Preparation of Thyme Oil Loaded Microcapsules for Textile Applications

Senem Karagönlü, Güldemet Başal , Figen Özyıldız, Ataç Uzel

***Abstract*—The aim of this work is to develop functional textile materials with antimicrobial properties. For this purpose thyme oil *(Thymus vulgaris L.)* was encapsulated by complex coacervation method using gelatin (GE) and gum arabic (GA) as wall materials. No crosslinking agent were used during the encapsulation process in order to eliminate their toxic effect. These capsules applied to a nonwoven fabric. The effect of various processing parameters, including the amount of oil and concentration of wall material on the encapsulation yield, particle size distribution and capsule loading was investigated and antimicrobial activities of microcapsules and fabrics were determined. Microencapsulation yield increased as the amount of oil increased, but shapes of capsules became irregular. When amount of wall material in solution increased, formation of capsules diminished. Antimicrobial activity test revealed that both microcapsules and fabrics with different concentrations of microcapsules showed antimicrobial activity against *E. coli, S. aureus* ve *C. albicans* microorganisms.**

***Index Terms*— antimicrobial activity, complex coacervation microencapsulation, thyme oil.**

I. INTRODUCTION

In recent years, textile industry has spent great effort to add new functionality to their product due to consumers high hygiene and comfort property expectations from regular textiles. Especially, antimicrobial textiles became very popular. They are recommended for the healthcare, work/uniforms, sports apparel and military. Antimicrobial textiles are produced by applying antimicrobial agents onto the surface of fiber, yarn, or finished textile, or incorporating them into the fiber. Several commercial antimicrobial fibers or finishes are currently available in the market, but they are mostly synthetic base and are not environmentally friendly. As consumers are becoming more conscious of using safer and healthier products, natural plant products with antimicrobial activity are gaining more attention. Essential oils from the roots, stems or barks, leaves, flowers, and fruits/seeds of plants can all have phytochemical components with antimicrobial property [1-6]. Thyme oil from thyme (Thymus vulgaris L.) plants is one of such essential oils. It is known for its antiseptic, antispasmodic, antitussive antimicrobial, antifungal, antioxidative, and antiviral properties and have been used in traditional medicine for the treatment of several diseases [7,8]. Essential oils can be used directly in the crude form and applied onto fabric, but for enhanced durability and controlled release of active agents that prolong the functionality of antimicrobial textiles microencapsulation technology is a better choice [9].

Microencapsulation can be described as a method to package micronized materials in the form of capsules which have size range from 1 µm to 1 mm [10]. In other words, microencapsulation is a technique to surround liquid droplets, solids or gases with a continuous film or a polymer material on a very small scale [10,11]. Microencapsulated materials are used in agriculture [12], pharmaceuticals [13], foods [14], cosmetics and fragrances [15], a variety of textile applications [16, 17, 18], papers, paints, coating and adhesives, printing applications and many other industries [19].

Microcapsules may be produced using several microencapsulation techniques for different purposes such as protection, controlled release, and compatibility of the core materials [10,11,19]. Coacervation which involves the phase separation of one or many hydrocolloids is one of the widely used techniques[19,20, 21]. This method can be divided into two types named simple and complex coacervation depending on number of colloids [22]. Complex coacervation, the oldest and most commonly used microencapsulation method, was firstly used at carbonless copying paper by Green at al [23]. The technique is based on interaction of two oppositely charged polymers (i.e. protein and polysaccharide) in an aqueous solution. Although interaction of two oppositely charged polymers is spontaneous, coacervation only occurs under specific conditions. As a result of the interaction, two different phase, polymer-rich phase and aqueous phase, are formed. While polymer-rich phase is known as coacervate, aqueous phase is known as equilibrium phase. Complex coacervation process is composed of 5 steps: dissolution of polymer, emulsion, coacervation, hardening and rinsing/filtering/drying [24, 25].

