POLYMERIC MICROCAPSULES FOR COSMETIC APPLICATIONS, BASED ON LEMON ESSENTIAL OIL (*CITRUS LIMON*)

Ciprian CHELARU[1], Mădălina IGNAT[2], Mădălina ALBU[3], Aurelia MEGHEA[4]

Microencapsulation is one of the latest technologies used for imparting an array of unique characteristics to a textile. The paper presents a new method for the synthesis of microcapsules based on natural polymers and lemon essential oil (μcLO). The new synthesized microcapsules were characterized by optical microscopy, gas chromatography, FT-IR (ATR) spectroscopy, DLS, SEM and their antimicrobial activity was tested against E. coli, after dispersing it on textile samples. They proved significant bactericidal effects against microorganisms such as E. coli.

Keywords: microcapsules, lemon essential oil, chitosan, gelatin, *E. coli*

1. Introduction

In the last years, encapsulation of cosmetic and personal care products ingredients has become very popular, attractive and the associated production processes technologically feasible. This is a consequence of the added value created by the generated products, but also since compounds' functionality can be effectively preserved. As a result, cosmetic technology is growing constantly in terms of raw materials, excipients and formulations of active agents. The use of oils in the perfumery, cosmetics [1], and agriculture or food industries is quite common due to their aromatic properties. In addition, some essential oils have biological activities that can be used in the preparation of pharmaceutical products and functional foods. Properties of essential oils can change depending on their origin and composition. Some oils have medicinal properties such as antioxidant activity, acting in fighting free radicals, anti-inflammatory and antimicrobial activities.

Microencapsulation provides an important tool for cosmetic and/or pharmaceutical industry, enabling protection and controlled release of several

[1] PhD student, Department of Inorganic Chemistry, Physical Chemistry and Electrochemistry, University POLITEHNICA of Bucharest, Romania, e-mail:cchelaru_cciprian@yahoo.com
[2] PhD eng, INCDTP-Leather and Footwear Research Institute Division, Bucharest, Romania
[3] PhD eng, INCDTP-Leather and Footwear Research Institute Division, Bucharest, Romania
[4] Prof., Department of Inorganic Chemistry, Physical Chemistry and Electrochemistry,University POLITEHNICA of Bucharest, Romania

active agents. The encapsulation of essential oils in core–shell or matrix particles has been investigated for various reasons, e.g., protection against oxidative decomposition and evaporation, odor masking or merely to act as support to ensure controlled release [2]. A large number of microencapsulation methods have been developed in order to be adapted to different types of active agents and shell materials, generating particles with a variable range of sizes, shell thicknesses and permeability, providing a tool to modulate the release rate of the active principle.

Plant essential oils have been used for a variety of purposes [3], such as perfumery, preservation of stored food crops [4]. Lemon essential oil (Citrus Limon) [5] is used around the world in a wide variety of applications such as pharmaceuticals, alternative medicine, natural therapies and food preservation. It has anti-catarrhal, blood circulation, capillary protector, antihypertensive, diuretic, and anti-bacterial and antifungal properties.

2. Experimental

Stability measurements were performed on Malvern Nanosizer ZS equipment. Microscopic images were recorded using an optical microscope, Leica CME. The morphology of microcapsules on textile samples was evidenced by SEM measurements, with Quanta FEG 650 equipment. Lemon essential oil was characterized by FT-IR spectroscopy (4200 JASCO with SPECAC ATR) and GC-MS gas chromatograph coupled with mass spectrometry (DSQ II - Focus GC - ThermoScientific). The antimicrobial activity of lemon essential oil was conducted against *E. coli*, after microcapsules dispersal on textile samples.

The investigations were carried out using the following materials: bovine gelatin processed in INCDTP-ICPI Division; chitosan processed in INCDTP-ICPI Division, lemon essential oil (*Citrus Limon*), homemade; acetic acid, Merck; sodium sulphate, Sigma–Aldrich; sodium hydroxide, Sigma–Aldrich; glutaraldehyde, Fluka. All chemicals were of the highest purity available and were used as received, without further purification or distillation.

3. Results and discussion

3.1 Microcapsules synthesis

Polymeric microcapsules synthesis involves an oil-in-water emulsion, followed by a coacervation [5] (precipitation) process. The detailed scheme for the synthesis of microcapsules based on natural polymers and lemon essential oil [6], (μcLO) is presented in Fig. 1.

