

Use of Encapsulated Antimicrobial Substances in Innovative Litter Plastic Bag designed for Medical Waste and Litter with Significant Antimicrobial Load

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Healthcare activity inevitably generates waste, which contains pathogens in sufficient concentrations and poses risks for workers who manage it, public health and environment. The main sources of such waste are health units, such as hospitals, pharmacies, diagnostic centres, as well as, research centres and laboratories concerned with medical procedures. According to World Health Organization, 10 % to 25 % of healthcare waste is infectious and hazardous. Therefore good waste management practices that will minimize not only the negative effects of this waste in human health, but also the risk of infections in waste collection and disposal sites, are necessary. In this framework, the development of a litter low density polyethylene (LDPE) bag with encapsulated antimicrobial substances from oregano essential oil (OEO) and organic extract of citrus bioflavonoids (flavomix) through extrusion was performed. The encapsulated agents were gradually released into the inner content of the bag, neutralizing the studied contained microbes. The encapsulated structures were evaluated using Differential Scanning Calorimetry (DSC). The encapsulation efficiency (EE), as well as the controlled release of the encapsulated compounds, were determined using Ultraviolet–visible spectroscopy (UV-VIS). The gradual release of the encapsulated antimicrobial agents was studied by storing the produced polymers under controlled conditions of temperature- radiation (20 °C- darkness and 45 °C- light) and humidity (relative humidity- RH 35, 70 and 95 %) for 60 days. The results showed that EE was higher than 70 %. The 60-days analysis exhibited that elevated temperature (45 °C) and humidity (95 % RH) values led to increased release. Finally, the produced films have the potential to be applied in medical waste and the gradual release of the antimicrobial agents will ensure the elimination of contained microbes.

1. Introduction

Healthcare waste (HCW) includes a wide range of materials, from used syringes to body parts, diagnostic samples, blood, chemicals and radioactive material (Hinduja and Pandey, 2018), generated by processes of healthcare units, such as hospitals and clinics, as well as, research centres and laboratories concerned with medical procedures (Ranjbari et al., 2022). Based on estimations provided by World Health Organization (WHO) (WHO, 2014), HCW streams are divided to non-hazardous and hazardous fractions, constituting 75-90 % and 10–25 % of the total HCW amount, respectively (Ranjbari et al., 2022). The non-hazardous fraction comprises of waste produced principally from the kitchen, the administration and the housekeeping within the healthcare facilities (Oyekale and Oyekale, 2017) and its treatment can be along with the municipal solid waste (Hinduja and Pandey, 2018). Contrarily, the hazardous fraction includes highly infectious or toxic radioactive materials (Oyekale and Oyekale, 2017) and it requires a separate and precise management; otherwise it can present various health and environmental threats (Hinduja and Pandey, 2018).

The increasing population index (Ranjbari et al., 2022), in conjunction with the rapidly aging population and the improved access to healthcare services, results in the fast growth of healthcare industry, which in turn leads to a steady global increment of HCW production by 2–3 % (Minoglou et al., 2017). As a consequence, its management is a major challenge worldwide since a significant percentage of HCW contains infectious and hazardous pathogenic agents. Specifically, inappropriate management of HCW and its included microbes

(Geetha et al., 2019) can not only lead to toxic effects, infections and transmission of diseases to the healthcare personnel, waste handlers, patients and the community, but also cause environmental pollution (Hinduja and Pandey, 2018). Therefore, efficient management practices that will minimize the microbial load and thus many negative effects of this waste are necessary.

Essential oils (EOs) derived from plants are known to possess high levels of antimicrobial activity (Sivropoulou et al., 1996). Numerous in vitro and in vivo researches have determined the antibacterial, antiviral and antifungal properties of EOs (Leyva-López et al., 2017). Among them, oregano essential oils (OEO) are the most studied (Leyva-López et al., 2017) since they are reported to manifest the broadest and highest activity against Gram-positive and Gram-negative bacteria, such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Rhizobium leguminosarum* and *Bacillus subtilis* (Sivropoulou et al., 1996), as well as against the pathogenic fungi *Candida albicans*, *Candida tropicalis*, and *Torulopsis glabrata* (Aliannis et al., 2001). The strong activity of OEO can be attributed to the existence of phenolic compounds, and mainly carvacrol and thymol, which are its major constituents at an approximate percentage of 50 % (Aliannis et al., 2001).