Especially, coacervation of gelatin and gum arabic has been widely used to encapsulate both solids and liquids because both compounds are non-toxic, natural, and biodegradable. [26, 27]. Mostly type A gelatin (with an isoelectric point of

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approximately pH 7-9) is positively charged at approximately pH 4.0. Gum arabic is negatively charged at this pH value and form emulsion with gelatin [26]. At the system, a cross-linking agent such as formaldehyde or glutaraldehyde can be used to harden wall of microcapsules. Gelatin/gum arabic based microcapsules have been studied by several authors. Kong et al [28] microencapsulated dodecanol (C12OH) using gelatin and gum arabic as wall material. They prepared capsules with different concentrations of gelatin and gum arabic using formaldehyde and glutaraldehyde as cross-linking agent. They studied the effect of the type of cross-linking agent on cross-linking efficiency and the encapsulation of C12OH. They found out that formaldehyde caused to lower crosslinking efficiency and higher C12OH encapsulation compared to glutaraldehyde.. Huang et al [26] encapsulated shikonin in gelatin/gum arabic complex coacervation system for pharmaceutical dosage form for various drugs. They used glycerol as crosslinking agent and studied the effects of various parameters such as the concentration of surfactant, concentration of gelatin and pH level on the particle size distribution. They found out that the optimum concentration for surfactant/oil ratio was 1/10 and gelatin/oil ratio was 1/5 at the pH levels of approximately 4-6 for the coacervation process. They also observed that microcapsules prepared by glycerol were no different from those of formaldehyde. Liu et al [29] encapsulated flaxseed oil within gelatin/gum arabic capsules via complex coacervation. They studied the effects of homogenization rates and total biopolymer concentrations on efficiency and particle size. They found that size of the microcapsules and amount of non-encapsulated oil increased as the total biopolymer concentration increased. The optimum conditions were 1:1 core-to-wall ratio, total biopolymer concentration of 2% (w/v) and homogenization rate of 9,000 rpm. Jouzel et al [30] encapsulated cod liver oil using gelatin/gum arabic. They tried to optimized the encapsulation process to obtain capsules with a size under 100 micron in diameter. Dong et al [31] used gelatin/gum arabic complex coacervation system to encapsulate peppermint oil. They investigated the effects of various processing parameters, including the core/wall ratio, wall material concentration, pH value and stirring speed on the morphology, particle size distribution, yield and loading. They obtained that the optimum wall material concentration was 1% and the core/wall ratio was 2:1. Also, ideal preparation conditions were pH 3.7 and 400 rpm of stirring speed. Other than these studies, several core materials including camphor oil [32], violet, lemon and peach perfumes [33], orange oil [20], paraffin [34], N- benzylmorpholine [35], indomethacin [36], limonene and menthol powder [37], capsaicin [38], flavor compounds [39] were encapsulated using gelatin/gum arabic as wall material via complex coacervation for several aims.

The main aim of this work was to develop antimicrobial textile materials for medical applications such as bandages or wound dressings. For this purpose thyme oil loaded microcapsules were produced by complex coacervation method. During the microcapsulation process the effect of oil amount and concentration of wall material on microencapsulation yield, particle size distribution and capsule loading was investigated. Antimicrobial activity of resulting microcapsules and the fabric treated with these microcapsules were determined..

II. MATERIALS AND METHODS

A. Materials

Gelatin (from porcine skin, type A) and gum arabic (from acacia tree) were purchased from Sigma-Aldrich. Thyme (*Thymus vulgaris L.*) oil was obtained from Mecitefendi Trade Limited Company. Tween® 20 (viscous liquid, $C_{58}H_{114}O_{26}$), sodium hydroxide (NaOH), hydrochloric acid (37%, HCl) and 2-propanol were purchased from Sigma-Aldrich.

