LWT - Food Science and Technology 201 (2024) 116281

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

How oregano essential oil can be transformed into a taste-masking controlled release solid formulation

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ARTICLE INFO

Keywords:
Oregano essential oil
Oral administration
Phenols protection
Tablets

ABSTRACT

Oregano essential oil (OEO) has antioxidant, antiproliferative, anti-inflammatory and antimicrobial activities. OEO could be administered orally for some gastrointestinal diseases, however, its oral use, is severely hampered by its irritant taste, which requires a high dilution in water before administration, and by its high susceptibility to light and humidity. Moreover, phenols have a high instability during digestion, and are rapidly metabolized, so that only a small fraction of the oral dose reaches the lower gut. Protection of OEO from degradation in the gastrointestinal tract is essential for its enhanced efficacy and sustained release The aim of this work has been the preparation and characterization of tablets based on hydroxypropyl methylcellulose (HPMC) granules containing OEO adsorbed on zeolite and coated with Eudragit® E PO to protect the oil from instability. Measurements of angle of repose demonstrated that OEO-containing zeolite granules were not cohesive and could be easily compressed. The obtained coated tablets had sufficient mechanical strength and were able to prevent the oxidation of polyphenols in the stomach, indeed only the 15 and 30 % of them was released in simulated jejunal and ileal fluid, respectively. These solid dosage forms protect phenols contained in OEO from degradation and volatilization, as suggested by TG analysis.

1. Introduction

Essential oils (EO) are synthesized by plants as a natural defense from parasites and microorganisms, and, to attract pollinator insects and signal processes. Different studies have demonstrated that EO can exert numerous beneficial effects on human health. A large variety of plants has been evaluated for their EO content. Among them, oregano species have been the most studied. Oregano essential oil (OEO) has antioxidant, antiproliferative, anti-inflammatory and antimicrobial activities (Leyva-López, Gutiérrez-Grijalva, Vazquez-Olivo, & Heredia, 2017). In particular, OEO has been considered as one of the most effective natural antioxidant in virtue of its high phenolic content (Jafari Khorsand et al., 2022). Indeed, carvacrol, *i.e.*, the most present monoterpene in OEO, is an excellent antioxidant and reduces colonocyte damage caused by ROS (Kamada, Seo, Chen, & Núñez, 2013). OEO could be administered orally for some gastrointestinal diseases (Hosny et al., 2021). Its oral use, however, is severely hampered by its irritant taste, which requires a high dilution in water before administration, and also by its high susceptibility to light and humidity. Moreover, phenols have a high instability during digestion, and are rapidly metabolized, so that only a small fraction of the oral dose reaches the lower gut, where parasites reside (Kohlert et al., 2000). The colonic parasites are responsible for the breakdown of the original structures of phenols into small metabolites which are then responsible for the health effects (Cardona, Andrés-Lacueva, Tulipani, Tinahones, & Queipo-Ortuño, 2013).

Therefore, protection of OEO from degradation in the gastrointestinal tract is essential for its enhanced efficacy and sustained release. In the last decades numerous reports have described different strategies for encapsulating EO such as the preparation of micro- and nanoparticles and the formation of inclusion complexes with cyclodextrins (Donsì, Annunziata, Sessa, & Ferrari, 2011; Kotronia et al., 2017; Pilicheva, Uzunova, & Katsarov, 2021). The conversion of an oil into a solid increases the oil physical and/or chemical stability (Zambito, Piras, & Fabiano, 2022). Dextrins, cyclodextrins or inorganic scaffolds, such as

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https://doi.org/10.1016/j.lwt.2024.116281
Received 29 January 2024; Received in revised form 27 May 2024; Accepted 29 May 2024
Available online 3 June 2024

silica supports, have widely been used for this purpose. The most important requirement for host supports is a large surface area, which facilitates the adsorption of essential oils (Mallard, Bourgeois, & Fourmentin, 2018). Zeolite has been found to possess such a property. In fact, zeolite, being an ion exchanger and an antimicrobial agent (Fabiano et al., 2019) is apt for different pharmaceutical applications, including delivery systems for bioactive molecules with low molecular weight. Although zeolite is already used in the food industry, *e.g.*, as additive and natural preservant, and as dietary supplement in form of powder, capsule or liquid, it is regulated differently around the world. In Canada, zeolite dietary supplements are regulated by Health Canada, in Australia by the Therapeutic Goods Administration (TGA), in the United States by the Food and Drug Administration (FDA), in European Union (EU) by the European Food Safety Authority (EFSA). In the EU was not consumed as a food before May 15, 1997, so it requires a pre-market authorization to be placed as food on the market, however its application in biomedicine is growing (Serati-Nouri et al., 2020). Despite remarkably high production costs, OEO has already reached the market as a diet supplement for humans, principally in the form of soft gelatine capsules (*e.g.*, 25 mg Oregano soft capsules from Lindens, Lindens Health and Nutrition, UK; 100 mg Athina® Oregano Öl soft capsules, De). However, further problems are brought by these formulations, *e.g.*, a poor stability which requires the addition of stabilizers, increased permeabilities to oxygen and humidity and possible interaction with other ingredients of capsules (Partheniadis, Vergkizi, Lazari, Reppas, & Nikolakakis, 2019). From a practical point of view, compression is the simplest method for the preparation of oral extended-release dosage forms. Tablets are not only less expensive than capsules, but also, they have a better stability and acceptance by patients. Their aesthetic qualities such as colour, texture and taste-masking ability depend on their film coating. This, therefore, has a fundamental role in the formulation of tablets. Eudragit® E PO that is an amino methacrylate copolymer insoluble at pH above 5, has been used for the coating of particles containing paracetamol and anhydrous caffeine. *In vitro* and *in vivo* studies have demonstrated that the coating with Eudragit® E PO can mask the bitter odour and flavour of solid dosage forms (Drašković, Medarević, Aleksić, & Parojčić, 2017). Another aspect that should be considered for the preparation of tablets is the release of the drug. Hydroxypropyl methylcellulose (HPMC) is a pharmaceutical ingredient used in oral, nasal, ophthalmic and topic fields. It is approved by FDA, enzyme resistant, soluble in water and stable in a wide range of pH (3–11) (Hirun & Kraisit, 2022). Drug release from HPMC includes various mechanisms (swelling, erosion, and diffusion). The ability of HPMC to form a hydrogel when it comes into contact with water could be used to incorporate, protect and release hydrophilic and hydrophobic compounds. Viridén, Abrahmsén-Alami, Wittgren, & Larsson, 2011 have studied the release of theophylline and carbamazepine from tablets containing microcrystalline cellulose, lactose and HPMC and have demonstrated that the release of the drugs was dependent on the erosion of HPMC especially in the case of carbamazepine, which is a poorly water-soluble molecule.