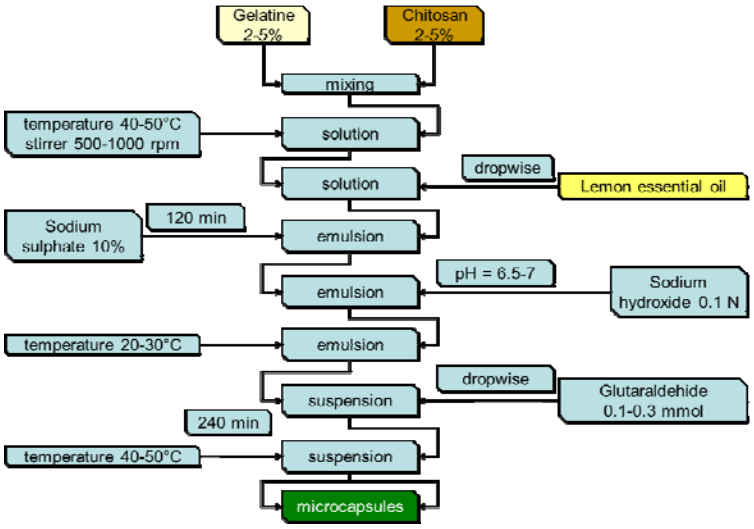


Fig. 1. Schematic preparation of microcapsules based on natural polymers and lemon essential oil (μcLO)

The solution containing microcapsules based on natural polymers and lemon essential oil [7] (μcLO) is presented in Fig. 2.

Fig. 2. Obtained microcapsules based on natural polymers and lemon essential oil (μcLO) suspension

The new obtained microcapsules were analyzed using optical microscopy and the microscopic images are presented in Fig. 3.

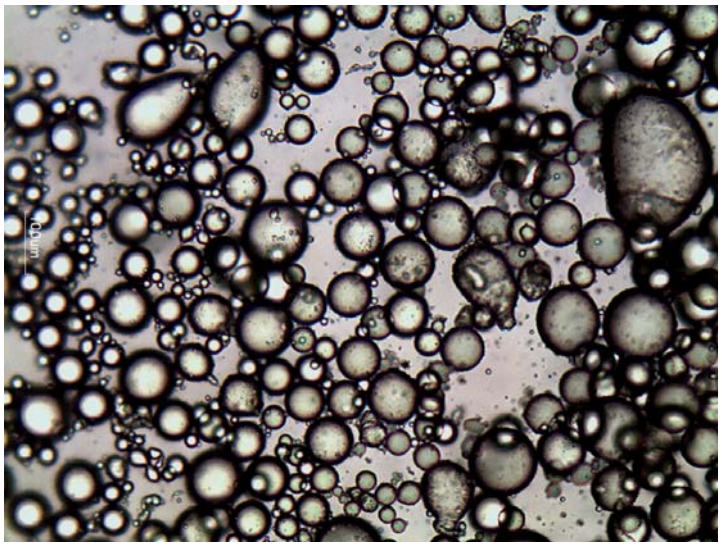
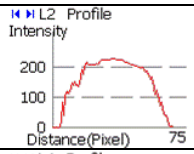
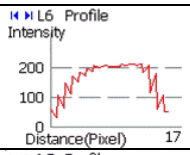
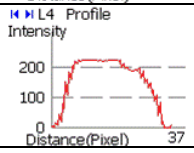
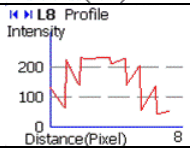


Fig. 3. Optical microscopy images for µcLO (x 100)

Microcapsules morphology profile is displayed in Fig. 8. Confirmation of spheroidal shape of microcapsules is provided by the individual graph profile of microcapsules. Profile graphs, along whit the specific dimension, are presented in the Table 1.

Table 1

Microcapsules shape graphs

No.	Profile	Size (µm)	No.	Profile	Size (µm)
1		91.13	3		20.26
2		40.02	4		8.67

3.2 Microcapsules components characterization

Due to the fact that some microcapsules components were homemade, they were characterized before being used for the synthesis.

Lemon essential oil (Citrus Limon) was obtained by steam distillation of lemons peel and kept at refrigerator for storage.

