FACULTY OF TEXTILE TECHNOLOGY

Iva Brlek

COSMETOTEXTILES - CARRIERS OF ACTIVE NATURAL SUBSTANCES TO THE SKIN

DOCTORAL DISSERTATION

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DOKTORSKI RAD

Mentor:

Prof. dr. sc. Sandra Bischof

Zagreb, 2023.

... To my Mom and Dad ...

Without their support I would not have started

and finished this adventure.

Tema doktorskog rada „**Kozmeto-tekstilije kao prijenosnici aktivnih tvari prirodnog porijekla na kožu**“ prihvaćena je na 7. redovitoj sjednici u akademskoj godini 2015./2016. Fakultetskog vijeća Sveučilišta u Zagrebu Tekstilno-tehnološkog fakulteta, održanoj 23. svibnja 2016., te odobrena od Senata Sveučilišta u Zagrebu na 5. sjednici Senata održanoj 15. studenog 2016., u akademskoj godini 2016./2017.

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SUMMARY

The main goal of this thesis was to determine the methodology best suited for the quantification of active substances on cosmetic textiles, before and after their release in washing cycles. Prior to development of methodology for quantifying active substances on cosmetotextiles, it was necessary to synthesise microcapsules. Active substances, α-tocopherol and immortelle essential oil were selected and three different types of microcapsules were synthesised (containing α-tocopherol, immortelle essential oil and mixture of α-tocopherol and immortelle essential oil). The synthesis was optimised and the best amount of active substance and ethyl cellulose, which plays the role of a membrane in the synthesised microcapsules, in rate 1:3, (oil: ethyl cellulose) was selected for further investigations.

Both, α-tocopherol and immortelle essential oil are very sensitive to external influences and encapsulation was a logical choice to prevent their decomposition, i.e. premature evaporation during and after the application to textiles. The synthesised microcapsules were analysed gravimetrically and microscopically to determine their morphology and to detect the presence of oil in the microcapsules. Antioxidant analysis was performed and demonstrated using Electron Spin Resonance (ESR) technique. As expected, α-tocopherol showed very strong antioxidant activity, but immortelle essential oil as an active substance showed significant antioxidant potential as well.

Microcapsules containing α-tocopherol and the ones containing immortelle essential oil were selected for application to textiles. Cotton fabric was selected as a potential cosmetotextile due to its natural origin, hydrophilic and pleasant characteristics in contact with the skin. Studies were also conducted on other textile materials (e.g. PES/cotton, silk, modal and liocel). Three different applications methods were carried out: impregnation, exhaustion and electrospinning. Exhaustion method has proven to be the most acceptable, as it allowed a uniform binding of the microcapsules to the textiles under controlled conditions of temperature, time and concentration of the chemicals, which was not the case with impregnation and electrospinning.

SEM confirmed the presence and unchanged morphology of the microcapsules after application to textiles, while HPLC qualitatively and quantitatively confirmed the presence of α-tocopherol on textiles. UV spectrophotometry confirmed qualitatively and quantitatively presence of immortelle essential oil on the treated textiles and the antioxidant content of the cosmetotextile containing microcapsules with α-tocopherol and immortelle essential oil. Remission spectrophotometry was used to analyse the whiteness before and after processing the textiles. The results revealed unsignificant change in the whiteness of the material after processing with microcapsules. Additionally, the presence of α-tocopherol on the treated textile was qualitatively confirmed by the drop test through a simple visual determination of colour change. Application of microcapsules containing 8 % (by weight of the material) has not achieved antibacterial and bactericidal properties in tests with cosmetotextile containing essential oil during testing with: *Klebsiella pneumoniae* (KP), *Acinetobacter baumannii* (AB) and *Staphylococcus aureus*, although immortelle has an antibacterial effect according to the literature. The dermatological test (Patch test) was performed on 50 subjects and all patients were negative for the tested textile samples. Since the tested patients were most likely to have sensitised skin, we could conclude that the tested textiles are hypoallergenic.

The textiles treated with microcapsules were subjected to a test for fastness to washing, rubbing and light to determine the binding of the microcapsules as well as their release during the usage. The cosmetotextile containing microcapsules of essential oil were found to have good light resistance (55.74 % of the active substance was still present on the cosmetotextile after the test), but they were not wash-resistant, as no essential oil could be detected on the textiles after 10 washing cycles. The obtained rubbing fastness results showed that the oil was gradually released when rubbed on the skin, which was a necessary precondition to classify prototype as a cosmetotextile.

Cosmetotextiles prototype containing microcapsules of ethyl cellulose and immortelle essential oil developed within the thesis can be used for clothing in close contact with the skin.

KEYWORDS: α-tocopherol, immortelle essential oil, microcapsules, cosmetotextiles

SAŽETAK

Glavni cilj ovog rada je utvrđivanje metodologije koja je najprimjerenija za kvantificiranje aktivnih tvari na kozmetotekstilijama, prije i nakon njihovog otpuštanja i pranja. Kako bi se mogla provesti istraživanja te razraditi metodologija za kvantificiranje aktivnih sredstava na kozmetotekstilijama za početak je bilo potrebno sintetizirati mikrokapsule. Za sintezu su odabrana aktivna sredstva α-tokoferol i eterično ulje smilja te su sintetizirane tri različite vrste mikrokapsula (one koje sadrže samo α-tokoferol, one koje sadrže samo eterično ulje smilja i one koje sadrže α-tokoferol i eterično ulje smilja). Provedena je optimizacija sinteze te je odabrana optimalna količina aktivnog sredstva i etil celuloze koja ima ulogu membrane u sintetiziranim mikrokapsulama u omjeru 1:3 (ulje:etil celuloza).

α-tokoferol i eterično ulje smilja su vrlo osjetljivi na vanjske utjecaje te je kapsuliranje bio logičan izbor za sprečavanje njihovog raspada, odnosno prijevremenog gubitka za vrijeme i nakon nanašanja na tekstil. Provedene su analize aktivnih sredstava prije sinteze u svrhu karakterizacije. Na sintetiziranim mikrokapsulama su provedene analize: gravimetrijska; mikroskopske (skenirajući elektronski mikroskop (SEM), konfokalni laserski skenirajući mikroskop (CLSM)) pomoću kojih je utvrđena morfologija mikrokapsula te dokazana prisutnost ulja unutar mikrokapsula; kvalitativne i kvantitativne analize (visokoučinkovita tekućinska kromatografija (HPLC) i UV spektrofotometrija); Fourier-ova transformacijska infracrvena spektrofotometrija (FTIR) analiza te antioksidativna analiza. Antioksidativna analiza je provedena na „Institutu Ruđer Bošković“ koristeći elektronsku spinsku rezonanciju (ESR) analizu te je dokazana antioksidativnost svih sintetiziranih mikrokapsula. α-tokoferol je pokazao veoma snažnu antioksidativnu aktivnost, ali i eterično ulje smilja kao aktivna supstanca je isto pokazala značajni antioksidativni potencijal.

Nakon provedenih ispitivanja sintetiziranih mikrokapsula, za aplikaciju na tekstil su odabrane mikrokapsule s α-tokoferolom i mikrokapsule s eteričnim uljem smilja. Pamučna tkanina je odabrana kao potencijalna kozmetotekstilija jer je pamuk prirodnog podrijetla, hidrofilan je i ugodan u dodiru s kožom. Istraživanja su provedena i na drugim

tekstilnim materijalima (PES/pamuk, svila, modal i liocel) kako bi se istražila mogućnost primjene i na drugim tekstilnim materijalima u budućim istraživanjima. Provedene su tri različite aplikacije mikrokapsula na tekstil: impregnacija, iscrpljenje i elektroispredanje. Aplikacija iscrpljenjem se pokazala najprihvatljivijom jer se na taj način omogućuje mikrokapsulama da se vežu na tekstil egalno i u kontroliranim uvjetima temperature, vremena i koncentracije kemikalija što kod impregnacije i elektroispredanja nije slučaj.

SEM-om je potvrđeno prisustvo i ne promijenjena morfologija mikrokapsula nakon aplikacije na tekstil, dok je HPLC-om kvalitativno i kvantitativno potvrđeno prisustvo α-tokoferola na tekstilu. UV spektrofotometrijom je potvrđeno kvalitativno i kvantitativno prisustvo eteričnog ulja smilja na obrađenom tekstilu te antioksidativnost kozmetotekstilija koje sadrže mikrokapsule s α-tokoferolom i eteričnim uljem smilja. Remisijskom spektrofotometrijom je analizirana bjelina prije i nakon obrade tekstila, a rezultati su pokazali da ne dolazi do značajnije promjene u bjelini materijala nakon obrade s mikrokapsulama. Jednostavnim vizualnim određivanjem promjene obojenja pomoću Testa kapi (Drop test) kvalitativno je potvrđeno prisustvo α-tokoferola na obrađenom tekstilu. Primjenom 8 % mikrokapsula (na masu materijala) nisu postignuta antibakterijska i baktericidna svojstva kozmetotekstilija s eteričnim uljem smilja tijekom ispitivanja sa: *Klebsiella pneumoniae* (KP), *Acinetobacter baumannii* (AB) i *Staphylococcus aureus* (SA) iako prema literaturi smilje ima antibakterijsku aktivnost. Dermatološki test (Patch test) je proveden na 50 ispitanika i svi bolesnici su bili negativni na testirane uzorke tekstila. S obzirom da su testirani bolesnici koji imaju senzibiliziranu kožu s velikom vjerojatnosti može se zaključiti da su ispitivane tekstilije hipoalergene.