The present study proposes a method for the processing of oregano essential oil into controlled release tablets. In literature, it has been reported the preparation of OEO tablets encapsulating the oil by spray-drying technique (Partheniadis et al., 2019), but, as already reported (Zambito et al., 2022), it is important to accurately set up the drying conditions to minimize the loss of volatile compounds. In this study, the preparation steps include HPMC granules containing OEO adsorbed on zeolite at room temperature, and coating by compression in presence of Eudragit® E PO. The thermal characterization of all the individual components (OEO, HPMC, zeolite, Eudragit® E PO), the OEO-zeolite, the compressed core, and coated tablets was carried out to determine their thermal stability and to assess both their stability and possible interactions among the constituents.

2. Materials and methods

2.1. Materials

Folin-Ciocalteau reagent, gallic acid, carvacrol, hydroxypropyl methylcellulose (HPMC) as well as all the inorganic salts and reagents were purchased from Sigma (Milan, Italy) and used as received. Oregano essential oil (OEO) was provided by Alidans S.R.L. Zeolite (clinoptilolite, average diameter <50 μm) was provided by Zeocel Italia (Pisa, Italy). Eudragit® E PO was donated by Evonik Nutrition & Care GmbH (Darmstadt, Germany). Caco-2 cell line was purchased from the American Type Culture Collection LGC standards (ATCC HTB-37, Milan, Italy) and propagated as indicated by the supplier. Complete Dulbecco's Modified Eagle medium (DMEM), fetal bovine serum (FBS), glutamine, penicillin and streptomycin were purchased from Sigma (Milan, Italy). Cell proliferation reagent (WST-1) was provided by Roche diagnostic (Milan, Italy).

2.2. Characterization of OEO

2.2.1. Determination of total phenolic and carvacrol content in OEO

The total phenolic content (TPC) in OEO was determined using the Folin-Ciocalteau colorimetric method, as already described (Viuda--Martos et al., 2010) slightly modified. Briefly, 50 μL of OEO in ethanol (25 mg/100 mL) was diluted 20-fold with distilled water. Thereafter, 1 mL of Folin-Ciocalteau reagent (10 mL/100 mL) and 950 μL of water were added. The mixture was incubated for 1 min and 1 mL of Na_2CO_3 (7.5 g/100 mL) was added. The final mixture was incubated for 2 h at room temperature in the dark followed by spectrophotometric analysis at 765 nm using a Lambda 25 PerkinElmer spectrometer. The results were expressed as mg of gallic acid equivalents (GAE) per g of oil, with reference to the calibration curve of gallic acid obtained in the same conditions. The carvacrol content in OEO was determined by HPLC (PerkinElmer, Walthaman, MA, USA) (Hajimehdipoor, Shekarchi, Khanavi, Adib, & Amri, 2010). An Aeris 3.6 μm, PEPTIDE XB-C18 Å, 250 × 4.6 mm column was used. The mobile phase (flow rate 1 mL/min) was acetonitrile:water (50:50) and UV detection was set at 275 nm. The carvacrol content was expressed as mg per g of OEO by referring to a standard calibration curve of carvacrol.

2.2.2. Cell treatment and oxidative stress

Cell viability of OEO was evaluated using the Caco-2 cell line. Caco-2 cells were seeded in 96-well culture plates at a concentration of 10^4 cells per well, incubated at 37 °C and 5% CO_2, and left to proliferate for 24 h prior the incubation with the samples. The culture medium from each well was removed and replaced with a medium containing OEO in ethanol and diluted with complete DMEM at concentrations in the range 3–50 μg/mL for 4 h. Cell viability was then assessed using WST-1 reagent diluted 10-fold and incubated at 37 °C and 5% CO_2 for 4 h. Measurements of formazan dye absorbance were carried out at 450 nm, with reference wavelength of 655 nm, using a microplat reader (BioTek 800/TS, Thermo Scientific). To evaluate the antioxidant activity of OEO, Caco-2 cells adhered to 96-well culture plates were incubated for 2 h with OEO at the concentration of 3, 5, 7, 10, and 12 μg/mL, which corresponded to phenol concentrations of 1, 2, 3, 4, and 5 μg/mL GAE in complete DMEM, respectively. Following this treatment, the cells were washed with phosphate-buffered saline (PBS) and subjected to oxidative stress induced by 0.5 mmol/L commercial H_2O_2 for 1 h. Caco-2 cells incubated with H_2O_2 without sample treatment were used as a reference for oxidative stress. Cell viability was assessed using the WST-1 reagent, and the cell viability percentages were calculated relative to untreated Caco-2 control cells which had not been exposed to H_2O_2 (Cioni et al., 2022).