In general, phenolic compounds are often linked to antimicrobial effects and are related to interactions with the cell membrane of microbes. Flavonoids are one of the largest class of polyphenolic secondary metabolites and they are synthesized by plants in response to microbial infection (Górniak et al., 2019). Especially citrus flavonoids have been found to be an antiviral and antimicrobial agent against a wide range of pathogenic microorganisms (Tripoli et al., 2007). Thanks to these properties, a natural, organic extract of citrus bioflavonoids (flavomix) was used in our study in order to study its contribution to the elimination of microbes contained in medical waste.

Nevertheless, the direct application of essential oils and extracts for the purpose of the study is not effective since they are thermolabile and highly volatile, which leads to rapid degradation of their active substances and, hence, to low persistence of their antimicrobial effects. A method that is capable of protecting essential oils from volatile losses and of offering a controlled and continuous release of their active substances, is encapsulation (Stramarkou et al., 2020).

Extrusion is a cost effective, continuous and environmentally friendly encapsulation technique that produces small particle extrudates, which can be easily utilised and release in a controlled way the bioactive compounds, improving their shelf life (Bamidele and Emmambux, 2021). The matrix material used in encapsulation can affect the process efficiency and the characteristics of the final product (Stramarkou et al., 2020). Low density polyethylene (LDPE) is widely used as a polymer matrix in antimicrobial packaging thanks to its acceptable flexibility, transparency, easy processability, thermal stability, environmental recyclability and low cost (Jokar et al., 2012).

The aim of this study is the development of a litter bag from LDPE with encapsulated antimicrobial substances that can be applied in medical waste and litter with significant antimicrobial load. The encapsulation of the antimicrobial agents from OEO and flavomix was performed through extrusion, while changing the antimicrobial agent concentration (%), the extrusion temperature (°C) and the screw rotation speed (rpm). The encapsulated antimicrobial agents were gradually released into the inner content of the bag, neutralizing the microbes of the contained waste. The produced polymers were stored under controlled conditions of temperature- radiation and humidity for 60 days in order to study the gradual release of the encapsulated antimicrobial agents. The encapsulated structures were evaluated in terms of their thermal behaviour using DSC. EE was determined using UV-VIS spectroscopy.

2. Materials and methods

LDPE, obtained from ACHAIIKA PLASTICS SA, was used as matrix for the production of the extruded pellets, whereas OEO, purchased from Organic Essential Oils-Zoi Filippou MEPE, and flavomix in powder, purchased from POLYPAN GROUP SA, Greece, were used as antimicrobial agents. Petroleum ether was purchased from Sigma Aldrich.

2.1 Extrusion

The development of the polymers with the encapsulated antimicrobial agents of OEO and flavomix was performed using a twin-screw extruder (Prism Eurolab, model KX-16HC, Staffordshire, UK). The extruder was initially preheated and its last zone was set at two studied temperatures: 180 and 200 °C. When temperatures in the extruder were stabilized, LDPE was introduced in the feeder, whereas OEO in liquid state and flavomix in solid state were introduced in the last zone so that the concentrations of antimicrobials in the final polymers are 10 and 20 %. The extruder operated at two screw rotation speeds: 50 and 70 rpm and screw torque was

recorded during the process. The various operation conditions that were selected after preliminary experiments are presented in Table 1.

Table 1. Extrusion conditions

Extrusion Parameters	Values
Essential Oil concentration (%)	10, 20
Temperature (°C)	180, 200
Screw Rotation speed (rpm)	50, 70

2.2 Determination of Encapsulation Efficiency (EE)

The determination of EE of the produced polymers was achieved by Soxhlet extraction and UV-Vis spectrophotometry. Specifically, the polymers with the encapsulated antimicrobial agents were extracted for 50 min using petroleum ether as solvent. The developed extracts, after reaching room temperature, were titrated and their absorbance was measured from 190 to 790 nm using the UV-Vis photometer (Espectrofotometro UV-M51, Bel Photonics, Italy) in order to initially calculate the amount of the non-encapsulated OEO and flavomix. The non-encapsulated agents were quantified by use of standard curve of pure OEO and flavomix diluted to different concentrations. EE was calculated using the following Equation.

$$EE (\%) = \frac{\text{encapsulated EO quantity (mL)}}{\text{EO initial quantity (mL)}} \cdot 100 \% \quad (1)$$

The measurements were carried out in duplicate.