Tekstilni materijali obrađeni mikrokapsulama su podvrgnuti ispitivanju postojanosti na pranje, trenje i svjetlost kako bi se utvrdilo vezivanje mikrokapsula, kao i njihovo otpuštanje tijekom upotrebe jer je to najvažniji preduvjet da se takav tekstil uopće može nazvati kozmetotekstilijom. Nakon provedenih testova na postojanosti utvrđeno je da kozmetotekstilije koje sadrže mikrokapsule s eteričnim uljem smilja imaju dobru postojanost na svjetlo (nakon testa je na kozmetotekstiliji ostalo 55.74 % aktivne supstance), ali nisu postojane na pranje jer nakon 10 ciklusa pranja više nije bilo moguće detektirati eterično ulje na tekstilu. Dobiveni rezultati postojanosti na trenje ukazuju da

se ulje postepeno otpušta prilikom trenja o kožu, što je u ovom slučaju poželjno jer se u protivnom razvijeni prototip tekstilije ne može kategorizirati kao kozmetotekstilija.

UV spektroskopija se pokazala najboljom za kvantifikaciju eteričnog ulja smilja na kozmetotekstilijama. Ova analiza je jednostavna, rezultati su ponovljivi i male količine ulja je moguće detektirati navedenom analizom.

Razvijeni prototip kozmetotekstilije pamučnog materijala s etil celuloznim mikrokapsulama koje sadrže eterično ulje smilja se može koristiti za odjevne predmete koji su u bliskom kontaktu s kožom (donje rublje, kratke majice, uske hlače, npr. tajice) te za maske za spavanje, posteljinu itd. u svrhu wellness tekstila.

KLJUČNE RIJEČI: α-tokoferol, eterično ulje smilja, mikrokapsule, kozmetotekstilije

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1 INTRODUCTION

Textiles and cosmetics were among the first products man created. However, joining textiles and cosmetics in the form of cosmetotextiles is a relatively new concept of applying them together and the investigations performed promise a high impact in the 21st century [1, 2]. The most interesting method of „storing" a cosmetic preparation is constructing a microcapsule, as it offers the means of controlled release of the active substance. Although microcapsules have been mentioned for some time in various applications in textile industry, they were not used for cosmetic applications before 1990s [3]. Application and review of microcapsules application on textile is presented in Figure 1.

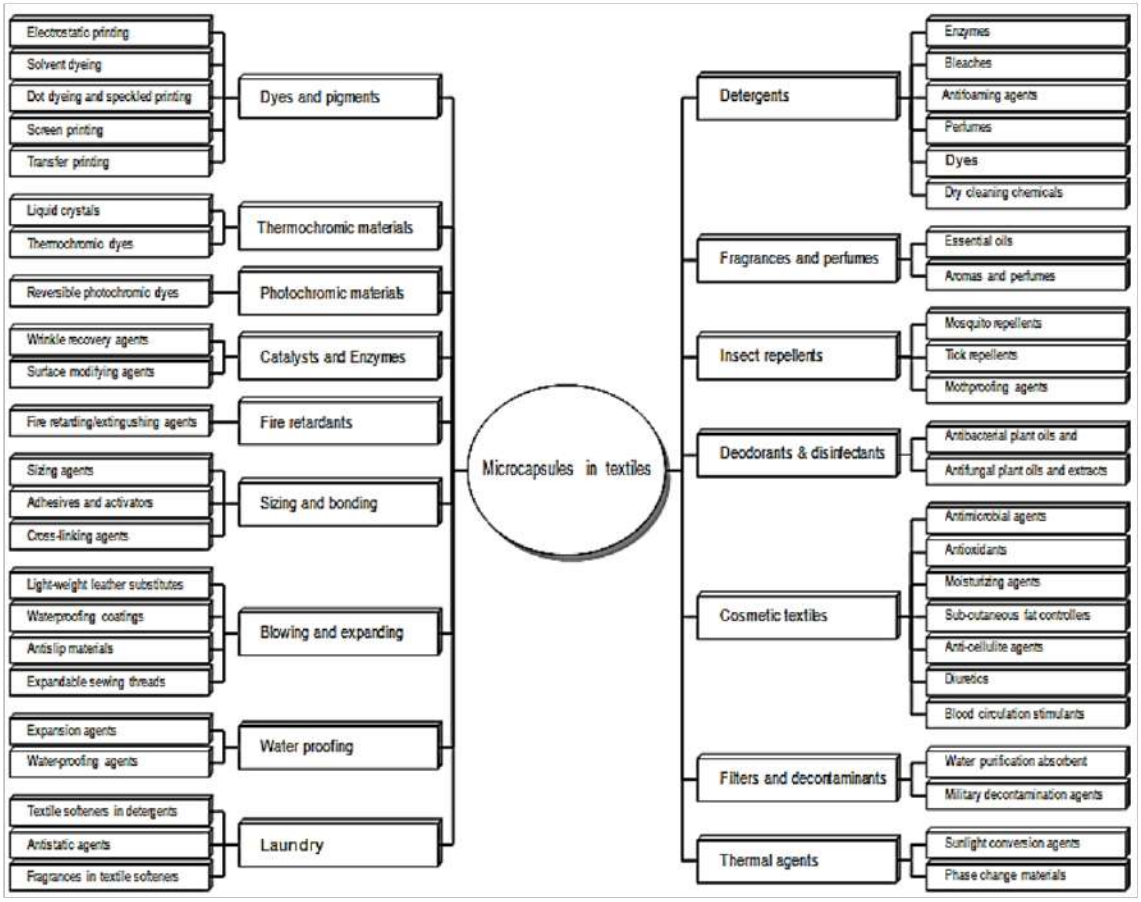


Figure 1 Review of microcapsules in textile application [4]

Apart from improving the odour and deodorising, microcapsules can also be used as a means of transferring odours for special purposes, e.g. for aromatherapy. Fragrances and aromas have been used in popular medicine for centuries. The expression „aromatherapy" was mentioned for the first time in late 1920s, by R.M. Gattefosse. The study of the relation between psychology and aromas (scents) that cause specific feelings and emotions was named aromachology in 1982. It is believed that the key of aromatherapy is in stimulating olfactory paths in the brain, particularly in the limbic system, as particular aromas (scents) cause the feelings of relaxation, excitement, sensuality, happiness, welfare or bliss [5].

One of the examples is microcapsules containing essential oils. These microcapsules are applied to textiles, which implies mechanical pressures and friction, with fragrance released or some other effect achieved, depending on the type of the essential oil used [3]. Cyclodextrins are often used in cosmetic applications, as they have a mild impact on skin, while they are able to create a complex with a cosmetic preparation, which can be attached to textiles using various techniques [6, 7].

Development of cosmetotextiles has been relatively slow and the main reason for that is still present today – inability to achieve targeted effectiveness and washing durability at the same time. One of the problems is the sensitivity, or instability, of numerous cosmetic compounds, e.g. perfumes or fragrances are highly volatile, while most vitamins are sensitive to high temperatures.

To develop and manufacture an efficient cosmetotextile product, such that would offer additional valuable properties to be realised in wearing, is by no means a simple task. Key targets are to keep the cosmetic preparation on the textiles after a washing cycle and enable controlled release of the active substance in it. However, the target of keeping the cosmetic preparation on textiles in washing cycles should not be overdone, as the primary function is to release it onto the skin, and it should not be endangered. Microencapsulation of highly volatile compounds, such as essential oils, together with a proper selection of the materials with adequate properties for the microcapsule walls, can achieve controlled release of cosmetic preparations, i.e. impermeability and at the same time sensitivity to

pressure or friction [3, 8]. The use of crosslinking agent (binder) is recommended when microcapsules are applied to a textile substrate, as it considerably improves washing fastness results [3, 9, 10].

Various approaches have been tried to solve the problem of controlled release. Historically, the focus of interest has been on preventing release in washing, while not enough attention has been paid to the mechanism of transferring the cosmetic preparation onto the skin [8, 11].

To be able to understand the importance of cosmetotextiles, it is necessary to be familiar with the biggest organ of our body – the skin. The average surface area of human skin is 1.8 m^2 and it constitutes 15 % of the overall body mass, which indicates that taking care of the skin is of utmost importance for our health. The skin is divided into three principal parts: epidermis, dermis and hypodermis. Epidermis is the target layer of the skin when cosmetics are concerned (Figure 2). It is the upper layer of the skin, above all the other layers, and is in a direct contact with the environment. Epidermis consists of cells that are in constant shuffling and change, until the upmost layer is constituted, called a horny layer (*lat. Stratum corneum*). Water content differs from layer to layer. In the basal layer (*lat. Stratum basale*) it is 75 %, while in the horny layer it reaches only 15 % [12-14]. Skin is healthy and attractive only when it is well balanced. Prerequisites for a healthy skin are: particular water content in the skin, ability of self-protection, skin elasticity, as well as the ability to renew cells [15].