2.3. Preparation of HPMC granules containing OEO-zeolite (HPMC-OEO-GRA)

The procedure for loading OEO into zeolite powder involved stirring 5 g of zeolite in 100 mL of water for 48 h, followed by centrifugation. The sediment, after drying at 40 °C under vacuum (Heraeus RVT360 vacuum oven, Heraeus S. p.a., Milan, Italy) was used to prepare OEO-containing zeolite (OEO-ZEO). To this purpose, 20, 12.5 or 7.5 mg OEO was added to 500 mg of purified zeolite to optimize the oil content in the final formulation. The resulting mixture was stirred 24 h, then, the mixture dried at 40 °C under vacuum. TPC in OEO-containing zeolite was determined before the exsiccation, by centrifuging 1 mL of dispersion at 9050 g for 5 min (Hetting Mikro 120 centrifuge, Hetting, Milan, Italy) then analyzing the supernatant to determine the percentage of TPC encapsulated in zeolite by difference. To prepare the final granules, the dried OEO-containing zeolite was mixed with HPMC in different ratios (5:5, 6:4, and 7:3) by adding 600 μL of water per gram of OEO-ZEO. The resulting mixtures were extruded to form 1.2 mm size HPMC-OEO-GRA which were dried at 40 °C under vacuum.

2.4. Measurement of HPMC-OEO-GRA angle of repose

The angle of repose of granules was measured following the procedure reported by Shah, Tawakkul, and Khan (2008). The funnel used had a 1 cm orifice and a 12 cm height from top of the funnel to the end of the orifice. The funnel was fixed 4 cm above the bench surface. Following the flow of 5 g of sample, the height of the granules forming the cone (h) and the radius (r) of the base were measured to calculate the angle of repose:

$$\theta = \tan^{-1}(h/r) \quad (1)$$

2.5. Preparation of core tablets (OEO-TAB) and Eudragit® E PO coated tablets (OEO-c-TAB)

The HPMC-OEO-GRA were compressed into flat-faced tablets of 0.6 mm diameter and 20 mg weight, with a hydraulic press (PerkinElmer, Walthaman, MA, USA) applying a force of 1000 kg for 15 s, to obtain the OEO core tablets (OEO-TAB). The OEO-TAB were compression-coated with Eudragit® E PO (OEO-*c*-TAB) maintaining core:coat weight ratio at 1:2. In detail, the tablets were prepared by first filling one half (20 mg) of the coat into the die cavity, then the core was positioned centrally on the power bed, the die cavity was filled up with the remaining coat (20 mg) and the mass was finally compressed as described above to give the final product (El Naggar, Mohamed, Borg, El-Sheakh, & Hamed, 2020).

2.6. Characterization of core (OEO-TAB) and coated tablets (OEO-c-TAB)

2.6.1. Weight variability

The weight variability test was carried out on OEO-*c*-TAB. Following the indications of the European Pharmacopoeia 11th edition (Ph. Eur. 11.0) the cores were not tested for uniformity of mass of single-dose preparations according to because their average mass was below 40 mg. Twenty coated tablets were weighed, the average weight was calculated, then the tablets were weighed individually. The percent deviation of each tablet from average weight was calculated by the following formula:

$$\%\ \text{Deviation} = \frac{\text{Average weight} - \text{Individual weight}}{\text{Average weight}} 100 \quad (2)$$

A tablet batch passed the test if no more than 2 tablets deviated from the average mass by more than the 10% and none deviated by more than 20%.

2.6.2. Resistance to crushing

Four OEO-TAB or OEO-*c*-TAB were randomly selected and oriented in the same direction as the force applied. The force needed to break the tablets was measured using Monsanto hardness tester (Farmalabor, Puglia, Italy), as reported by Sivakumar, Venkataraman, Natarajan, Ganeasn, & Ali, 2009.

2.6.3. Determination of tablet disintegration time

The tablet disintegration time was determined according to the general method of Ph. Eur. 11.0 in a calibrated single basket tablet disintegration tester (Pharma Test Apparatebau AG, Hainburg, Germany). The disintegration medium was 800 mL of water for OEO-TAB and HCl 0.1 M for OEO-*c*-TAB. One dosage unit was placed in each of the six tubes, placing the disk on each tablet. At the end of specified time the basket was lifted from the fluid and the dosage units were observed.

2.6.4. In vitro drug release

Drug release from OEO-TAB and OEO-*c*-TAB were measured at 37 ± 0.1 °C by the USP rotating basket method (150 rpm) with 100 mL of dissolution medium, (apparatus, DT light Series, Erweka). The release media were simulated gastric fluid (SGF, 0.1 mol/L HCl pH 1.2), simulated jejunal fluid (SJF, Na_2HPO_4 0.2 mol/L, with pH adjusted to 6.8 with NaOH 0.2 mol/L) and simulated ileal fluid (SIF, SJF adjusted to pH 7.4 with NaOH 0.2 mol/L) without enzymes. These were supposed to simulate pH values of stomach, small intestine, and colon respectively. It has been reported that the average residence time of a formulation in stomach is 2 h and that in small intestine is 3 h (Krishnaiah, Reddy, Satyanarayana, & Karthikeyan, 2002). Consequently, the release medium was changed during the experiment as follows: SGF for 2 h, SJF for 2 h and SIF for 2 h. At predetermined time intervals, 1 mL of dissolution medium was replaced with 1 mL of fresh medium, pre-thermostated at 37 °C and analyzed for TPC after purification with Amicon® Ultra-15, MW cutoff (MWCO) 30-kDa filters (Merck Millipore, Toronto, CN, USA). The cumulative percentage of TPC was calculated. All the experiments were performed in triplicate.