B. Preparation of Microcapsules

Microcapsules were prepared by complex coacervation method according to a modified method published by Jouzel et al [17]. First, solutions of gelatin and gum arabic at three different concentrations were prepared by dispersing 4, 10, or 16 g polymer into 200 ml of deionized water at room temperature (~ 22 °C) for 30 min. Subsequently, temperature of aqueous solution was raised to 50 °C to dissolve polymer and pH was adjusted to 7.0 with 3 M NaOH. Solution was stirred at 50 °C for 15 min.

During the preparation of microcapsules, gelatin solution (2,5,or 8 % w/v) was taken to a three- neck flask. 10, 20 or 30 ml of thyme oil was dispersed in gelatin solution at 50 °C. The mixture was stirred by a mechanic stirrer at 400 rpm at 50 °C for 30 min to form stable oil in water emulsion. Both gum arabic and gelatin are surface active microcapsules were formed in the absence of surfactants. Gum arabic solution (2, 5, or 8 %, w/v) was added to emulsion drop by drop. Emulsion was stirred at 400 rpm for 15 min. Then, pH of emulsion was adjusted to 4.0 by adding 1 M HCl solution in order to stabilize polymers. At this pH value, gelatin was positively charged while gum arabic was negatively charged. In order to allow coacervation, emulsion was stirred at 400 rpm for 90 min. At the end of the coacervation, heater was switched off and 600 ml of cold deionized water was added. After temperature of coacervate had been lowered to room temperature, system was cooled to 5-10 °C with ice bath. Consequently, gelatin and gum arabic covered the solidified oil droplets and formed microcapsules. The system was stirred at 400 rpm for 2 h. After 2 h of hardening, wet microcapsules were taken to the refrigerator overnight. Finally, microcapsules were collected, rinsed with 2-propanol, filtered and dried at room temperature. Chemical cross-linking agents

such as formaldehyde or glutaraldehyde was not used due to their toxic effect. A flow chart of the microencapsulation process by complex coacervation is given in Figure 1.

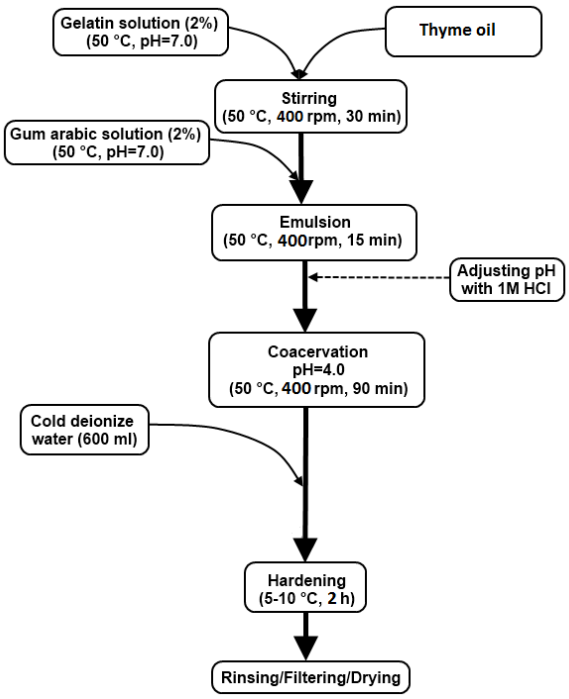

Figure 1. Flow chart of the microencapsulation process by complex coacervation

In the present study, a replicated 3^2 full factorial design was employed to determine the effect of amount of oil and concentration of wall material on microencapsulation yield, particle size distribution and capsule loading. The levels of independent parameters are given in Table 1. As a result, 9 experiments with a replication were run randomly (Table 2).

Table 1. Process parameters and levels used in the experimental design.