Human skin is an impermeable barrier which protects the organism from foreign, outside, substances, including bacteria, fungi, viruses, allergens, dust and some other big molecules. The uppermost layer contains more than 90 % of multilayer keratocytes. The horny layer, the outer epidermis layer, offers a highly efficient barrier for the controlled entry of the cosmetic product. However, due to the unique composition and structure it also constitutes a significant barrier on the skin, protecting the body from the impacts of the environment and prevents dehydration [16].

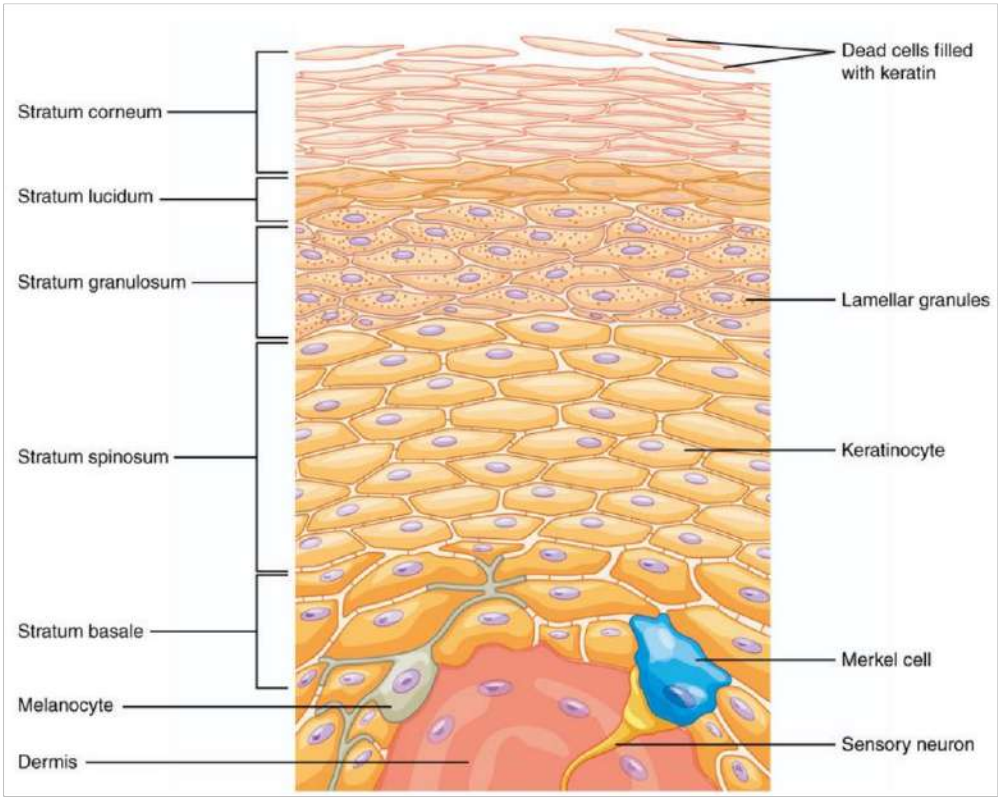


Figure 2 Schematic of epidermis [14]

Because of that big importance of taking care of skin this research was made. It was examined controlled release of active substances synthesised in microcapsules and applied on textiles so cosmetotextiles were made. Also, the stability on washing cycles, on light and on rubbing of cosmetotextiles was tested. This research is very important because cosmetotextiles have big future perspectives but that specific influences (washing cycles, stability on light and stability on rubbing) causing problems.

In this research microcapsules with essential oil of immortelle and with vitamin E were synthetized. There are numerous scientific papers about the essential oils' encapsulation; however, there is a major gap in encapsulated immortelle oil research. Encapsulation of vitamin E isn't something new, but the optimisation of synthesis and analyses of microcapsules carry out after synthesis has significant contribution to science.

1.1 Research hypothesis

Research hypotheses were:

1. It is possible to achieve targeted efficiency of cosmetotextiles by applying active, natural products based on vitamins or essential oils on textiles.

This study confirms that it is possible to achieve targeted efficacy of cosmetotextiles by applying active, natural products based on vitamins and essential oil to textiles. The natural vitamin and essential oil-based products used in this study are α-tocopherol (vitamin E) and essential oil of immortelle. Different textiles were used for this purpose: cotton fabric, PES /cotton fabric, silk fabric, modal fabric and liocel - nonwoven textile. The presence of the active substance α-tocopherol was detected on all the textiles mentioned using the Drop test (pages 135 and 136). The targeted effects of the cosmetotextiles were the antioxidant activity and the dermatological test of the cosmetotextiles with the essential oil of immortelle. The antioxidant activity of EC microcapsules was investigated using the ESR method. It was proved that the synthesised microcapsules contain active substances that have good antioxidant activity (pages 101-105). The antioxidant activity of cosmetotextiles was also investigated using the UV spectroscopic ABTS method. The percentage inhibition of the cosmetotextiles after 15 and 60 minutes showed that the free radicals were reduced in contact with the antioxidant. The results were presented on pages 133-135. The synthesis of microcapsules using ethyl cellulose as the membrane of the microcapsules and selected active substances, proved to be a good combination, as the membrane stores the active substance and protects it from external influences. On the other hand, the properties of the active substances remain unchanged. The cosmetotextiles with the essential oil of immortelle were examined in a dermatological test (Patch test) on 50 test persons. All patients reacted negatively to the tested textile samples. Since the tested patients most likely have sensitised skin, so it is concluded that the tested textiles are hypoallergenic (page 141).

2. Synthesized microcapsules will be attached to textile substrate and gradually released from cosmetotextiles.

In this research, it is confirmed that synthesised microcapsules containing essential oil of immortelle can be attached to textile substrate and gradually released from cosmetotextiles. For this purpose, the following fastness tests were performed: washing cycles, rubbing and light test. Rubbing test and test on light were performed for cosmetotextiles with EC microcapsules containing α-tocopherol. HPLC analysis was performed to quantify α-tocopherol. The results of the HPLC analysis showed that cosmetotextiles with EC microcapsules containing α-tocopherol lose some of the active substances on the cosmetotextiles after the rubbing test and the light test. After the rubbing test, which simulated the rubbing of cosmetotextiles on the skin, the result was positive (5.54 % of the active substance was released) (page 127), as this type of cosmetotextile would otherwise not be applicable and cannot be called a cosmetotextile. The light fastness showed as that external influences affect the release of the active substance and after 41 hours of sun exposure, 83.37 % of the active substance is still present on the cosmetotextiles (page 127).

UV spectroscopy analysis was used to quantify the essential oil of immortelle on cosmetotextiles. For cosmetotextiles with EC microcapsules containing immortelle oil, the rubbing test and the light test gave the following results: 73.77 % of the active substance was released after the rubbing test (page 132) and 55.74 % was still present on the cosmetotextiles after 41 hours of sun exposure (page 132). A wash test was also carried out for this type of cosmetotextile. The washing fastness of the cosmetotextiles produced shows acceptable results; ten washing cycles is the limit that can be achieved with acrylic binder for this application on cotton. After one washing cycle, 31.15 % of the active substance remains in the cosmetotextiles. After ten washing cycles, 11.89 % of the active substance remains in the cosmetotextiles (8 % microcapsules were applied to the mass of the material) (page 132).

3. Developed methodology will allow quantitative determination of the amount of active products in the cosmetotextiles, which is directly related to the effectiveness of treatment and washing durability.

The methodology developed allows the quantitative determination of the amount of active product (substance) in the cosmetotextiles. HPLC analysis was used for quantitative determination of α-tocopherol on cosmetotextiles containing EC microcapsules containing α-toc (page 127) and UV spectroscopy was used for quantitative analysis of essential oil of immortelle (EOI) on cosmetotextiles containing EC microcapsules containing EOI (page 132). It was directly related to treatment efficacy and wash resistance (see hypothesis 2).

2 THEORY PART

2.1 *Cosmetotextiles*

According to a manual [17] based on a European Commission (EC) regulative No 1223/2009 for cosmetic products [18], textile can be a "carrier" that delivers active substances or mixtures of substances to the human skin. The active substance(s) can be delivered to different parts of the human body, especially the skin, in a reasonable time and duration. They have specific functions, such as cleansing the skin, adding fragrance, changing the appearance of the skin, correcting odours or, most importantly, keeping the skin healthy.

Cosmetotextiles are complexes consisting of cosmetic preparations, or their mixtures, and textile substrates. Cosmetic preparation, or mixture of preparations, can be of natural or synthetic origin. For an active preparation or a mixture applied to a textile substrate to be considered an active substance at all, it is necessary to possess the ability to be released to the skin. Preparations that are not released to the skin are not considered cosmetic products, nor are the textiles with active substances applied to them that are not released to the skin, classified as cosmetotextiles [17].