2.7. Thermal gravimetric analysis (TGA)

The thermal stability of OEO, zeolite and OEO-ZEO, OEO-TAB, OEO-*c*-TAB, HPMC and Eudragit® E PO, was performed using a TA Instruments Thermobalance model Q5000 IR (TA Instrument, New Castle, DE, USA) using a previously developed method (Mezzetta, Guazzelli, & Chiappe, 2017). Weight standards (100 mg and 1.00 g) were used for weight calibration and nickel standards were used for temperature calibration. All standards were supplied by TA Instruments Inc. For the drying procedure, the sample (10–15 mg) of OEO or zeolite or OEO-ZEO was heated to 40 °C in a platinum crucible and maintained in N_2 flow (90 mL/min) for 30 min. The samples were then heated from 40 °C to 800 °C at a heating rate of 10 °C/min under nitrogen (90 mL/min) and held at 800 °C for 3 min. Measurements of OEO-TAB, OEO-*c*-TAB, HPMC and Eudragit® E PO were carried out at a rate of 10 °C/min, from 30 °C to 900 °C under nitrogen flow (25 mL/min).

The mass change was recorded as a function of temperature and time. TGA experiments were performed in duplicate.

2.8. Statistical data treatment

All the results were expressed as mean values and standard deviation (SD) and the statistical differences between means was assessed by the ordinary one-way ANOVA test, followed when necessary by student's *t*-test or Turkey test, using GraphPad Prism 10.0 software. Differences were considered significant for p values lower than 0.05.

3. Results and discussion

3.1. Characterization of OEO

3.1.1. Determination of total phenol and carvacrol content in OEO

Chemical composition of OEO was provided by Alidans S.R.L. as shown in Table 1. Because the antioxidant activity of OEO is attributed to its major components, *i.e.*, phenolics (Rodriguez-Garcia et al., 2016), TPC and carvacrol content in OEO were determined and considered in subsequent studies. The TPC in OEO was found to be 420.6 ± 23.0 mg/g, and the carvacrol content was 376.1 ± 3.4 mg/g (corresponding to 0.036 ± 0.003 g/100 mL). These data are in agreement with those reported in the literature, where a TPC in OEO ranging from 135.8 to 502.6 mg/mL (Tuttolomondo et al., 2013) and a carvacrol content in the range from 0.028.1 to 0.073 g/100 mL (Sari et al., 2006) are reported. Such a high range for either TPC or carvacrol content depends on genotype and cultivation conditions (Azizi, Yan, & Honermeier, 2009), while the apparent difference found between carvacrol content determined by HPLC and that reported by the producer could be attributed to different quantification methods (Figiel, Szumny, Gutiérrez-Ortíz, & Carbonell-Barrachina, 2010).

3.1.2. Cell viability assay and in vitro cellular assessment of antioxidant properties

Before evaluating the OEO protection from oxidative stress, the OEO cytotoxicity on Caco-2 cells was assayed. Caco-2 cells were incubated 4 h with OEO in the 3–50 μg/mL concentration range with no cytotoxic effect on cell viability up to 50 μg/mL (Fig. 1 a), indeed the concentrations tested were not significantly different from control cells. For the oxidative stress assay, five different concentrations of OEO were evaluated, set on the basis of relevant GAE equivalents and corresponding to 1–5 μg/mL. Gallic acid, a well-known natural antioxidant, was used as a reference. The oxidative treatment with H_2O_2 resulted in a drastic decrease in cell viability (43%) with respect to untreated and unstressed control cells (Fig. 1 b) (Turkey test). Preincubation of Caco-2 cells with nontoxic concentration of OEO showed that OEO protected against hydrogen peroxide induced cytotoxicity in a concentration-dependent protective effect, probably thanks to the high radical scavenging activity of carvacrol (Coccimiglio, Alipour, Jiang, Gottardo, & Suntres, 2016). These results were also consistent with the research of Llana-Ruiz-Cabello et al., 2015 who identified a comparable pattern in the responses of carvacrol. Carvacrol participates in one or more secondary reactions, resulting in a modest reduction of free radicals. The OEO had the same ability effect on H_2O_2 as that of GA at all the concentrations tested. Different reports have shown that the essential oils present in plants are natural antioxidants reducing cell damage caused by reactive species. In particular, carvacrol, a major component, has remarkable antioxidative properties (Imran et al., 2022).