Parameter	Lower Value	Medium Value	High Value
Amount of oil (ml)	10	20	30
Wall material concentration (%)	0.2	0.5	0.8

Table 2. Experimental design

	Amount of oil (ml)	Wall material concentration(%)
1	10	2
2	20	2
3	30	2
4	10	5
5	20	5
6	30	5
7	10	8
8	20	8
9	30	8

C. Morphology and Particle Size of Microcapsules

The structure, shape and formation of microcapsules was investigated by optical microscope (Bresser® LCD Micro) connected with a camera with 10x magnification. To measure size of the microcapsules, images of moist microcapsules obtained by optical microscope were used. Sizes of the microcapsules were measured using Image J software as pixels and transformed to microns using a scale. 50 measurements were taken for each treatment and mean particle size was obtained from values of diameter of 50 microcapsules. Particle size distribution was analyzed by SPSS 18.0 statistical analysis software.

D. Determination of Microencapsulation Yield

Microencapsulation yield of thyme oil was determined by following equation:

$MY(\%) = (W_1 / W_2) * 100$

MY: microencapsulation yield

W_1: weight of the microcapsules obtained

W_2: initial weight of the materials used (combined weight of core and wall materials)

E. Determination of Oil Loading

Oil loading was determined by High-performance liquid chromatography (HPLC) using an Agilent 1100 system. Working condition for HPLC is given in Table 3. During analysis Zeković et al.'s [40] work on thyme (*Thymus vulgaris L.*) oil extract was used as a reference. Oil loading in microcapsules were determined by the amount of carvacrol, one of the phenolic compounds in thyme oil. The standard solution of carvacrol was prepared at a concentration of 10 mg/mL, injected in to the HPLC system and chromatographed at a UV wavelength of 276 nm. The thyme oil used in experiments was mixed with 2 proponal and this solution was diluted to get a calibration standard solutions of 0.5, 1.0, 2.5, 3.5, 5.0 mg/mL, in mobile phase. Each of these calibration standard solutions was injected in to the HPLC system separately and chromatographed at a UV wavelength of 276 nm. The calibration curve for carvacrol was constructed by plotting the respective peak areas against the thyme oil concentrations.

To release the oil inside microcapsules by breaking capsule walls, 0.75 g microcapsules in 10 ml 2 proponal solution were centrifuged at 1000 rpm for 15 minutes. The resulting samples were filtered through 0.45 µ membrane filter and 20 µL of solution injected into HPLC system. The thyme oil content of the microcapsules was calculated using the calibration curve.

Table 3. Working conditions for HPLC

Column	C18 LiChrospher 100 analytical column
Column lenght	250 mm
Column diameter	4 mm
Mobile phase	acetonitrile-water (50:50; v/v)
Flow rate	0,8 ml/min
Temperature	30 ºC
Absorbance	276 nm
Sample volume	20 µl

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F. Statistical Analysis

The effect of amount of oil and concentration of wall material on microencapsulation yield, particle size distribution and capsule loading were statistically evaluated by applying one-way ANOVA at 0.05 level using a commercially available software package SPSS 18.0

G. Impregnation of microcapsules on textile substrate

Microcapsules were applied to a polyester nonwoven fabric (40 g/m2) using a laboratory scale

Foulard (Ataç FY350) at a cylinder pressure of 2 psi and speed of 2 m/min to get a pick up of 100% on weight of fabric. Four padding baths were prepared with different microcapsule concentrations: 10, 20, 30 ve 40 g /l .

After padding fabrics were air dried at room temperature. The padding bath did not contain any binder since the initial trials revealed that the curing at high temperatures damages the microcapsules. Besides the application area for the final product thought to be disposable items like surgical gowns, bandages and gauzes, which do not need laundering.

H. Antimicrobial activity assays

Antibiotic resistant test strains were used for all antimicrobial assays including *Escherichia coli* O157H7 (RSKK232), Methicillin resistant *Staphylococcus aureus* RSKK 95047and *Candida albicans* DSMZ 5817. Antimicrobial activity of the thyme oil was determined by means of the disc diffusion method described by the National Committee for Clinical Laboratory Standards (NCCLS 2003). All tests were performed in Mueller-Hinton agar (MHA, Oxoid) and Sabouraud Dextrose agar (SDA, Difco) for bacteria and *C. albicans* respectively. Ten micro liter of thyme oil in prepared in different concentrations of Dimethyl sulfoxide (DMSO) were injected into sterile discs of 6mm in diameter for disc diffusion assay (Murray et. al, 1995). Gentamycine (10µg) and Nystatin (100U) were used as positive reference standards to determine the sensitivity of the tested strains.