The European Committee for Standardization (CEN) appointed a working group (WG) in 2005, task to deal with the problems of cosmetotextiles, CEN/TC 248/WG 25 [1]. The working group WG 25 was responsible for the development of standards associated with cosmetotextiles. WG 25 identified some areas where standardisation was necessary and appointed accordingly five sub-groups to work in different areas of cosmetotextiles. The standard was accepted by the European Committee for Standardization CEN/TR 15917:2009: Textiles – Cosmetotextiles [19]. Necessary normative references cited in the above standard are:

- HRN EN ISO 3175-1:2010: Textile– Professional care, dry-cleaning and wet cleaning of fabrics and garments – Part 1: Assessment of performance after cleaning and finishing (ISO 3175-1:2010; EN ISO 3175-1:2010)

- HRN EN ISO 3758:2008: Textiles. Care labelling code using symbols (ISO 3758:2005; EN ISO 3758:2005)
- HRN EN ISO 6330:2003/A1:2009 enpr: Textiles – Domestic washing and drying procedures for textile testing (ISO 6330:200/Amd 1:2008; EN ISO 6330:2000/A1:2009)
- HRN EN ISO 22716:2008: Cosmetics–Good Manufacturing Practices (DPP) – Guidelines for good manufacturing practice (ISO 22716:2007; EN ISO 22716:2007)

It is recommendable to use the above normative references and harmonise them with the regulations such as Oeko-Tex® 100 and Oeko-Tex® 1000. This would ensure high quality of textiles prior to applying a cosmetic preparation onto it, as well as high standard of cosmetic textiles as a finished product. It is necessary to perform individual tests on cosmetic preparations in chemical industry, while, after the product has been completed (cosmetic textile product), it is also necessary to test the product using general biological tests, similar to antimicrobial tests conventionally performed. Cosmetic textiles are supposed to pass through all the tests following the standard HRN EN ISO 10993-10:2013: Biological evaluation of medical devices, Part 10 Tests for irritation and skin sensitization (ISO 10993-10:2010; EN ISO 10993-10:2013) as well as the OECD methods (OECD 405, 406, 407 & 471) [1, 12].

Various literature references [1, 9, 20, 21] classify cosmetotextiles using different bases and concepts, most often using the impacts on human body as a basis of classification, or the method of applying them onto textile substrates.

On the basis of the impact on human body, cosmetotextiles can be divided into:

- anti-cellulite cosmetotextiles [12],
- skin hydration cosmetotextiles [21, 22],
- energising cosmetotextiles [21],
- fragrance and perfume containing cosmetotextiles [9, 23- 25],
- refreshment and relaxation cosmetotextiles [20, 26],

- revitalisation cosmetotextiles [27-29],
- UV protection cosmetotextiles [28, 30],
- anti-age cosmetotextiles [29].

2.2 Microcapsules

Microcapsules are particles ranging in size from 1 to 1000 μm, containing an active substance (in liquid or solid phase), surrounded by natural, semi synthetic or synthetic polymer membrane. They consist of two parts, the core and the membrane [15, 31, 32]. Microparticle structure can generally be described in various ways: as a microcapsule with a single core surrounded by a layer – envelope, i.e. a wall of material; as a microsphere with dispersed core in a continuous matrix network; or as a more complex structure, i.e. multilayer microcapsule, or multishell microsphere (Figure 3) [33].

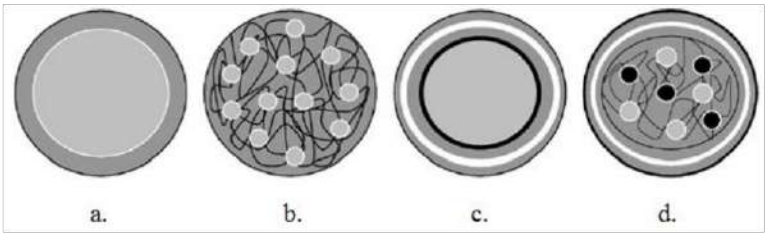


Figure 3 Different morphologies of microparticles: a. microcapsule, b. microsphere, c. multilayer microcapsule and d. multishell and multicore microsphere [33]

The core of a microcapsule is most often composed of materials in the form of a solution, dispersion or emulsion. Compatibility of the core material and the membrane is an important factor in improving microencapsulation effectiveness, thus core material is often pretreated to ensure better compatibility. Core size plays an important role in diffusion, permeability and/or controlled release. A wide range of materials can be

encapsulated, for various purposes. These can be essential oils, vitamins, pigments, dyes, monomers, catalysts, curing agents, flame retardants, softeners etc. [31].

Numerous active substances are sensitive to heat, prone to oxidation or change. Microencapsulation or bonding into complexes enables the protection of sensitive active substances from detrimental influences, such as degradation by oxidation or by polymerisation during drying and / or thermal treatments and garment storing. These processes prevent evaporation of volatile compounds and prolong their lifetime, which is of high importance when using perfumes and essential oils [12, 16, 34].

There are many previous reports on the encapsulation of essential oils, and some of them are based on the following themes: Control of the size distribution of the capsules, finding that the essential oil was entrapped in the microcapsules instead of being adsorbed on the surface, influence of the weight ratio of the core and wall material, control of the main variables (transfer rate of the sample, drying air flow and temperature) to obtain the best powder yield and oil content [35- 42].

When widely used, microcapsules have a number of interesting advantages:

- protection of unstable, sensitive materials, from the environment in which they are used,
- easier processibility (increased solubility, dispersibility and flowing properties),
- self-preservation through prevention of decomposition reaction (oxidation, dehydration),
- controlled, continuous or gradual release,
- masking odours and tastes,
- immobilisation of enzymes and microorganisms,
- controlled and targeted delivery of medicines,
- handling liquids as if they were solids [31].

Selection of the material for microcapsule membrane is highly important and is mostly dictated by the physical properties of the environment where the microcapsule is used, as some systems prompt for firmer capsules so as to prevent or regulate premature release of the active substance [5, 9, 24, 43-47]. In the Table 1 are presented most often used membrane materials for microcapsule formation.

Table 1 Review of most often used materials for membrane [48]

Water-soluble resins	Water-insoluble resins	Waxes and lipids	Resins
Gelatin	Ethyl cellulose	Paraffin	Shellac
Gum arabic	Polyethylene	Carnauba	Cellulose acetate phthalate
Starch	Polymethacrylate	Spermaceti	Zein
Polyvinylpyrolidione	Polyamide (Nylon)	Beeswax	
Carboxymethylcellulose	Poly [Ethylene-vinyl acetate]	Stearic acid	
Hydroxyethylcellulose	Cellulose nitrate	Stearyl alcohol	
Methyl cellulose	Silicones	Glyceryl stearates	
Arabinogalactan	Poly (Lactide-co-glycolide)		
Polyvinyl alcohol			
Polyacrylic acid			

Ethyl ether of cellulose (EC) is used for membrane material in synthesis in this research. Ethyl cellulose is prepared from wood pulp or cotton by treatment with alkali and ethylation of the alkali cellulose with ethyl chloride. The article of commerce can be specified further by viscosity [49]. It is frequently used as a hydrophobic polymeric coating material for extended drug release applications [49- 51]. Microcapsules can be

prepared with EC using various methods such as phase separation, coacervation, solvent evaporation, either by addition of a non-solvent, or of an incompatible polymer [51, 52].

Different methods are used for the encapsulation of microparticles, depending on the type of substance encapsulated and the polymer used [53]. They can be divided into physical and chemical methods (Table 2) [16, 31, 48, 54- 57].

Table 2 Microencapsulation methods

Physical methods	Chemical methods
Spray drying	Coacervation Phase separation
Spray chilling	Solvent evaporation
Rotary disk atomization	Solvent extraction
Fluid bed coating	Interfacial polymerisation
Stationary nozzle coextrusion	Simple and complex coacervation
Multiorifice - Centrifugal Process	In situ polymerisation
Submerged nozzle coextrusion	Liposome technology
Polyacrylonitrile coating (PAN)	Nanoencapsulation
Air-Suspension Coating	Matrix polymerization

A widely used method for encapsulating water-insoluble substances in water-insoluble polymers is the solvent evaporation method [36, 37, 53, 54, 56, 58-60]. Spraying and coacervation are also commonly used techniques for encapsulating active substances. Both respect the concept of "green chemistry" by using mainly plant proteins and other renewable and biodegradable sources. It is important to note that both techniques do not use organic solvents throughout the process [33].

The selection of microencapsulation technique to be used for a particular process depends upon the size, biocompatibility and biodegradation of the particles, physical-chemical properties of the core and membrane, the application of microparticles, the mechanism proposed for the release of the active core, as well as upon the costs of the process [33]. The selection of the technique also depends upon the following parameters:

- microcapsules purpose;
- inertness towards the encapsulated agent and towards the membrane;
- process conditions in order to prevent capsulated substance to be released too early;
- optimal concentration of the active substance for encapsulation;
- the mechanism of releasing active substance from the microcapsule (e.g. pH, pressure, solubility, time and agitation (stirring);
- what manner of release is preferred: continuous, immediate or controlled;
- particle size and density requirements for stability of the encapsulated agent;
- costs of the capsules, substance in it and/or application, as compared to the cost of the final product. High cost of microencapsulation is still one of the outstanding problems for the market, although in many cases the cost of the final product is quite proportional with its value [15, 31].

