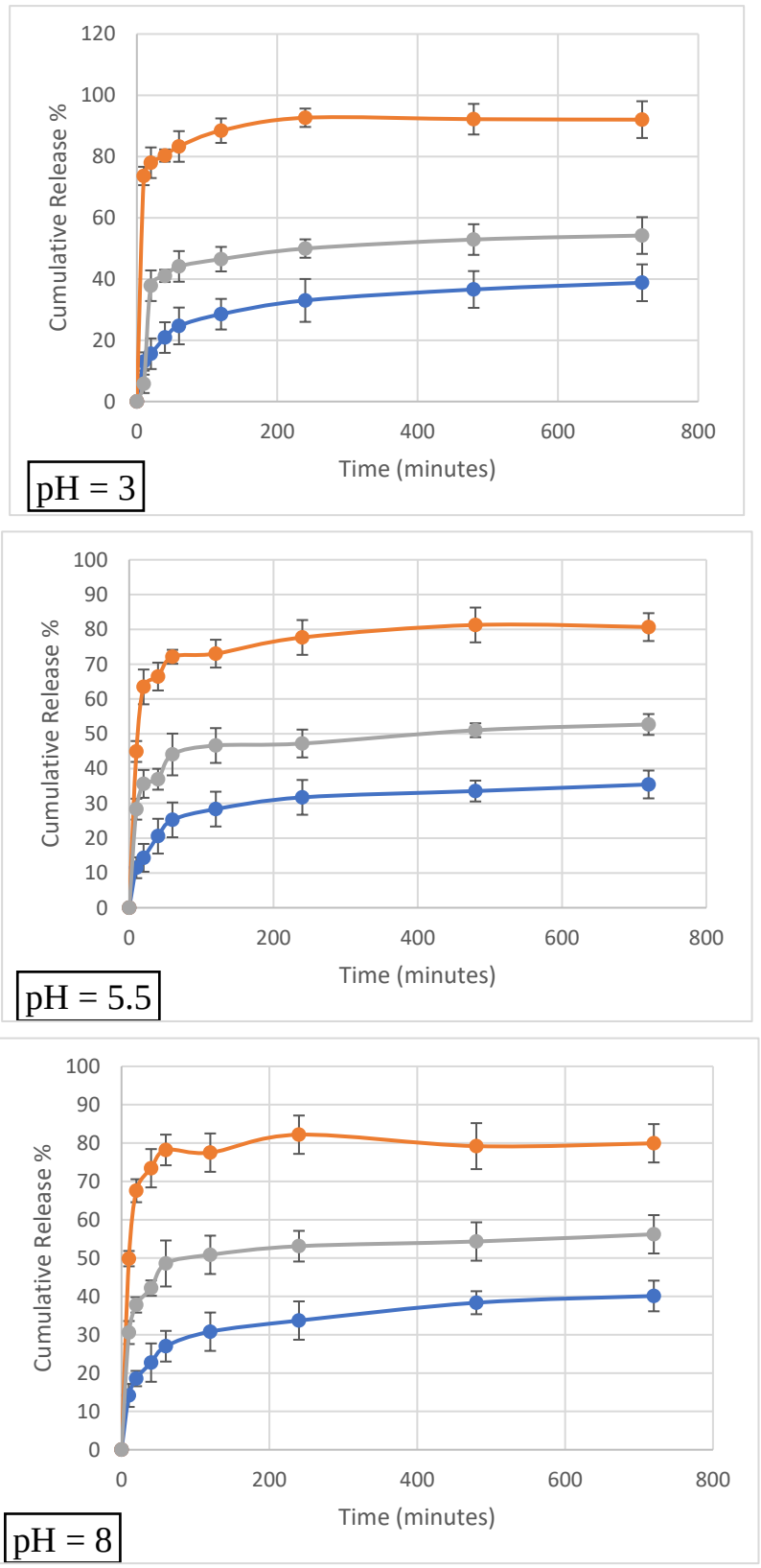

Figure 4. Release profiles of CEO from CEO-HP-β-CD (blue), CS-CEO (orange) and CS-CEO-HP-β-CD (grey).

The pH of the release medium does not appear to significantly affect the release profiles of CEO from the inclusion complex. The release profiles at pH 3 and 8 appear to be

quite similar, reaching a cumulative release of 38 and 40% of the CEO, respectively, and 35% at pH 5.5.