3.2. Preparation of HPMC-OEO-GRA

The use of essential oils, like OEO, in their typical liquid form has limited applications because of their susceptibility to degradation caused by environmental stresses, storage conditions, presence of volatile compounds. In addition, OEO has limited water solubility and intense flavour that add limitations to its use. Numerous encapsulation strategies have been developed and reported in literature to overcome these limitations, such as the encapsulation in micro- or nanoparticles or cyclodextrins (El Asbahani et al., 2015). However, some of these strategies are inadequate for the encapsulation of EO, since high temperatures or pressures can alter the essential oil themselves (Lai, Wissing, Müller, & Fadda, 2006) and often complicated preparations are described. Although the encapsulation of EO in cyclodextrins is one of the most efficient strategies to improve their physiochemical properties (Rakmai, Cheirsilp, Mejuto, Simal-Gándara, & Torrado-Agrasar, 2018; Zambito et al., 2022), if the guest has a wrong size, it will not fit properly in the cyclodextrin cavity, consequently, the stability of the complex will decrease (Ayala-Zavala, Del-Toro-Sánchez, Alvarez-Parrilla, & González-Aguilar, 2008). In this contest, zeolite represents a useful alternative. The natural zeolite clinoptilolite is the only zeolite registered in the EU that can be used in the oral treatment (Hao et al., 2021) and has a great potential for the absorption of EO (Yi, Liu, Su, & Xue, 2022). Therefore, zeolite was chosen for encapsulating OEO. Zeolite (500 mg) was loaded with different amounts of OEO, namely 7.5, 12.5 and 20 mg. With 7.5 and 12.5 mg of OEO, the percentage of TPC encapsulated in zeolite was below the quantification limit. With 20 mg of OEO, the percentage of TPC encapsulated was around 15.53 ± 1.43 according to the results obtained with other fragrant molecules as well as cationic species (Li et al., 2020). This result suggests that although the TPC percentage entrapped in zeolite is not high it, nevertheless, is comparable with data reported in literature (Abdelhameed, Alzahrani, Shaltout, & Emam, 2021; Ruiz-Rico et al., 2017). Then it can be stated that EO encapsulation with zeolite can be used to stabilize and slow down the release of highly volatile compounds (Li et al., 2020). To further increase the stability and extend the shelf life of OEO, we prepared OEO-containing zeolite granules. Indeed, granules are known to have advantages over powders, in particular, a lower specific surface area in virtue of which they are physically and chemically more stable than the corresponding powders (Ali, Suliman, Elhaj, & Suliman, 2019).

Table 1
Chemical composition of OEO.

Component	Content (g/100 g)
Carvacrol	60–75
p-Cymene	5–15
Gamma-terpinene	5–10
Thymol	0–5

3.3. Measurement of angle of repose

HPMC-OEO-GRA were prepared mixing the dried OEO-ZEO with HPMC in different ratios (5:5, 6:4, and 7:3). To select the best granules, measurements of angle of repose were carried out. The angle of repose (θ) was calculated from Eq. (1) and the results are presented in Fig. 2. As reported (Carr, 1965) a value of θ in the 31–35° range indicates "good" flow, whereas a value < 30° indicates "excellent" flow. Although the HPMC-OEO-GRA 5:5 had a θ < 30°, this value is not significantly different from that of HPMC-OEO-GRA 6:4 or HPMC-OEO-GRA 7:3, indicating that all granule preparations are not cohesive. Because powder cohesion is one the great obstacles to compression (Jones-Salkey, Chu, Ingram, & Windows-Yule, 2023). This result point out that the bulk properties of the HPMC-OEO-GRA make them suitable to be directly compressed.

Considering that the OEO dose in commercial products is high (*e.g.*, Lindens) and that the flowabilities of the granule preparations were not different from each other, only the formulation with the highest content in OEO and the lowest content in HPMC (OEO-containing zeolite:HPMC 7:3) was further investigated.

3.4. Characterization of OEO-TAB and OEO-c-TAB

3.4.1. Tests of weight variability and resistance to crushing

There is a growing interest in developing tablet formulations of EO to increase oil stability and commercial benefits (Ying et al., 2016), especially because the final commercial product should maintain the quality in term of stability in various conditions during production, storage, and transportation. The more common excipients used in solid oral dosage forms could interact with the bioactive compound affecting the quality attributes of the final dosage forms including its stability (Crowley & Martini, 2001). So, the choice of the excipients is critical to preserve the effectiveness of the actives. Chewable tablets containing essential oils

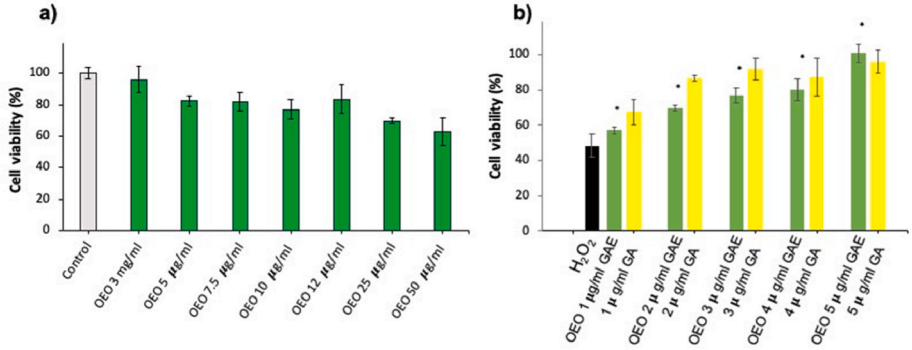

Fig. 1. *In vitro* cell evaluation of Caco-2 cell line treated with OEO. Cytotoxicity screening after 2-h treatments with OEO (a); protective effects OEO from H_2O_2-induced oxidative stress, reported as cell viability % after 2-h treatments with 0.5 mmol/L H_2O_2 (b) (black: H_2O_2-induced oxidative stress control; green: OEO, yellow: gallic acid). Data are expressed as the % of viable cells compared to 100% of control (untreated cells). Means ± SD, $n = 6$,*significantly different from H_2O_2.