Factors influencing microencapsulation efficiency

The efficiency of encapsulation for microparticles, microcapsules or microspheres depends on various parameters (Figure 4). Favourable parameters are low polymer solubility in organic solvents, high solubility of organic solvents in water, high polymer concentration, low ratio of dispersed phase/continuous phase (DP/CP), as well as a rapid effect of removal in a solvent. All of these are prerequisites for rapid stiffening (curing) of microparticles, which results in high encapsulation efficiency [56].

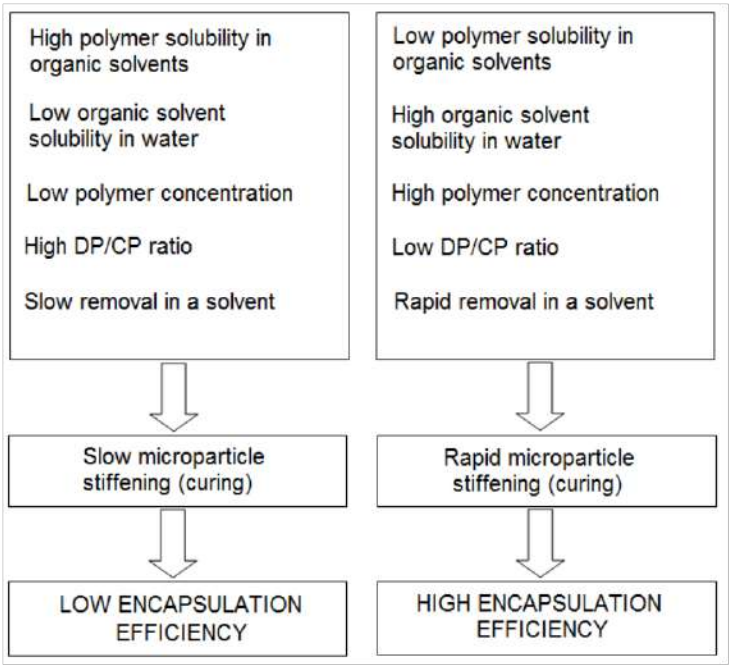


Figure 4 Factors influencing encapsulation efficiency [56]

It can be said that cosmetic products are any substances or preparations intended to come into contact with various parts of the human body (skin, hair, lips and external genital organs) or with teeth and mucous membranes in the oral cavity. Their function is only or mainly to cleanse, perfume or protect them, to keep them in good condition, to change their appearance or to improve body odour. The EU has replaced the Cosmetic Directive 76/768/EEC from 11.7.2013 by the Regulation (EC) No. 1223/2009, entitled "Cosmetic Products Regulation" [18].

Various active substances can be applied on textile substrates using different application techniques at various production stages, with the aim of achieving optimal cosmetic effect. The selection of application process or technique depends on the nature of cosmetic preparation and on the nature of textiles in question, as well as on the amount of the active substance to be applied. The procedures of applying active substances onto textile substrates can be divided into:

- encapsulation using binder (impregnation, exhaustion etc.), grafting [9, 60, 61],

- complexation using additional chemicals for binding [62],
- coating [12],
- adding active substance in the manufacturing process (e.g. coextrusion) [12].

Table 3 gives a comparison of the selected processes of applying active substances onto textiles, based on the area of application, the ability of binding the preparations, washing fastness and transfer to the skin of the wearer.

The development of cosmetotextiles until now has been mostly aimed at finding and constructing various active substances and inventing methods of their application. However, major issue is still monitoring the mechanism of the active substances controlled release. This puts into the focus of interest various technical challenges, such as the selection of adequate active substance, uniform distribution of the agent, inconspicuous integration into the fabric to be used. The aim is to achieve the effect with as minor loss of the active substance during the textile care and washing cycles. Microencapsulation is most often used for a purposeful, i.e. controlled release of active substances [12].

Basic active substances have their origin in inorganic and synthetic chemicals, animal (chitosan, squalane) and vegetable derivatives (essential oils, vitamins, aloevera, various fruits, flowers, etc.) [1, 22].

Various plant preparations can be used in preparing cosmetic products, depending upon the target effect on the skin, e.g. revitalisation, hydration, skin protection, reduction and prevention of acnes, spots, eczema. They offer achieving the targeted effect for a particular cosmetic purpose, or more of them as preparations are most often active in more than one area. For example, green tea acts as a free radical scavenger, i.e. has antioxidative properties and revitalises the skin in this way, improves microcirculation in the skin and additionally offers protection from UV irradiation [15, 28].

Table 3 Application properties of microcapsules depending of action method [12]

Process	Area of application	Ability of receiving the preparation	Washing fastness	Transfer to the skin
Microencapsulation (grafting)	Very wide, but primarily to compounds not soluble in water	High	Good, depending on the type of bonding agent	Good
Complexation (e.g. cyclodextrin)	Highly specific, for a limited range of preparations	Medium to high	Good	Limited
Coating	Wide. Not applicable for sensitive and volatile agents	Very high	Good, depending on the fibre type and bonding agent	Good
Coextrusion	Limited to extremely robust preparations	Low to medium	Excellent	Insignificant

Essential oils are often used for cosmetic preparation. They are volatile complex of natural compounds, with characteristic fragrance, obtained from aromatic plants as secondary metabolites. They are mostly obtained by the processes of evaporation or hydro distillation. Being familiar with their antiseptic, e.g. antibacterial, antiviral and antifungal properties, Arabs in Middle Ages used these oils for embalming, food preparation and as antimicrobial analgetics, in treating various inflammations, as spasmolytics, as well as local anaesthetics. The properties expected from essential oils have not been changed much until the present days. However, we know more about the mechanisms of their activity, especially in the antimicrobial area [63].

Essential oils are known for their numerous biological effects, among which antibacterial activity has gained special attention [8, 22, 64, 65]. Some of the essential oils exhibit a wide scope of other effects on human organism, some of them improving blood

circulation in the brain, some act as tranquilisers or refresh tired organism. This is why essential oils-based aromatherapy has been widely accepted in the course of past few decades. Aromatherapy is conducted by inhaling essential oil vapours from fragrant lamps or by contact of the oil with the skin. Developing products that would enable another manner of continuous aromatherapy is a challenge and constitutes a significant area of development in the application of natural bioactive substances for the improvement and preservation of health [64]. Due to the above properties, they are quite often used for cosmetotextiles [66].

One of the challenges is the selection of proper textile substrate, so as to achieve optimal bonding as well as the release of the essential oil in the course of using cosmetotextiles. Additionally, careful selection of final finishing processes to be implemented on the fabric or garment, e.g. bleaching, dyeing, etc., can also be of key importance, as these processes can have a detrimental effect on the durability and effectiveness of the essential oils used in finishing [66].

Essential oils often used for cosmetic application are lavender, rosemary, tea tress (melaleuca alternifolia), grapefruit, bergamot, etc. [3, 67]. Essential oils obtained from lavender (*lat. Lavandulaangustifolia*), clary sage (*lat. Salvia sclarea L.*), sandal (*lat. Santalum*) and sweet orange (*lat. Citrus sinensis*) are efficient and secure for the purpose of easing anxiety disorder [30]. Rosemary (*lat. Rosmarinus officinalis L.*) is a plant of high medical and aromatic value. Rosemary essential oil functions as an antiproliferative, antioxidative and antibacterial agent [68]. Lavender oil is used as a remedy itself and as an additive to other remedies and in cosmetics, while inhaling lavender and rosemary increases the activity of bonding free radicals and reduces the level of cortisol in saliva [26, 69]. Fragrant stimulation by grapefruit essential oil impacts positively autonomous neurotransmission and blood pressure [70]. Bergamot (*lat. Citrus bergamia, Risso*) is a fruit well known by its essential oil (BEO) that is used in aromatherapy to ease the symptoms of stress-cause anxiety and mild mood disorders, while the impact of this oil on pains caused by cancers is being investigated [71]. Although essential oils have been used in traditional medicine throughout history, due to their high potential as anticancer therapeutically agents, there is still no sufficiently

detailed explication of their mechanisms and investigations in this area are currently underway [72].

For this research, Mediterranean immortelle essential oil and α-tocopherol (vitamin E) were chosen as the active substances' for application on textile.

Mediterranean immortelle (*lat. Helichrysum italicum* / Roth / G. Don) belongs to the Asteraceae family, genus Helichrysum. Detail category classification of immortelle is presented in Table 4 [73].