Agar well method (Bell and Grundy, 1968) was found convenient and used for assessing the antimicrobial activities of the microcapsules containing thyme oil since the granular nature of the microcapsules. 100 µl of suspension containing 10^6 cfu/ml bacteria and 10^5 cfu/ml yeast were spread onto sterile petri dishes containing MHA and SDA. 6 mm wells were excised aseptically and filled with 50 mg microcapsules containing thyme oil. Plates were kept at 4°C for 2 h and then incubated at 35°C for 24 h for bacteria and 48 h for yeast. The results were recorded by measuring the zones of growth inhibition surrounding the wells.

Antimicrobial assessments bioactive and control fabrics were carried out according to AATCC(American Association of Textile Chemists and Colorists)Test Method 100-2004.The nonwoven fabrics loaded with microcapsules containing thyme oil was used as bioactive fabrics and the untreated fabrics are used as controls. The bioactive fabrics impregnated with different concentrations of microcapsules ranging from 10 – 40 g/L and control fabrics with no additives were tested. All test materials were sterilized by using ethylene oxide before the assay by using Ethylene Oxide sterilization system (Axis). The contact time was set as 1 hour. A diluted microorganism suspension with about $2\text{-}4\times10^8$ colony forming units per milliliter (cfu/ml) concentration was used. 25µl microbial suspension was loaded onto the circular swatches of control and test fabrics that are 4.8 ± 0.1 cm in diameter in the presence of a nonionic wetting agent (Triton X-100) in a sterilized glass jar. After 1 hour contact time 0.02N sodium thiosulfate was added in excess to quench the biological growth. The mixture was then vortexed vigorously for 2 min. An aliquot of the solution was serially diluted, and dilutions were plated onto agar media plates. The same procedure was applied to nonwoven fabrics with no additives as a control. Viable microbial colonies on the agar plates were counted after incubation at 37°C for 24-48 h. The reduction rate in the number of microorganisms was calculated using the following formulae;

$$R(\%) = (B - A) / B \times 100,$$

Where R is the percent reduction rate, A the number of bacteria recovered from the inoculated fabrics over the desired period of contact, and B is the number of bacteria recovered from the inoculated fabrics membranes at zero contact time. Final reduction rate was reported as Log reduction. All antimicrobial assays were done in triplicates.

III. RESULTS AND DISCUSSION

A. Morphology of microcapsules

Structure, shape and wall/core construction of microcapsules were observed with optical microscope. Figure 1 shows the optical microscope image of microcapsules. The spherical shapes are oil droplets and the dark clear phases are the complex coacervates encapsulating the oil droplets. It is clear from the photographs microcapsules have spherical shape with only one and continuous core surrounded by a continuous shell and relatively uniform size distribution.

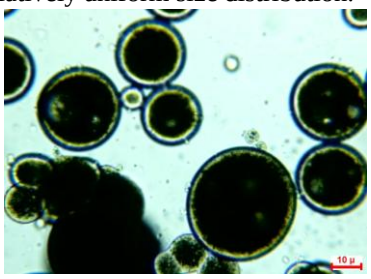

Figure 2. Optical microscope image of microcapsules (10 ml of oil, 2 % of wall concentration)

B. Effect of oil amount and wall material concentration on encapsulation yield, particle size and oil loading

Average encapsulation yield, particle size and oil loading values obtained from all experiments are displayed in Table 4. Wall material concentration of 8% did not result in any microcapsules. Similarly, no microcapsules were formed when oil amount was 10 ml and wall material concentration was 5%. In addition, 5 % wall material concentration and high amount of oil content at 2% wall material concentration caused severe agglomeration. As a result, particle size was measured only at 2% wall material concentration with 10 and

20 ml oil content.