2.3 Differential Scanning Calorimetry (DSC)

The thermal transitions of the produced polymers with the two encapsulated antimicrobial agents and the non-encapsulated polymers were studied by Differential Scanning Calorimetry (DSC) (Perkin Elmer DSC 6, CT, USA), using Pyris 6 software. Nitrogen gas was used at a flow rate of 20 mL/min to create an inert atmosphere. 10 mg of each sample were sealed in an aluminium sample carrier, which was inserted into the DSC. A blank, sealed sampler was used as a reference. The temperature program that the samples were subjected to consisted of three cycles:

- Heating from 0 °C to 250 °C at a rate of 5 °C/min (1st heating cycle)
- Cooling from 250 °C to 0 °C at a rate of 5 °C/min
- Heating from 0 °C to 250 °C at a rate of 5 °C/min (2nd heating cycle)

The measurements were carried out in duplicate.

2.4 Evaluation of shelf life

The optimal polymers were stored under controlled conditions of temperature- radiation (20 °C- darkness and 45 °C- light) and humidity (RH 35, 70 and 95 %) for 60 days. During storage, EE of the two antimicrobial agents, namely OEO and flavomix, was studied over time at regular intervals (2, 5, 10, 20, 30 and 60 days) and was calculated as described in Section 2.2. The measurements were conducted in duplicate.

2.5 Statistical analysis

One-way and factorial analysis of variance (ANOVA) was applied in order to analyse the differences between the produced polymers. Tukey's range test ($\alpha=0.05$) was applied and all the statistical tests were performed with STATISTICA software (version 13.6, StatSoft®Inc., Palo Alto, USA).

3. Results and discussion

The encapsulation efficiencies for the polymers with the encapsulated OEO and flavomix produced under various conditions of OEO and flavomix concentration, screw rotation speed and temperature, are shown in Tables 2 and 3, respectively.

The encapsulation rate of OEO polymers is on average for all studied conditions equal to 67.4 %, while the corresponding rate of the polymers with flavomix is higher by 3.5 % and equal to 70.9 %. The difference between the EEs of the two antimicrobial agents is probably due to their form. Specifically, flavomix was in solid powder form, while OEO was added to the extruder as a liquid extract. At high operating temperatures, small amounts of oil may evaporate and there may be losses. This is also confirmed by the fact that the polymers produced at lower temperatures and screw rotation speeds showed higher EE. In general, the best production conditions for both antimicrobials were at screw speed of 50 rpm and extrusion temperature of 180 °C. The abovementioned combination of conditions was also the most easily manageable without the occurrence of any interruption during the process.

Based on literature, natural polymer films and LDPE films containing antimicrobial agents from essential oils (Radfar et al., 2020) and seed extracts (Shojaee-Aliabadi et al., 2013) with the same or even lower encapsulation efficiency show notable antimicrobial activity. In fact, thanks to the addition of the agents, which consist of high percentages of phenolic compounds like OEO and flavomix that were used in our study, the films of Shojaee-Aliabadi (Shojaee-Aliabadi et al., 2013) and Radfar (Radfar et al., 2020) could inhibit the growth of Gram-negative *E. coli* and *S. aureus*, the two most common found microbes in medical waste (Saini et al., 2004; Sohrab Hossain et al., 2013).

Table 2. Encapsulation efficiencies (EE, %) of LDPE polymers with 10 and 20 % OEO produced at screw rotation speeds of 50 and 70 rpm and extrusion temperatures of 180 and 200 °C. Values not sharing the same superscript are significantly different ($p < 0.05$).

OEO concentration (%)	Screw Rotation speed (rpm)	Temperature (°C)	EE (%)
10	50	180	70 ^{a,b} ± 1.1
		200	63 ^{c,d} ± 0.5
	70	180	66 ^{c,e} ± 1.2
		200	60 ^d ± 1.0
20	50	180	75 ^f ± 0.9
		200	68 ^{b,e} ± 0.9
	70	180	73 ^{a,f} ± 1.2
		200	64 ^c ± 1.0

Table 3. Encapsulation efficiencies (EE, %) of LDPE polymers with 10 and 20 % flavomix produced at screw rotation speeds of 50 and 70 rpm and extrusion temperatures of 180 and 200 °C. Values not sharing the same superscript are significantly different ($p < 0.05$).