Table 4 Taxonomic affiliation of Mediterranean immortelle (Helichrysum italicum / Roth / G. Don) [73]

Category classification	Term
Regnum	Plantae
Phylum	Magnoliophyta
Classis	Magnoliopsida
Ordo	Asterales
Familia	Asteraceae
Genus	*Helichrysum*
Species	*Helichrysum italicum* (Roth) G. Don

The etymology of the term *Helichrysum* is not fully understood, however, the most common interpretation is that the name *Helichrysum* comes from the Greek words *helios* (sun) and *chrysos* (gold). Second, a rarer interpretation of the term *Helichrysum* suggests

that the term may also refer to a single climbing plant of golden flowers (*helix*), named after the eternal flower. The French name, *Immortelle* (immortal) suggests the property of immortelle to retain its appearance and colour for a long time, since the flower heads are surrounded by scaly palms that do not wither [73].

The genus *Helichrysum* belongs to several hundred species distributed around the world. Most species are perennial shrubs. In the Mediterranean area, the genus is represented by about 25 indigenous species, and we find them in Albania, Bosnia and Herzegovina, Montenegro, France, Greece, Croatia and Italy. They are also represented in Northwest Africa and Asia Minor. Mediterranean immortelle (*lat. Helichrysum italicum* / Roth / G. Don) has the greatest economic value. The scientific nomenclature of the species was established by G. Don, who first described it in 1830s [74]. Given the variability of the species, it was agreed that *Helichrysum italicum* G. Don would be used as a synonym for the following names: *Helichrysum angustifolium* Lam D.C., *Gnaphalium angustifolium* Lam. and *G. italicum* Roth. Mediterranean immortelle is also known by the following folk names: bilobrada, cmilj, cmilje (Cro), smilj, smilje, margiž, uzkolistni smilj i bela brada. Botanical sketch of *Helichrysum italicum* (Roth) G. Don is presented at Figure 5 [73].

Figure 5 Botanical sketch *Helichrysum italicum* (Roth) G. Don [75]

The leaves and flowers of immortelle are the most commonly used parts in the treatment of health disorders such as colds, allergies, liver, coughs, skin and gallbladder diseases, inflammations, infections and insomnia. Immortelle is a medicinal plant with promising pharmacological activities. However, most of the traditionally claimed uses have not yet been scientifically proven. Medical studies are needed to further confirm these data and promote immortelle as an important tool in the treatment of various diseases [76-78].

A wide range of extracts can be prepared from *Helichrysum italicum*, and the products obtained differ in chemical composition. Research is mainly focused on the analysis of essential oil, which can be obtained from all green parts of the plant. The composition and content of essential oil in the plant depends on the stage of development of the plant, climatic and pedological conditions, the method of isolation and the chemotype of the plant itself. The fragrance of aromatic herbs comes from terpene compounds (isoprenoids) which are the main constituents of essential oils [73]. Mediterranean essential oils are characterized by a high content of α-pinene (22 %) and appreciable amounts of γ-curcumin (10%), β-selin (6 %), neryl-acetate (6 %), and β-caryophyllene (5 %) [79, 80]. The proportion of each component in the oil depends on the location of the plant [81].

Immortelle contains very little essential oil, less than 0.05 % so it takes more than a ton of plants to get a kilo of essential oil. Immortelle oil has a sweet, layered and extremely perfume scent, is pale yellow in colour, has a very complex chemical composition with many compounds of similar structure and similar physicochemical properties, which significantly complicates their identification [73, 82].

Table 5 The most common compounds in immortelle essential oil [73]

Common compounds	Amount / %
α-pinene	8.76 – 27.23
neryl-acetate	5.75 – 50.79
2-methylcyclohexyl pentanoate	7.81 – 17.76
α-cedrene	5.35 – 16.62
caryophyllene	3.41 – 6.73
limonene	3.01 – 6.18
nerol	1.74 – 5.47

Chemical composition of immortelle essential oil at different stages of plant development was investigated. Plant material was collected in the Municipality of Dugopolje. Analyses of essential oil isolated from fresh plant during different vegetation periods of plant development were performed by gas chromatography-mass spectrometry (GC-MS). A total of 67 compounds were identified, which belong to monoterpene (C10), sesquiterpene (C15) and non-terpene compounds, i.e. their hydrocarbons, alcohols, esters, acids, carbonyl compounds and oxides. Table 5 shows the values (%) of the most common compounds in immortelle essential oil [73].

The major gap is the spectrophotometric analysis of immortelle oil. In general, the UV/VIS spectrophotometric method has been cited by many researchers as the preferred method for the determination of essential oils because it is relatively inexpensive, rapid, accurate and repeatable [37, 40]. According to the literature, pinene has an absorption

peak at 265 nm, which is confirmed in this thesis by the UV spectrophotometric analysis of immortelle essential oil [83].

Tocopherol is a chemical name for vitamin E that belongs to the group of amphipathic and lipid-soluble compounds that are easily oxidized when subjected to heat, light and alkaline conditions [84].

Tocopherols consist of a polar (hydrophilic) chromanol ring and an apolar (hydrophobic) 16-carbon side chain attached to the ring via the C-2 atom having saturated phityl side chains. They occur as four vitamins (α, β, γ and δ) that differ each other by the number and position of methyl groups in the chromanol ring [84]. α-tocopherol (α-toc) belongs to the group tocopherols that are consisting of a series of related benzopyranols (or methyl tocols). Hydrophobic part of a molecule (C-16) side chain, that is saturated with 20-carbon phytyl tails (including the pyranol ring), with variable numbers of methyl groups attached to the benzene ring. The side-chain methyl groups of natural tocopherols are responsible for R,R,R stereochemistry. The four general constituents of the two classes are termed α (5,7,8-trimethyl), β (5,8- dimethyl), γ (7,8-dimethyl) and δ (8-methyl) (Figure 6) [84- 86].

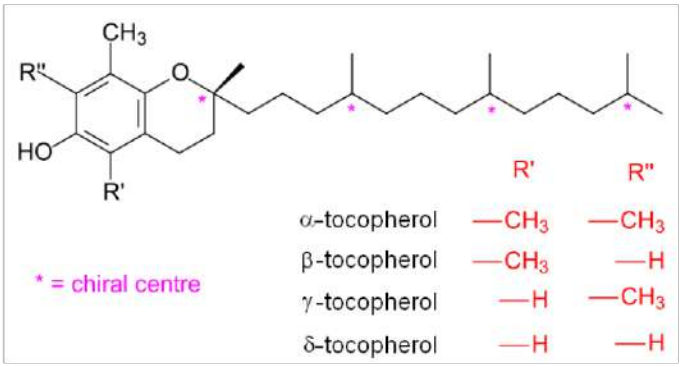


Figure 6 Structure and composition of tocopherols [85]

In the cosmetic industry, α-toc is used as antioxidant and lipid-soluble compound with active substance. It has moisture binding capacity in aliphatic cosmetic creams, lotions, emulsions, body and face oils for dry skin care as well as for decorative cosmetics like lipsticks. α-toc is also successfully applied for various skin diseases. It belongs to the group of lipid-soluble vitamins and is found in nature in many vegetable oils and plant tissues [84- 86].

Vitamin E is well known as a youth source and for the purpose of slow release α-tocopherol was applied to textiles resulting with the anti-ageing properties [87]. Microencapsulated vitamin E significantly increase the skin moisture and elasticity while reducing the skin wrinkles and roughness [86]. Previous successful research in the field of encapsulation of α-toc using the solvent removal method has been carried out [60]. Temperature and light sensitivity of α-toc is a reason why commercial products are prepared also like complex, e.g. with cyclodextrin [88, 89]. In that form it is stable and adequate for application on textiles enabling controlled release during the wearing of cosmetotextiles [90].

2.3 Textile substrate

Cosmetic preparations can be applied to all types of textiles: woven, knitted or nonwovens. Depending on the nature of the fibres used, the textile substrate can be biodegradable or non-biodegradable. Individual fibres can also contain biologically active substances, medical substances in their structure and active substances that can be covalently bonded to functional groups of the textile substrate involved. Nonwoven textiles manufactured by electrospinning have also been used, and offered the possibility of applying medical and cosmetic preparations for various biomedical and health-care applications [48].

When textiles with the ability of releasing active substance are considered, the prerequisite for their use is that preparations giving health-care or wellness properties have previously been applied to the fibres or fabric. Textiles can be treated with bioactive substances with adequate physical or chemical modifiers present, so as to improve and enhance covalent bonding of these substances to the textile. Generally, active substances are adsorbed, coated, encapsulated, or covalently conjugated onto the textile substrate [2, 12].

When using nonwoven textiles obtained by electrospinning of nano fibres, active substances can be added into the solution prior to spinning. Fibre diameter and orientation can be controlled by adjusting parameters of the electrospinning, e.g. electric potential, polymer solution flow, the distance between the drum and filament collector, with the aim of obtaining targeted mechanical properties and targeted rate and manner of releasing the active substance from the substrate [12].