Statically both the effect of oil amount and wall material concentration and their interaction on encapsulation yield were found to be significant. Encapsulation yield increased with increasing amount of oil. This might be attributed to inadequate amount of oil droplets in emulsion at low oil ratios resulting excess wall material which did not contribute to microencapsulation process. When wall material concentration increased from 2% to 5% microencapsulation efficiency increased but also microcapsules showed a tendency to agglomerate . It is likely that the viscosity of the dispersed phase increased due to the increase of the concentration of gelatin and acacia solutions and this increase made the coalescence of emulsified dispersed droplets easier and caused the microcapsules to conglutinate to large blocks. A further increase in wall material concentration resulted in no microcapsules.

Size of microcapsules was measured only when microcapsules were obtained at the wall material concentration of 2% and oil content of 10 and 20 ml. The limited data obtained from the experiments suggests that with the increase of oil amount, the mean diameter of microcapsules and their relative oil content increased and the particle size distribution became wider. These were consistent with the observation made by Yu et al. and Pakzad[42, 43] . The presence of more oil in the oil-water emulsion caused more and bigger oil droplets to be formed . As a result, total surface area which wall material surrounds decreased and oil/wall ratio of microcapsules increased.

Table 4 Average values of microencapsulation yield, particle size and oil loading for each experimental condition

Oil amountt (ml)	Wall concentration (%)	Microcapsulation Yield (%)	Microcapsule Size (µ)	Oil Loading (%)
10	2	74,72	25,37	36,38
20	2	75,93	37,60	56,27
30	2	82,74	Agglomeration	64,35
10	5	0	No microcapsule	No microcapsule
20	5	85,83	Agglomeration	43,89
30	5	88,49	Agglomeration	44,58
10	8	0	No microcapsule	No microcapsule
20	8	0	No microcapsule	No microcapsule
30	8	0	No microcapsule	No microcapsule