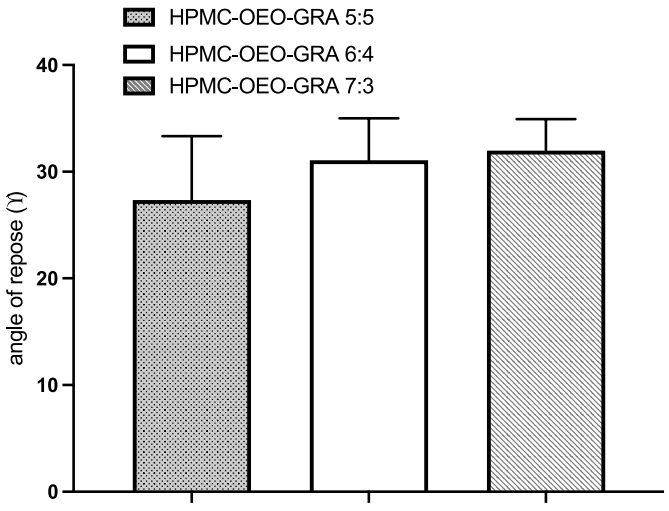

Fig. 2. Angle of repose (deg) of HPMC granules containing OEO-zeolite HPMC-OEO-GRA, prepared under different OEO/HPMC ratio. Means ± SD, $n = 3$.

were successfully prepared by Sachan, Sachan, Kumar, Sachan, and Gangwar (2010), but they contain lactose, which is harmful for lactose intolerance. Zeolite has been found to be safe in a wide range of applications (Sharma, Sutar, Xiao, & Zhang, 2023). Thanks to their porous structure, zeolites are compressible and thus suitable for the preparation of tablets. Moreover, since OEO has an irritating taste, it is convenient to use a coating able to mask OEO taste and aroma. Eudragit ® E PO, a cationic copolymer that dissolves at pH < 5 while remaining intact at neutral pH, offers a potential application for taste masking, as it dissolves in the stomach without interfering with the release kinetics of the actives (Xu, Bovet, & Zhao, 2008). Tablets from zeolite granules containing OEO coated with Eudragit® E PO were successfully prepared by compression.

Physical properties of coated tablets and cores are reported in Table 2. The results of the weight variability tests demonstrate that the weight of the coated tablets was almost uniform, indeed they respected the admitted SD of ±10%. No oil leakage should occur during compression (Partheniadis et al., 2019), and, in fact, no leakage was indicated by these results.

Table 2
Results of quality control tests. *RSD (%) = relative standard deviation. Means ± SD, $n = 4$. *significantly different from each other.

Tablets	Weight (mg), n = 20 RSD* (%)	Hardness (kg) n = 10
OEO-TAB	–	2.04 ± 1.02
OEO-*c*-TAB	50.06 ± 6.50 (1.42%)	4.59 ± 0.72*

Although no defined values have been established for tablets hardness, a force of about 4 kg has been considered satisfactory for tablet manufacturing (Allen & Ansel, 2013). Hence, only the coted tablets ensure a sufficient mechanical strength. The hardness test is informative; however, it is considered an important parameter in the definition of the features of the productive process because tablets are subjected to mechanical shocks during production, packing, transportation, and distribution and therefore, they should have an appropriate mechanical resistance (Giordani & de Melo, 2012). As alternative to compression, 3 d printed tablets containing essential oil have been recently proposed. Although the powerful and customized perspectives of 3 d printed tablet, the variability and mechanical strenght requisites are still open issues (Koshovyi et al., 2023; Shetta, Ali, Sharaf, & Mamdouh, 2024).

3.4.2. *Disintegration time*

The dosage unit disintegration time is an important property, as it can be the rate determining step in the process of drug absorption. The coating with Eudragit® E PO affected the tablet disintegration time (Drašković et al., 2017), indeed, the OEO-TAB disintegrated within 15 min in water, whereas the OEO-*c*-TAB disintegration time was 30 min in HCl 0.1 M. The slower disintegration of the latter could be ascribed to the lag due to the dissolution of the coating. It should also be considered that Eudragit® E PO is a pH dependent polymer, so the coated tablets could prevent the release of the bioactive in saliva (pH 6.8–7.4) and release it in the gastric environment (pH 1.0–1.5). Thus, the coating with Eudragit® E PO offers a more suitable approach to taste masking than the addition of artificial flavours (Cerea, Zheng, Young, & McGinity, 2004).

3.4.3. *In vitro drug release*

In vitro release of total phenols from OEO-TAB and OEO-*c*-TAB is reported in Fig. 3. There are no differences in the release of phenols from OEO-TAB or OEO-*c*-TAB indicating that such release is not influenced by the coating. It has been reported that extracts from plants are made up of several compounds that could be modified through the digestive tract, so it is important to design a suitable pharmaceutical formulation. Water-based oral formulations could limit the solubility of some bioactive and lead to microbiological contamination. Dry powders are exposed directly to gastrointestinal enzymes and stomach acidity, thus limiting the efficiency of the actives (De Torre, Vizmanos, Cavero, & Calvo, 2020). Therefore, tablets represent an oral formulation more advantageous than liquid forms or not compressed powders. Indeed, as shown in Fig. 3, only the 10% circa of total phenols was released in SGF whereas in SJF and SIF such release was 15 and 30% respectively. These results demonstrate that both formulations prevent the oxidation that occurs in the stomach and simultaneously counteract the interactions between phenols and bile acids and pancreatic secretions that could led to a