Lemon essential oil (*Citrus Limon*) [7] was characterized by GC-MS (Fig. 4) and FT-IR (Fig. 5)

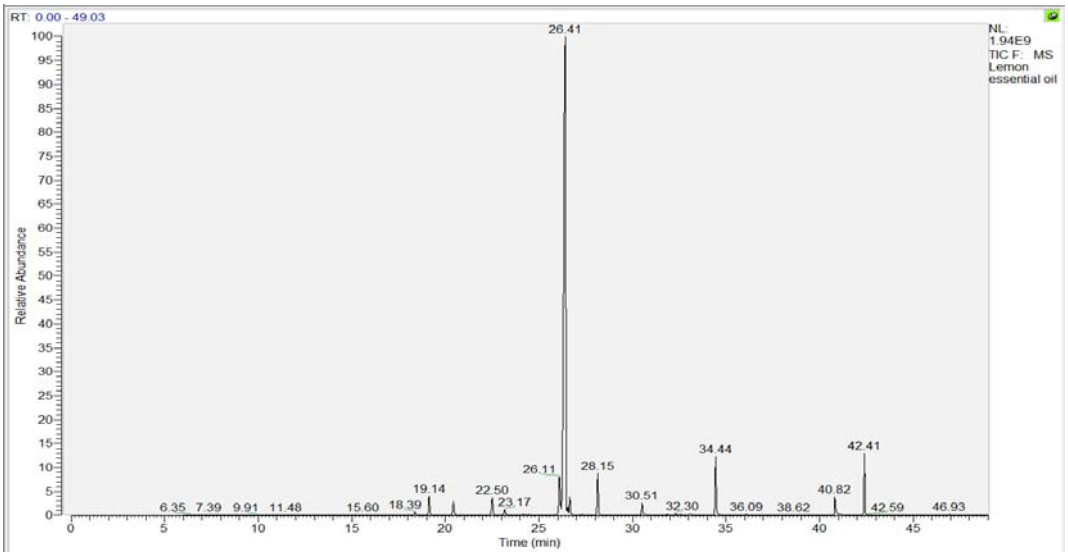


Fig. 4. Lemon essential oil (*Citrus Limon*) chromatogram

Table 2

Chemical composition of Lemon essential oil

No.	Retention time (min)	Compound	Area (%)	No.	Retention time (min)	Compound	Area (%)
1	18.39	Tricylene	0.29	8	26.66	1.8 Cineole	1.37
2	19.14	α-Pinene	1.76	9	28.15	γ-terpinene	3.32
3	20.44	Camphene	1.32	10	30.51	Linalol L	1.02
4	22.50	2-β-Pinene	1.68	11	34.44	4-Terpineol	4.30
5	23.17	β-pinene	0.52	12	40.82	Geranyl acetate	1.53
6	26.11	Cymol	3.94	13	42.41	Caryophyllene	4.03
7	26.41	dl-Limonene	74.92				

As the essential lemon oil (*Citrus Limon*) is a mixture of compounds, GC-MS technique [8] was used to determine the components, which are shown in Table 1. It can be seen that the major component is dl-Limonene - 74.92%.

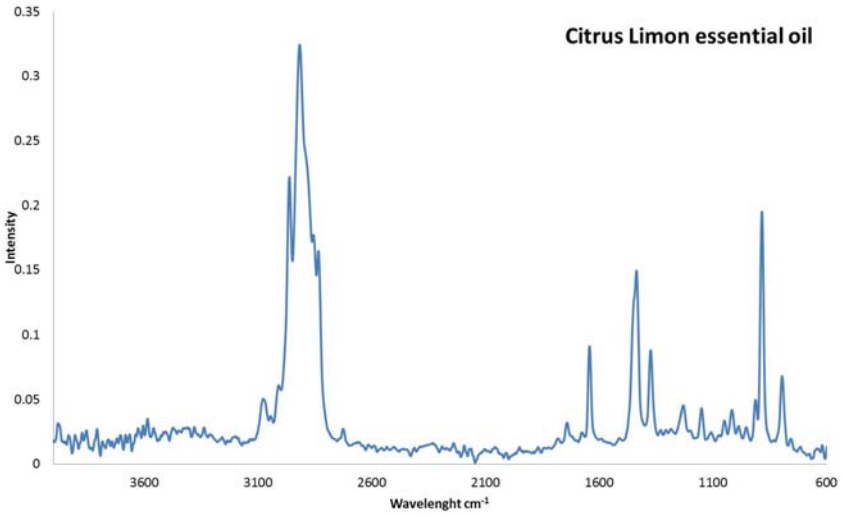


Fig. 5. - Lemon essential oil (*Citrus Limon*) FT-IR (ATR) spectra

The FT-IR analysis was used to assess the functional groups present in the lemon essential oil (*Citrus Limon*).