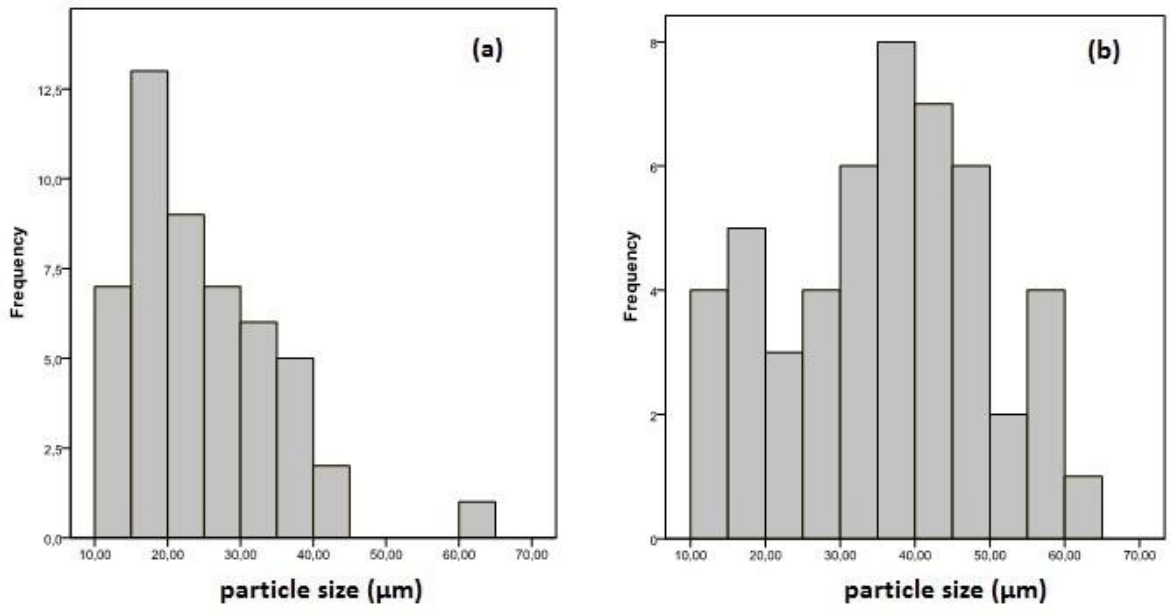

Figure 3. Particle size distributions of microcapsules prepared with a) 10 ml oil and b)20 ml oil at %2 wall material concentration

C. Antimicrobial activity

Antimicrobial activity of the thyme oil was determined by means of the disc diffusion method. Gentamycine (10µg) and Nystatin (100U) were used as positive reference standards to determine the sensitivity of the tested strains. Thyme oil was found active against all test microorganisms in all tested concentrations (Table 5). The strongest effect was seen for *C. Albicans* for all concentrations of DMSO. The inhibition zone didn't appeared in the discs without thyme oil. It is clear that thyme oil has a strong antimicrobial activity, capable of compete with standard antibiotics.

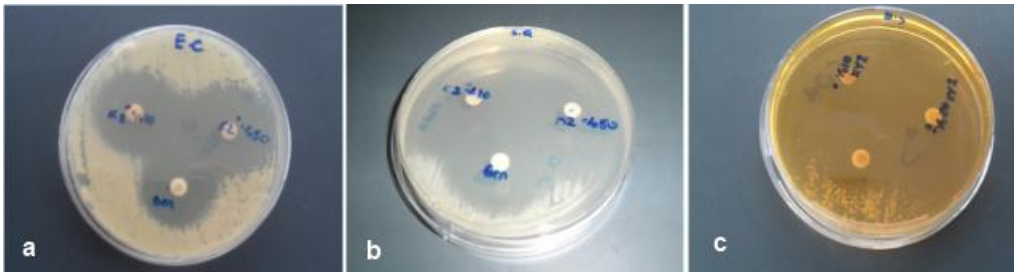

Figure 4 Growth inhibition zone of thyme oil a) *E. coli* b)*S. aureus* c) *C. Albicans*

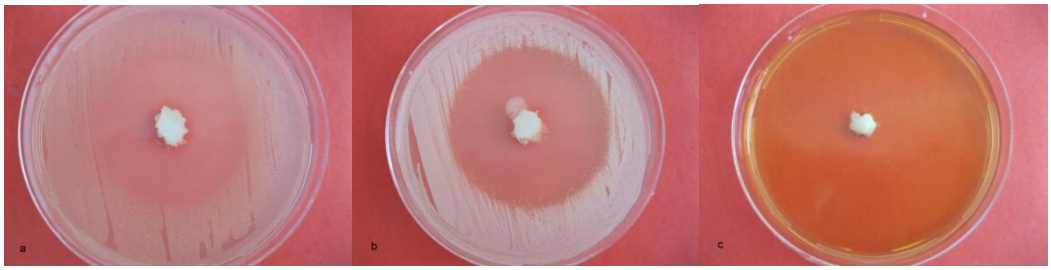

Figure 5 Growth inhibition zone of microcapsules with thyme oil a) *E. coli b)S. aureus* c) *C. Albicans*

Table 5. Antimicrobial activity of the *Thymus* sp. oil against test microorganisms

	Inhibition zone (mm)							
	***Thymus* sp. Oil**			**Antibiotics**		**DMSO**		
Microorganisms	**10% DMSO**	**25% DMSO**	**50% DMSO**	**Gentamycine (10µg/disc)**	**Nystatin (100U/ disc)**	**10% DMSO**	**25% DMSO**	**50% DMSO**
Escherichia coli O157H7(RSSK 232)	40	35	35	21	Nt	0	0	0
Methicillin resistant *Staphylococcus aureus* (RSKK047)	48	35	35	23	Nt	0	0	0
Candida albicans (DSMZ 5817)	60	75	71	Nt	18	0	0	0

*Nt; not tested.