Flavomix concentration (%)	Screw Rotation speed (rpm)	Temperature (°C)	EE (%)
10	50	180	73 ^{a,c} ± 0.9
		200	71 ^{a,b} ± 1.0
	70	180	69 ^b ± 1.1
		200	68 ^b ± 1.1
20	50	180	74 ^a ± 1.2
		200	73 ^{a,c} ± 0.6
	70	180	70 ^{c,b} ± 1.3
		200	69 ^b ± 1.1

The thermal transitions of the encapsulation complexes and of the plain LDPE polymer were evaluated through DSC in order to investigate whether the addition of OEO and flavomix affects the thermal behaviour of LDPE. Two heating cycles were carried out since the aim of the first one was the removal of the polymers thermal history. As calculated from the second heating cycle, the melting point (T_m) of LDPE, i.e. the temperature at which the solid is converted to an isotropic liquid, was 116.7 °C, while the melting points of the polymers with the encapsulated antimicrobials of OEO and flavomix are presented in Tables 4 and 5.

Table 4. Melting points (T_m , °C) of LDPE polymers with 10 and 20 % OEO produced at screw rotation speeds of 50 and 70 rpm and extrusion temperatures of 180 and 200 °C. Values not sharing the same superscript are significantly different ($p < 0.05$).

OEO concentration (%)	Screw Rotation speed (rpm)	Temperature (°C)	Melting point (T_m) (°C)
10	50	180	110.6 ^a ± 1.2
		200	110.3 ^a ± 0.8
	70	180	110.3 ^a ± 0.6
		200	109.9 ^a ± 0.4
20	50	180	109.4 ^a ± 1.1
		200	109.0 ^a ± 1.3
	70	180	109.2 ^a ± 1.1
		200	109.2 ^a ± 0.9

Table 5. Melting points (T_m , °C) of LDPE polymers with 10 and 20 % flavomix produced at screw rotation speeds of 50 and 70 rpm and extrusion temperatures of 180 and 200 °C. Values not sharing the same superscript are significantly different ($p < 0.05$).

Flavomix concentration (%)	Screw Rotation speed (rpm)	Temperature (°C)	Melting point (T_m) (°C)
10	50	180	116.8 ^a ± 0.9
		200	116.7 ^a ± 0.8
	70	180	116.1 ^a ± 1.1
		200	116.6 ^a ± 1.2
20	50	180	116.2 ^a ± 1.2
		200	116.7 ^a ± 1.0
	70	180	116.2 ^a ± 1.1
		200	116.1 ^a ± 0.7

The melting point of the polymer after the encapsulation of OEO was decreased by 7.0 °C, while the T_m of flavomix polymer was decreased slightly by 0.6 °C. The reduced T_m values after the addition of OEO are a consequence of polymer and essential oil interactions. The operating parameters do not affect the thermal behaviour since the differences among T_m of the polymers produced when employing different operating conditions is in the range of the standard deviations. In addition, the two different concentrations of the antimicrobials (10 % and 20 %) decreased to a negligible degree T_m values (1.1 °C for OEO polymers and 0.3

°C for flavomix polymers) as T_m is a characteristic property of the polymer and polymer content did not alter. This was also observed in our previous study of glass transition temperature (T_g) of PLA with encapsulated rosemary essential oil (Stramarkou et al., 2020).

Finally, the shelf life of 20 % OEO and 20 % flavomix polymers (produced at screw speed of 50 rpm and extrusion temperature of 180 °C) was determined by storing them under controlled conditions of temperature-radiation (20 °C-darkness and 45 °C-light) and relative humidity (RH 35, 70 and 95 %) for 60 days and calculating EE over time at regular intervals (2, 5, 10, 20, 30 and 60 days). The results of OEO and flavomix polymers are demonstrated in Figure 1a, 1b and 1c, 1d, respectively.