In Fig. 5 is presented the infrared spectrum of lemon essential oil (*Citrus Limon)* with the following assessment of the main vibration bands, displayed in the Table 3:

Table 3

Main vibration bands in the infrared spectrum of *Citrus Limon*

No.	Wave numbers	Characteristic group	
1	3076 cm^{-1}	=C-O-C	strong symmetric stretching band
2	2856 and 2917cm^{-1}	C-H	symmetric and asymmetric stretching band
3	2727 cm^{-1}	H-C=O	absorption band specific to aldehydes
4	1742 cm^{-1}	C=O	stretching absorption band specific to aldehydes
5	1375 cm^{-1}	CH_3C-H	bending bands present in alkanes and alkyls
6	1231 cm^{-1}	=C-O-C	asymmetric stretching bands present in ethers
7	1151 cm^{-1}	C-O	stretching bands of groups present in alcohols
8	1018 cm^{-1}	=C-O-C	stretching band of ethers groups

The image recorded for bovine gelatin obtained from leather is presented in Fig. 6.

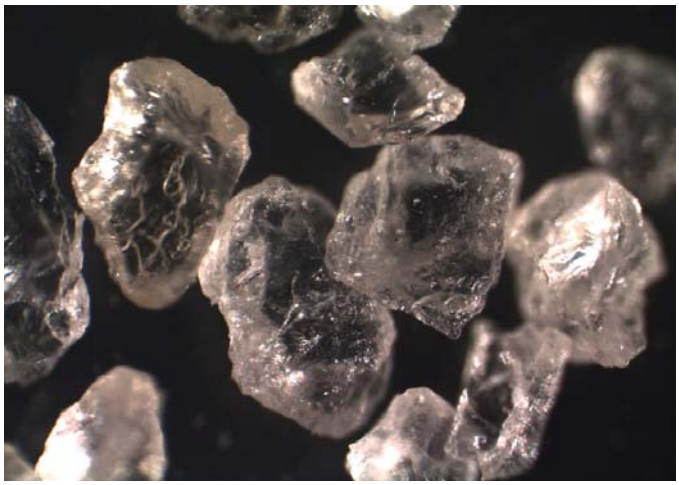


Fig. 6. Optical microscopy of bovine gelatin (x40)

Microscopic images showed, in case of bovine gelatin, white, translucid grains with irregular shapes.

Chitosan [9] was obtained from shellfish and characterized by FT-IR and SEM.

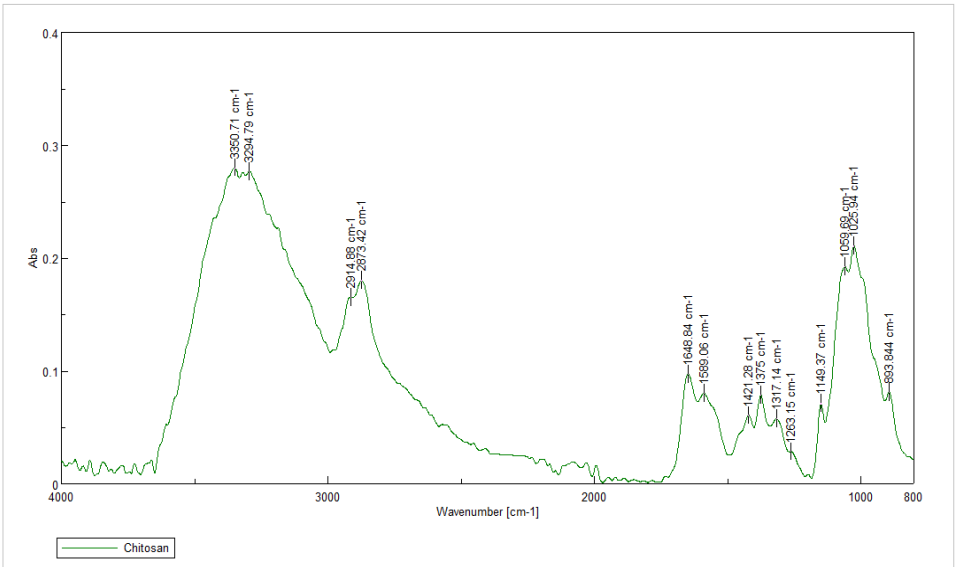


Fig. 7. Chitosan FT-IR (ATR) spectrum

The FT-IR analysis was used to assess the functional groups present in the chitosan. Fig. 7 presents the infrared spectrum of chitosan.