3.8. DPPH Radical Scavenging Ability

Clove essential oil is known for its significant antioxidant profile, including DPPH and ABTS radical scavenging [37] and lipid peroxidation inhibitory activities [3], which can be attributed to its high eugenol content.

The antioxidant activity of the CEO and CEO-HP-β-CD inclusion complex was tested using DPPH assay. The DPPH radical scavenging method has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances, including heterocyclic compounds and essential oils.

In the present study, CEO exhibited potent DPPH scavenging ability with IC_{50} values of 25.1 μg/mL and 17.7 μg/mL at 30 min and 60 min, respectively. The CEO-HP-β-CD complex was dispersed both in water and in DMSO and tested for its antioxidant activity. The CEO-HP-β-CD complex dispersed in water presented 87.8% DPPH scavenging ability at a concentration of 100 μg/mL of CEO, while free CEO presented 71.4% scavenging ability at the same concentration at 60 min. Similar antioxidant activity was achieved by the dispersion of the CEO-HP-β-CD complex in DMSO (87.5% at 60 min). It was also determined that HP-β-CD had no significant antioxidant activity. These results are displayed in Table 4.

Table 4. DPPH radical scavenging ability of CEO, HP-β-CD and CEO-HP-β-CD complex.

	DPPH Radical Scavenging Ability	
Sample	**(%) 100 μg/mL/30 min**	**(%) 100 μg/mL/60 min**
CEO	71.2 ± 1.0	71.4 ± 1.2
HP-β-CD	no	no
CEO-HP-β-CD (H_2O)	85.8 ± 0.8	87.8 ± 0.8
CEO-HP-β-CD (DMSO)	84.4 ± 0.4	87.5 ± 0.2

It is noteworthy that the CEO-HP-β-CD complex exhibited higher antioxidant activity than the free CEO, both at 30 min and at 60 min. In addition, free CEO was tested only in DMSO, while CEO encapsulated in HP-β-CD exhibited significant antioxidant activity both in DMSO and in an aqueous medium, indicating the enhanced aqueous solubility and biological activity achieved by the encapsulation of CEO in HP-β-CD.

4. Conclusions

In the present work, the inclusion of clove essential oil into hydroxypropyl-β-cyclodextrin using the kneading method and the subsequent incorporation of the inclusion complex in chitosan film was studied. Eugenol was the major component of the studied CEO (more than 70%). Inclusion efficiency of the complexes was 50%, while the nanoparticles possessed 214.40 ± 15.81 nm mean diameter, PDI 0.498 ± 0.11, and a satisfactory ζ-potential (−27.5 ± 3.25 mV). FT-IR and TGA studies provided evidence of the successful encapsulation of CEO in the HP-β-CD cavity. The antioxidant activity of free CEO (71%) and the CEO-HP-β-CD inclusion complex was tested using the DPPH assay, indicating the enhanced radical scavenging ability of the essential oil after its encapsulation in HP-β-CD (87%). The release profile of CEO from the inclusion complex and the prepared films was studied during a period of 12 h in aqueous solutions of different pHs (3, 5.5 and 8) and temperatures. The results showed that in all cases, a burst release takes place during the first hour, as expected for cyclodextrin inclusion complexes. The release rate was not significantly affected by the pH of the medium. However, it is worth noting that the release of CEO from the chitosan film containing the pure essential oil reached 83% in the first hour, whereas the corresponding value from the chitosan film containing the inclusion complex was only 44% during the same period of time.

The results indicate that the incorporation of clove essential oil into chitosan films in the form of inclusion complexes with HP-β-CD can be considered as an attractive approach in the development of bioactive, biocompatible films for a variety of applications such as active packaging, wound healing patches or dental supplements.

Author Contributions: Conceptualization, A.D.; data curation, A.A., A.R.N.P., A.K., E.K., S.G. and S.L.; methodology, A.A., A.R.N.P., A.K., E.K., S.G. and S.L.; writing—original draft preparation, A.A., A.R.N.P., S.G., A.T. and A.D.; writing—review and editing, A.A., A.R.N.P., A.T., A.K., E.K. and A.D.; supervision and project administration A.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

Conflicts of Interest: The authors declare no conflict of interest.

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