Similarly, microcapsules containing thyme oil showed considerable antimicrobial activity against all test microorganisms (Table 6). These results were found compliant with disc diffusion results and are in close agreement with the results of the study conducted by Guarde et al. They showed that Thymol (T) and carvacrol (C) showed a significant AM activity against Escherichia coli O157:H7 and Staphylococcus aureus [41].

Antimicrobial activity of the microcapsule impregnated fabrics were evaluated using AATCC test method 100-2004 and bioactive fabrics were found effective against test microorganisms in all tested concentrations, as well. (Table 7). Results showed that even the lowest concentraion (10 g/l) provided sufficient level of antimicrobial activity to the fabric.

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Table 6. Antimicrobial activity of the thyme oil loaded microcapsules against test microorganisms.

Microorganisms	Inhibitionzone[a] (mm)		
	Microcapsules	Gentamycine (10µg/disc)	Nystatin (100U/disc)
Escherichia coli O157H7(RSSK 232)	43	20	Nt
Methicillin resistant*Staphylococcus aureus* (RSKK 95047)	53	23	Nt
Candida albicans (DSMZ 5817)	85	Nt	18

[a]. Inhibition zones are the mean values of three different experiments and contain the diameter of the wells (6 mm). Nt; not tested.

Table 7. Antimicrobial activity of the microcapsule impregnated fabrics against test microorganisms

Microorganisms	IMC (log_{10}cfu/ml)	FMM (log_{10}cfu/ml)						
		C	% R	MK1	MK2	MK3	MK4	% R
E. coli	6,96	6,95	3,22	2<	2<	2<	2<	99,99>
S. aureus	7,04	6,99	10,9	2<	2<	2<	2<	99,99>
C. albicans	4,95	4,91	8,43	2<	2<	2<	2<	99,99>

cfu; colony forming unit, C; control fabrics with no additives, MK1; bioactive fabric impregnated with 10gr/ltmicrocapsule containing solution, MK2; bioactive fabric impregnated with 20gr/lt microcapsule containing solution; MK3; bioactive fabric impregnated with 30gr/lt microcapsule containing solution, MK4; bioactive fabric impregnated with 40gr/lt microcapsule containing solution, R; Reduction rate, IMC;initial microorganism concentration, FMC: final microorganism concentration.

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IV. CONCLUSION

The purpose of this study was to prepare antimicrobial agent loaded microcapsules for medical textile applications. For this purpose, thyme oil with antimicrobial activity was encapsulated successfully via complex coacervation method using gelatin and gum arabic as wall materials. The formation of microcapsules were observed through optical microscope images. Resulting microcapsules had spherical shape with a continuous core, surrounded by a continuous shell. The effects of the amount of oil and wall material concentration on the encapsulation yield, mean particle size and oil loading were investigated. Generally, encapsulation yield increased with increasing amount of oil. Similarly, increasing wall material concentration resulted in higher encapsulation yield up to a certain concentration. After that no capsules were formed. As expected, as the amount of oil increased the size of microcapsules and their oil content increased, but they tended to agglomerate. High wall material concentration caused agglomeration as well. For the present study it was concluded that 10 ml of oil and 2% wall concentration resulted in best outcome in terms of microcapsule size. At these conditions, encapsulation yield was 74.72%, mean size of microcapsules was 25.37 micron and oil loading was 36.38%. Antimicrobial activity tests revealed that thyme oil loaded microcapsules had a strong antimicrobial activity, against E. coli, S. aureus ve C. Albicans. Subsequently, prepared microcapsules were grafted onto a nonwoven fabric at four different concentrations to impart antimicrobial characteristics to this fabric. Results showed that even the lowest concentration (10 g/l) provided sufficient level of antimicrobial activity to the fabric.

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