N-H and O-H stretching, as well as the intramolecular hydrogen bonds, display a strong band in the region 3294–3350 cm^{-1}.

C-H symmetric and asymmetric stretching absorption bands are present at 2914 cm^{-1} and 2873 cm^{-1}.

Xylan, glucans and carrageenans are polysaccharides which also have these bands a location that are characteristics for polysaccharides and are found in their spectra.

The presence of bands at 1648 cm^{-1} (C=O stretching of amide I) and 1317 cm^{-1} (C-N stretching of amide III) indicate the presence of residual *N*-acetyl groups.

At 1553 cm^{-1} a location that corresponds to N-H bending of amide II, is visible a very small band. This is the third band characteristic of typical N-acetyl groups.

A band at 1589 cm^{-1} corresponds to the N-H bending of the primary amine.

The CH_2 bending and CH_3 symmetrical deformations were confirmed by the presence of bands at around 1421 cm^{-1} and 1375 cm^{-1}, respectively.

C-O-C bridge is present in the FT-IR (ATR) spectra with band at 1149 cm^{-1}, that can be attributed to asymmetric stretching.

C-O stretching displays bands at 1059 and 1025 cm^{-1}.

Since the chitosan used in this study is of animal origin, there is always the possibility of contamination by glycosaminoglycans (GAGs), which are another type of polysaccharides found in these organisms.

GAGs are sulfated, and the presence of sulfate groups covalently bonded to the polysaccharide may be confirmed in the infrared spectra by the presence of very strong bands in the region around 1260 cm^{-1} – 1270 cm^{-1}.

In the spectrum obtained from chitosan (Fig. 7), the signal at 1263 cm^{-1} is very small and, therefore, does not correspond to sulfate groups, thus ruling out contamination of chitosan by GAGs.

This signal at 1263 cm^{-1} was assigned to the bending vibrations of hydroxyls present in chitosan. The signal at 893 cm^{-1} corresponds to the CH bending out of the plane of the ring of monosaccharide.

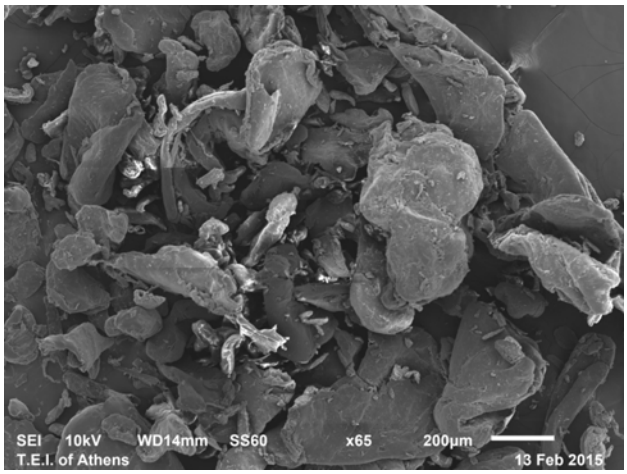


Fig. 8. SEM image for chitosan powder (x65)

3.3 Stability studies

In order to determine the stability of the microcapsules, Zeta Potential measurements were made and also microscopic images were recorded.

Zeta Potential was used as a stability indicator for microcapsules existing in the dispersed system. If all species present in suspension have high, either negative or positive Zeta potential values (outside the range -30 ÷ +30 mV), they tend to repel each other and to avoid aggregation. Therefore, Zeta potential values presented in Tabel 4 suggest that this dispersion has a high stability, but tends to decrease over time.

Table 4

Zeta potential values for water dispersed microcapsules

Sample	Time	Zeta Potential, mV
(µcLO)	Initial	-35,7
	1 day	-30,2
	3 days	-27,4
	5 days	-24,1
	7 days	-18,2

Microscopic images were recorded for the same sample, kept at room temperature (Fig. 9).