2.4 Technology of bonding active substance to textile substrate

The earliest concept of microencapsulation appeared in 1930s, when the technique of spray-drying was for the first time used [55]. The technology of microencapsulation was used by NASA in early 1980s to control garment thermal properties, particularly for space suits. Phase-change materials (PCMs) were encapsulated, with the aim to reduce extreme temperature differences the astronauts were exposed to during their space missions [91]. The use of microencapsulation was not restricted to NASA but included all the parts of men's activities and needs [92, 93]. Microcapsules have been intensively investigated for the last 25 years in various areas: medical, bio-medical, agricultural, food industry, cosmetic and textile industry [31, 59, 94- 96].

Microencapsulation is a technique used to integrate some substances (in liquid, solid or gaseous state) within an membrane (envelope, wall, shell or capsule) in order to get a spherical shape product, of micro- or nanometre size. The membrane protects the active substance and their biological, functional and physicochemical properties within, i.e. the core, from outer influences. So, the microencapsulation technology is mostly used as a protective measure [31, 33, 97- 99]. The main advantage of microencapsulation technology is that it can protect the active substance from dangerous environmental influences such as oxidation, heat, acidity, alkalinity, moisture or evaporation [35, 37- 40, 55].

Grafting of microcapsules

Although covalent grafting of microcapsules is most often described, especially on natural fibres, the most often encountered type of bonding includes crosslinking agents (binders), specifically adapted for cosmetic and textile systems, with the accent at compatibility to the skin. A binder is required for the fixation of microcapsules on the textile. It forms the continuous film, adherent to the substrate, and holds the microcapsules on the textile [9]. Binders can be water-soluble polymers (e.g. starch and modified starches, carboxymethyl cellulose), synthetic latexes (e.g. styrene-butadiene, polyvinylacetate or acrylate latexes), aminoaldehyde resins polyacrylic, polyurethane-vinyl-acetates, polyurethanes, silica, etc. [9, 100]. Its function is to fix the microcapsules

in the textile preventing them to be released during the washing cycles. To bond microcapsules complexes or particles applied efficiently, a definite amount of binder is necessary, generally from 0.25 % to 6 % (of the dry substance to the fabric mass) in order to reduce their loss during the laundering. The rate of active substances release can be controlled by varying the amount and type of the binder used. Higher amounts of binder, covering the microcapsule completely, offer better protection from breaking in use and slow down the release of active substances onto the skin. Additionally, binders and some other additives can offer additional properties to the article of clothing involved, such as soil resistance and moisture resistance [12, 100, 101].

In a technique of microcapsules application to textile materials, microcapsules can be initially introduced in the textile material without a binder. A dispersant is introduced to disperse the microcapsules around and through the textile material, and thereafter the binder is added to promote the adhesion of microcapsules to the textile material. Secondly, microcapsules can be applied during the finishing process of textiles fabrication using a foulard in which the textile is impregnated by means of a finishing bath containing microcapsules, a softener and a self-crosslinking agent [9].

Electrospinning

Electrospinning is a simple but powerful process for making very thin polymer fibres [102]. At the end of the 1500s, Sir William Gilbert explained the behaviour of electrostatic and magnetic emanation. He found that by affecting the water droplet by electrostatic field, the water gets cone and hopper shape, and a droplet extrudes from the head of the hopper. This formed the first process of electro-spraying. Electrospinning can be viewed as a kind of electro-spraying. As with electro-spraying, the raw material of electrospinning is linked to a high-voltage power supply to enhance the liquid electrostatic potential [103].

Working conditions (solution, process and ambient parameters) are very important because each of those parameters can affect the fibre morphology. By proper control of those parameters, electrospun fibres with desired morphologies and diameters can be fabricated [104]. Nanofiber capsules including drugs to control drug delivery system can

also be prepared by electrospinning. Different kinds of artificial and natural polymers have been successfully electrospun into small and fine fibres. Many kinds of drugs such as antibiotics, anticancer, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) have been used with electrospun fibre [103]. In this research, electrospinning will be used with the aim to apply microcapsules on textiles.

Complexation

Complexation involves chemical bonding of two or more species, ions or molecules [105]. If it is employed in the area of cosmetotextiles, i.e. to bond a substance to a textile substrate, complexation usually deals with creating inclusive compounds, e.g. cyclodextrins with a high content of organic substances.

Cyclodextins are natural cyclic oligosaccharides created during the enzymatic decomposition of starch. The best known and available to the industry are the forms of α-, β- and γ- cyclodextrins, with 6, 7 and 8 D- glucoside units of hollow structure, possessing polar –OH groups and hydrophobic inner part. D- glucoside units are covalently bonded to carbon atoms C1 and C4 [15]. The diameter of the hollows varies from 0.5 to 0.85 nm [15, 106, 107]. These hollows can be used for placement of active substances. It is not necessary that the whole of the active substance is stored into the hollow to create a complex of the active substance (so called guest) and the hollow, i.e. cyclodextrin (so called host), a part of it is quite sufficient [108, 109].

β-CD is most often used on textiles, due to its simple manufacture, pronounced diameter of the hollow, low costs and simple manner of bonding to textile surface. Commercially available β–CD derivative is monochlorotriazine. Cyclodextrins are important auxiliaries, environmentally friendly, as they exhibit no toxicity and are completely biodegradable. Cyclodextrins can make inclusive compounds with a number of other organic substances. Textile fabrics can be also manufactured with new functional properties, with considerably reduced rate of active substance release [106, 107, 110].

These materials can captivate compounds such as body odours, using an inclusion complex, or can be used to release fragrances or deliver cosmetic substances onto the skin [107].

There are various ways of bonding CD to a textile surface. Physical methods consist of soluting CD derivative with hydrophobic chains in a polymer solution prior to fibre forming. CDs tend to migrate to the surface, forming hollows available for inclusions. Chemical methods include: (1) CD derivative synthesis with ionic side groups, which interact with ionic groups bonded to the fibre, and (2) synthesis of CD reactive derivatives that are split on the textiles with the help of a curing agent [6, 15, 111].

Complexes can be formed (created) using a bonding (curing) agent. Literature references indicate reactive polyurethanes as the most appropriate for this purpose, although, in practice, water-soluble polymers are often used as well, such as starch and modified starch, carboxy methyl cellulose, synthetic latex, styrene-butadiene, polyvinyl acetate or acrylic latex and aminoaldehyde resins [6, 12].

The other cyclodextrin-based approach is the application of cell-shaped molecules, consisting of six to eight glucose units, obtained by enzymatic decomposition of starch. They more readily create protective complexes with various molecules, including those that have a cosmetic effect, e.g. menthol, caffeine, or α-tocopherol (vitamin E) [12, 112]. The investigations of vitamin E stability, performed on cotton and viscose fabrics, showed that the stability of vitamin E functions was directly dependent on pH values of the bath in textile care. The highest stability was achieved when pH was <8, while the stability was not satisfactory with pH ≥8 [113].

Coating

Coating is one of the simplest methods for direct application of an active substance onto a textile surface. Coating can be done by immersing textiles into a solution containing the active substance, or by coating with micro/nano particles or capsules. The efficiency of active substance application will depend on the type of the fibres used and on the active substance used. For example, active substances with higher affinity will readily form a

thin layer on the surface of the polymer. Coated fabrics will quite often release considerable amount of active substance immediately after in vivo implantation. This disadvantage is quite detrimental if the goal is to release the active substance gradually, i.e. in a longer period of time. To avoid the problem, the textile is treated with a coating consisting of microcapsules and a bonding agent. This considerably reduces initial sudden release and freeing of the active substance, which depends exclusively on the nature of the micro/nanocapsule used. One of the possibilities in treating synthetic fibres (e.g. PA fibre) is to pre-coat the fibre with a solution of the active substance, with the process repeated to achieve controlled release of the active substance during a prolonged period of time. Another example is coating fibres with silver nanoparticles, with the aim of obtaining an antibacterial surface. Efficiency is improved by applying ultrasound and ionic irradiation to the fibres. Free radicals created on the fibre surface after the exposure to energy radiation form stable covalent bonds with silver nanoparticles, creating an effective antibacterial surface [12].

Coextrusion

Application of active substances during the fibre preparation is one of the most efficient methods of bonding active substance with textile. Such an approach utilizes a homogeneous polymer–active substance solution to fabricate fibres with suitable means. Hence both the active substance and polymer have to be dissolved in a common solvent. This approach allows the required amount of active substance loading and uniform active substance distribution throughout the fibre structure without any extra effort. In the case of poorly soluble active substance in the chosen solvent system for dissolving a polymer, active substance–polymer suspensions can be created by dispersing fine particles followed by agitation or exposing the system to ultrasonic waves. Several polymeric active substance loaded systems were created using non-degradable polymers such as polyethylene, polyurethanes and polyethylene vinyl acetate copolymers, and also biodegradable polymers including poly(lactic acid), poly(glycolic acid), poly(orthoesters) and poly(phosphazenes). The coextrusion of cosmetic substances, aloe vera and vitamin E, into fibres has been done for polyamide yarn [12].

2.5 **Active substances release**

After the active substance has been incorporated into the textile material, it is important to define and understand the mechanism of its release. Primary aim is sometimes to release the active substance completely at a particular moment, while sometimes it is supposed to be slowly released, so that the product retains its functionality as long as possible.