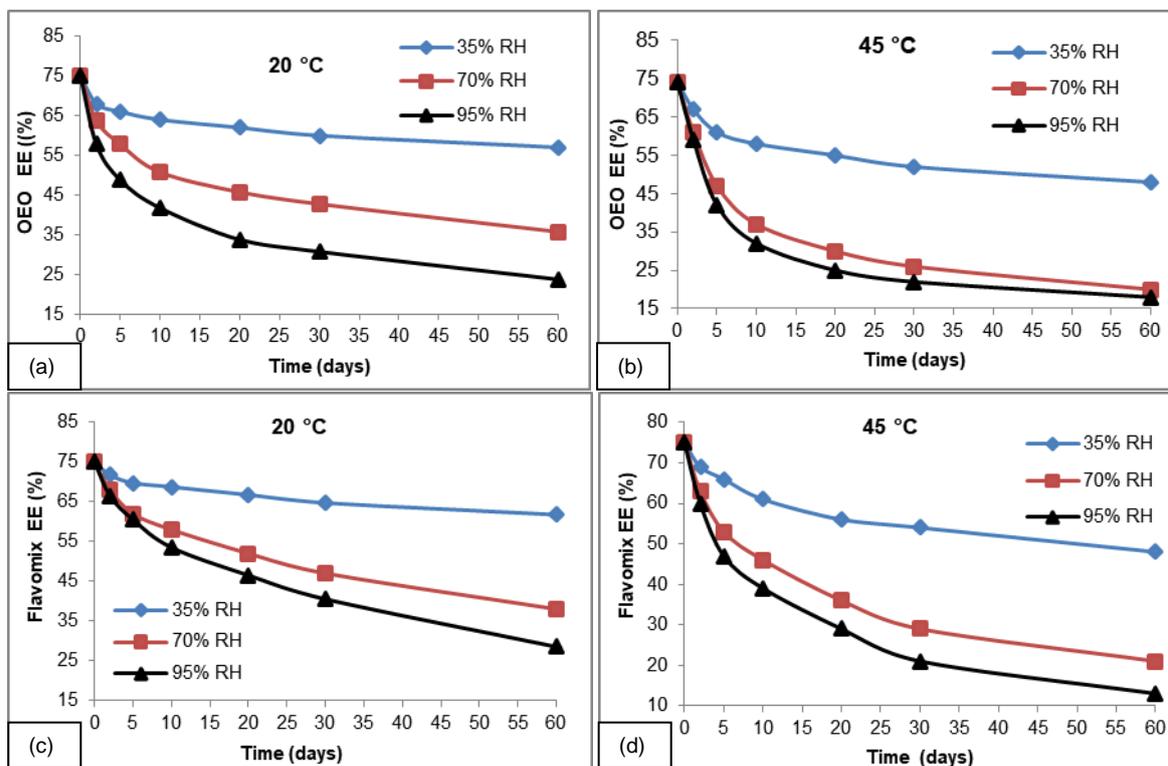


Figure 1. Encapsulation efficiency (EE, %) of (a) LDPE polymers with OEO stored at 20 °C and darkness, (b) LDPE polymers with OEO stored at 45 °C and light, (c) LDPE polymers with flavomix stored at 20 °C and darkness, (d) LDPE polymers with flavomix stored at 45 °C and light, during 2, 5, 10, 20, 30 and 60 days.

The rise of relative humidity and temperature-radiation in the storage environment, leads to a decrease in EE of both antimicrobials from the polymeric matrix. Temperature and light play a very important role in the release of the antimicrobials, with polymers at 45 °C having an average EE value of 28.0 % on the 60th day, whereas the respective value at 20 °C is 40.8 % Humidity during storage is also a crucial factor and this is evident at 45 °C from the beginning of storage. In the case of OEO polymer, the average differences between 35 % and 70 % and 35 % and 98 % RH are 20 % and 23.8 %, respectively. Comparing the behaviour of the two antimicrobial agents, OEO polymer demonstrates higher and faster release rate in the first days of storage, especially at high humidities and temperature, while in the following days, the rate is decreased reaching almost a steady state. This may be attributed to the liquid form of OEO, which in the first days is greatly affected by high temperatures and humidity and is released in large quantities. On the contrary, in the case of flavomix polymer, the release is continuous during the 60 examined days.

4. Conclusions

In conclusion, LDPE films with encapsulated antimicrobial substances from OEO and flavomix through extrusion under various conditions were developed. The ideal production conditions for both antimicrobials were at addition of 20 % antimicrobial agent, screw speed of 50 rpm and extrusion temperature of 180 °C. The studied storage conditions heavily affected the release rate of the antimicrobials agents from the polymeric matrix during time, with extreme RH and temperature achieving their faster release. Finally, the produced films

have the potential to be applied in medical waste and the gradual release of the antimicrobial agents will ensure the reduction of contained microbes.

Acknowledgments

The work was co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH– CREATE – INNOVATE (project code T2EDK-04762).

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