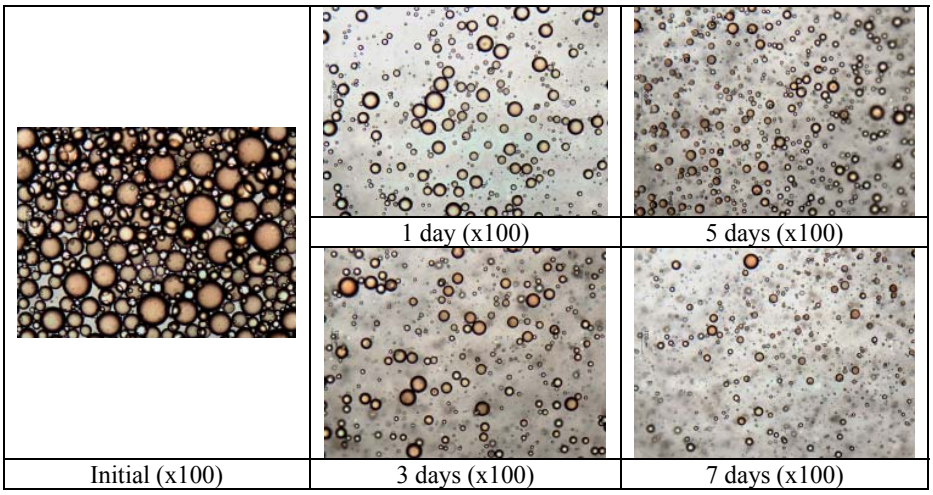


Fig. 9 Microscopic images for microcapsules dispersion over time

3.4 Antimicrobial activity of µcLO

The antimicrobial activity [10] of microcapsules based on natural polymers and citrus lemon essential oil (µcLO) after being spread on textiles, was investigated against *Escherichia coli* using fingerprinting method [11]. The test was performed in sterile Petri dishes (100 mm diameter) containing an appropriate solid sterile media (*MacConkey Broth)* which is used for the selective enrichment of *E. coli.*

The control of microbial contamination seeks to determine the total number of aerobic microorganisms or the absence of pathogen/pathogenic condition by using fingerprinting method for determining the total number of aerobic germs (NTGA) and the total number of fungi and yeasts (NTFL).

Results are presented below, as compared with the control sample (C).

Table 5

Antimicrobial activity of µcLO treated samples

Sample	Total number of aerobic germs (UFC/cm^2)	Total number of fungi and yeasts (UFC/cm^2)
C	1 UFC/ cm^2	Absent
Treated with µcLO	1 UFC/ cm^2	Absent

Experimental results for antimicrobial activity [12] of µcLO treated samples are presented in Fig. 10.

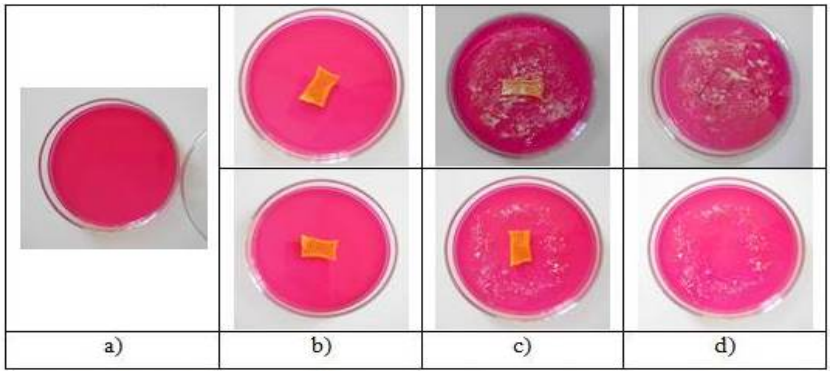


Fig. 10. Photographic images for: a) *MacConkey Broth* medium; b) *MacConkey Broth* medium with control sample (up) and treated sample (down)-initial; c) after 5 days; d) *MacConkey Broth* medium after samples removal

From Fig. 10 it can be observed that after 5 days, in case of control sample, the whole medium is contaminated by *E. coli*, while for the treated sample appeared an inhibition area.

4. Conclusions

Emulsification followed by coacervation showed to be a useful technique for synthesizing microcapsules based on natural polymers and lemon essential oil (µcLO). The microcapsules obtained present a good stability over time, confirmed by microscopic and DLS analysis. The microscopic investigation showed that most of the microcapsules are spherical, with size ranging from 40 to 100 µm.

The antimicrobial activity study demonstrates that lemon essential oil presents significant bactericidal effects against microorganisms such as *E. coli.*

Aknowledgement

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