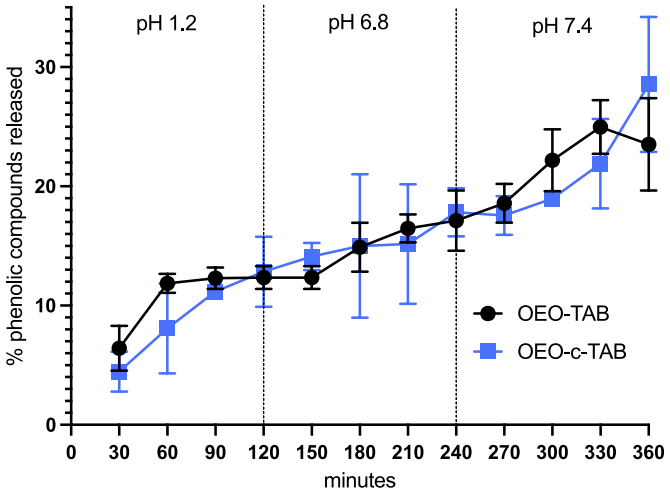

Fig. 3. Phenolic compounds released from core (OEO-TAB) and Eudragit® EPO coated (OEO-*c*-TAB) tablets. Samples were immersed, in sequence, in simulated gastric fluid (SGF), pH 1.2, simulated jejunal fluid (SJF), pH 6.8, and simulated ileal fluid (SIF), pH 7.4. Means ± SD, $n = 3$.

reduced bioavailability (Sirovec et al., 2022). In addition, EO possess antimicrobial activity that makes them potential modulators of the intestinal microbiota. It should be considered that most of the EO molecules do not arrive to the colon, because they are assimilated in the small intestine. Considering that most of the intestinal microbiota reside in the colon, then the release of EO in the colon is crucial to modulate the colon pathophysiology (Spisni et al., 2020). Based on the results obtained in this study, OEO-TAB and OEO-*c*-TAB prevent the instant release of the entrapped phenols through the digestive tract, thus phenols could reach intact the lower gut where the parasites live. Moreover, according to the present results the release of OEO was not modified by Eudragit® E PO, hence it can be deduced that coated tablets represent a suitable pharmaceutical form to mask the unpleasant taste of OEO (Shahzad et al., 2011).

3.5. TG analysis

To evaluate a possible taste masking effect of Eudragit® E PO a sensory measurement could be carried out. However, an essential oil could contain approximately 200 components related to its odour and flavour, so the absence of one component due to the use of different part of plants could led to a change of its aroma. Moreover, a wide number of psychological errors (*e.g.*, expectation, suggestion, stimulus error etc.) as well as the control of the climate or the condition of the panelists must be controlled (Mani-López, Lorenzo-Leal, Palou, & López-Malo, 2017). Thermal analysis is widely used in the pharmaceutical field and makes it possible to assess many important drug properties (*e.g.*, transition temperature and glass transitions), to study isomerization phenomena and decomposition kinetics, and to determine the purity and compatibility of the pharmaceutical components of a drug easily and accurately (Guo et al., 2020; Ramos, 2021; Ramos, 2022; Rojek & Wesolowski, 2021; Saber, Attia, & Salem, 2014). TG analysis of the pure substances, core and coated tablets was carried out to get an insight into possible taste making effect of Eudragit® E PO. The results obtained are reported in Fig. 4 (top and bottom) and Table 3. Moreover, the thermal stability also allowed the evaluation of potential interactions between the tablet components, which could lead to potential incompatibility issues between them. Indeed, thermal analysis is a powerful tool for such studies: the thermal degradation profile of the whole system is different from that obtained by the weighted sum of the degradation profiles of the different components. A shift of a particular mass loss can be associated to a stabilization/destabilization of a components due to the presence of

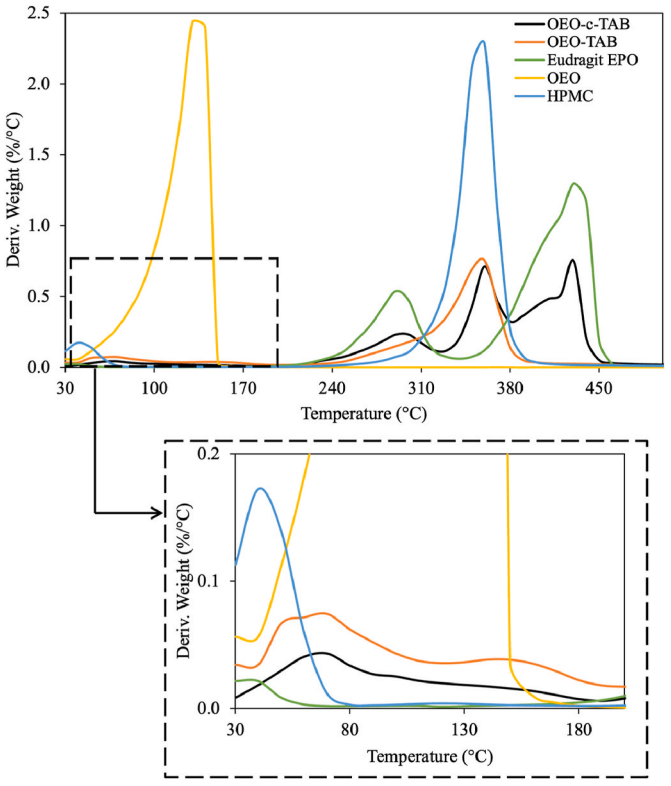

Fig. 4. Thermogravimetric (top) and derivate thermogravimetric (bottom) curves of Zeolite, OEO, HMPC, Eudragit® E PO, OEO adsorbed on Zeolite (OEO-ZEO), core tablets (OEO-TAB) and Eudragit® E PO compression-coated tablets (OEO-*c*-TAB) obtained under nitrogen flow at a heating rate of 10 °C/min.

interactions, as well as the appearance of new mass losses can be related to the formation of different components (Rojek & Wesolowski, 2017; De Mendonça, De Barros Lima, Aragão, & Gomes, 2014). The thermal stability of OEO, zeolite and OEO-ZEO was determined in the range of 30–900 °C. Comparing the thermal degradation profiles of the pure substances it can be observed that OEO has the lowest thermal stability. In accordance with the work of Hosseini, Zandi, Rezaei, and Farahmandghavi (2013), the OEO shows a single mass loss due to its evaporation (González-Rivera et al., 2016; Duce, Vecchio Ciprioti, Spepi, Bernazzani, & Tine, 2017) with maximum degradation rate at 136 °C. The thermogravimetric curve of zeolite shows a continuous mass loss with an overall weight loss of 10.3% over the entire temperature range investigated. The weight loss can be attributed to the evaporation of water, both weakly and strongly bonded, or formed during the dehydroxylation process, taking place while heating (Narasimhulu, Gettu, & Babu, 2014). The thermogram of the OEO-ZEO is similar to that of plain zeolite, with the presence of a 2% mass loss in the temperature range 100–200 °C that could be attributed to the OEO encapsulation. The thermal degradation of HPMC occurs through two steps, the first at temperature below 100 °C due to evaporation of water, and the second in the 250–350 °C temperature range with a maximum degradation rate at 356 °C, corresponding to thermal decomposition of the polymer (Wang, Dong, & Xu, 2007).

In the thermal degradation profile of OEO-*c*-TAB is possible to observe the degradation steps of each component separately, thus indicating the absence of interaction between them (Macêdo, Gomes do Nascimento, & Veras, 2002; Tita, B., Ledeti, Bandur, & Tita, D. 2014). When OEO is encapsulated in coated tablets, its thermal degradation appears to be different as the peak observed in the DTG curve (Fig. 4, bottom) has a different shape compared to that of OEO in the core tablet. In particular, the peak observed in the DTG curve related to the evaporation of OEO in the coated tablet is broader than the peak observed in

Table 3
Experimental temperatures and weight loss percentage of thermal degradation steps. Zeolite, OEO, HMPC, OEO adsorbed on Zeolite (OEO-ZEO), Eudragit® E PO, core tablets (OEO-TAB) and Eudragit® E PO compression-coated tablets (OEO-*c*-TAB) samples.

n° step	Degradation step temperature (°C)/weight loss (g/100 mL)						
	Zeolite	OEO	HPMC	OEO-ZEO	Eudragit® E PO	OEO-TAB	OEO-*c*-TAB
1	64 °C	–	43 °C	70 °C	42 °C	T_{max} = 66 °C T_s = 47 °C	T_{max} = 67 °C T_s = 38 °C
	2.8 %	–	4.4 %	3.1%	0.4 %	4.4 %	3.5 %
2	64–900 °C	136 °C	–	126 °C	–	154 °C	–
	7.6 %	100 %	–	6.0 %	–	2.7 %	–
3		–	–	126–900 °C	293 °C	–	T_{max} = 296 °C T_s = 229 °C
		–	–	3.1 %	27.1 %	–	14.2 %
4		–	356 °C		–	358 °C	361 °C
		–	88.5 %		–	41.8 %	20.9 %
5		–	–		T_{max} = 434 °C T_s = 406 °C	447 °C	T_{max} = 429 °C T_s = 411 °C
		–	–		69.9 %	1.9 %	29.7 %
6		–	–		–	566 °C	508 °C
		–	–		–	1.4 %	2.1 %
Residue (900 °C)	89.6 %	–	7.1 %	87.8 %	2.6 %	47.8 %	29.5 %

the DTG curve of OEO in the core tablet. In addition, the OEO evaporation produces a continuous mass loss up to 180 °C indicating that the evaporation process of OEO is affected by the coating which makes spread it over a wider temperature range. These results suggest that the addition of Eudragit® E PO allows the masking of the irritating taste of OEO (Tran et al., 2023).

4. Conclusions

OEO has high antioxidant properties, is non-toxic and able to protect cells from oxidative stress thanks to the presence of phenols, in particular carvacrol. Although OEO is already commercialized in gelatine capsules, their production cost is high and have several limitations, like a poor stability and possible interaction with other ingredients of capsules themselves. Moreover, it is not simple to transform an oil into a solid dosage form, which could be suitable form producing, powders, granules, tablets, etc. The adsorption of OEO into zeolite allowed the preparation of granules the were compressed into tablets. The coating with Eudragit® E PO increases the oil stability to both degradation and volatilization. The coated tablets have sufficient mechanical strength and are able to modulate the release of phenols. Thus, Eudragit® E PO coated tablets containing OEO represent a powerful tool for the oral administration of essential oils derived from plants, as they protect them from degradation because they are resistant to gastric and intestine *in vitro* simulation. Beside OEO, the proposed formulation and preparation steps could be adopted for other plant derived essential oils. Considering the obtained stabilization of the oil and the masking effect Eudragit® E PO, the proposed formulation could lead to a better compliance toward essential oil oral intake, expanding the beneficial effects of natural compounds to a wider audience of consumers.

CRediT authorship contribution statement

Chiara Migone: Writing – review & editing, Investigation, Formal analysis, Data curation. **Anna Maria Piras:** Writing – review & editing, Validation, Data curation. **Ylenia Zambito:** Writing – review & editing, Validation, Data curation. **Celia Duce:** Writing – review & editing, Validation, Data curation. **Elena Pulidori:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Lorenzo Guazzelli:** Writing – review & editing, Validation, Conceptualization. **Andrea Mezzetta:** Writing – original draft, Investigation, Data curation. **Angela Fabiano:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

Declarations of interest: none.

Data availability

No data was used for the research described in the article.

Acknowledgments

Thanks to Alidans s. r.l. and Zeocel Italia for kindly providing Oregano essential oil and zeolite.

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