Controlled and gradual release of the active substance can also be initiated by mechanical actions (e.g. by friction), by soluting, biodegradation, diffusion, heat, changes in pH or by enzymatic activity. The selection of the mechanism and membrane of the microcapsule depend upon the end-use of the product, having in mind its physical and chemical stability, concentration, particle size, release mechanism and manufacturing costs. This is why it is possible to use microcapsules for various applications: in food industry, biomedicine, for pharmaceuticals, cosmetics, as well as in textile industry, agriculture and catalysis [32, 33].

Active substances diffuse through the microcapsule envelope at a particular rate (Figure 7). Release of the active substance can be classified according to some other mechanisms, such as erosion (the product is gradually dissolved in the membrane envelopes), diffusion (e.g. oil diffuses out of the system), extraction (mechanical forces in chewing or treatment of the increased oil surface) or fracture (system container fractures under the impact of mechanical or osmotic forces). Membrane fracture can be initiated in various manners: mechanically, by solution, by melting, thermally or UV/VIS irradiation, by biodegradation, enzymatic degradation or wall swelling [32].

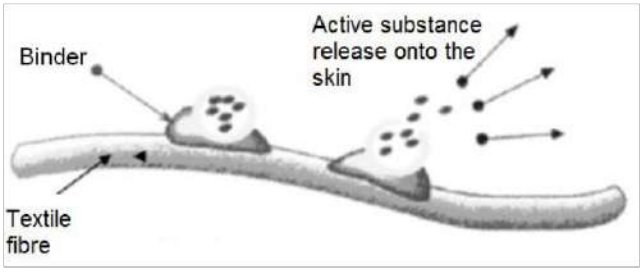


Figure 7 Diffusion and controlled release of active substance [32]

Some properties of the polymer network used, such as the chain, flexibility, mobility, sorption and desorption properties, plasticising degree or potential interaction between the polymer and the active substance involved, impact the rate of diffusion in the polymer matrix and the rate of releasing the active substance, i.e. osmosis. [16, 32, 48, 114, 115].

2.6 Efficiency of cosmetotextiles

Cosmetic product efficiency is defined by the amount of active substance necessary to achieve the target effect on the skin. For example, effects such as humidity and weight loss ask for a considerable amount of active substances on the skin to show measurable results. Fragrances, on the other hand, require a small amount of the active substance to achieve the targeted effect. They can have a prolonged effect, depending upon the microcapsule and textile construction. It is extremely complex task to prevent water-soluble substances from dissolving during washing. The application of products for regeneration of textiles, i.e. sprays containing active substances, cosmetotextile efficient performance can be prolonged. For example, cosmetotextiles with the weight-loss effect that is lost in wearing and washing can be renewed by the re-application of the active substances. Another aspect that should be taken into account is the cosmetotextile design. Design and construction of the textile substrate, garment design and finishing should be aligned to achieve maximum efficiency. Heavier textile substrate could offer prolonged and retained cosmetic effect for some 5 to 10 days of wearing, e.g. skin moisturising, if

hand washing or mild machine washing are employed [12]. Another example are cosmetotextiles offering weight-loss effect as a result of wearing/activity of adequate cosmetotextiles. This will probably exhibit good results if they are constructed as elastic tights with a particular degree of non-medical compression or as tight jeans. Such a construction offers good contact between the textile and problematic areas of the skin, probably also gives a massage effect and enables adequate transfer of the cosmetic substance from the textile substrate onto the skin [12].

Cosmetotextiles can be evaluated either subjectively or objectively when testing various effects. Chemical properties can be tested, together with toxicity, the presence of active substance, efficiency, scent analysis, durability or marking [1].

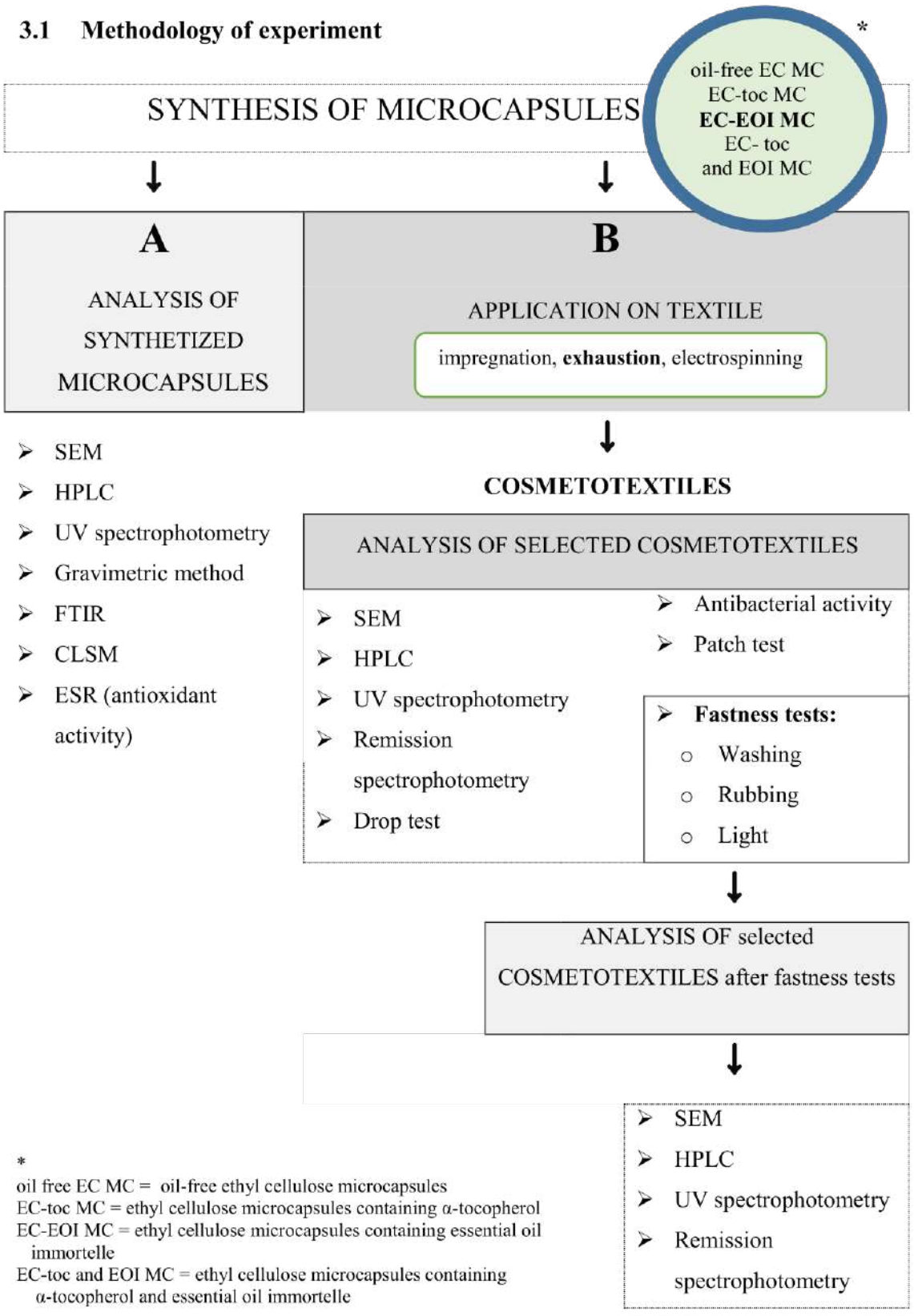
Objective methods of evaluating cosmetotextiles test the skin and include: corneometry (Corneometer®), test of skin hydration effect; *in vivo* optic technique of human skin geometry (FOITS, Dermatop®), used to test the effect of skin roughness and determine the changes at trans-epidermal water loss (Tewameter®), test skin barrier function. The effects such as cooling and weight-loss (anti-cellulite effect) are evaluated using subjective methods, such as testing the end-users through questionnaires and/or interviews [12].

Also, other tests of cosmetotextiles are objective but they are used for textile substrate: washing test, rubbing test and light test.

Washing test
• Washing test are very usually used for cosmetotextile analyse. Some of examples are: • Neroline-loaded microcapsules were impregnated on polyamide textile substrate and effect of cleaning cycles on impregnated textile substrates was studied, according to the ISO 105-C10 standard of 2010, in order to improve the lifetime of scent textiles [43]. • Vitamin E microcapsules were applied on dyed cotton fabrics and washing test was carried out according to the AATCC test method 61-1989 [112]. • Melamine formalin microcapsules containig mint flavour applied on cotton fabrics were subjected to washing test in accordance with ISO Standard 105 C01 [116].
Rubbing test
• Vitamin E microcapsules were applied on dyed cotton fabrics and rubbing test was carried out using a standard Crockmeter according to the AATCC test method 8-2005 [112]. • Melamine formalin microcapsules containig mint flavour applied on cotton fabrics were tested by rubbing procedure using a standardized Crockmeter [116]. • Microencapsulated Tea Tree oil were applied on footwear materials (leather and textile) and microcapsule rubbing fastness was performed according to the Standard EN ISO 105-X12:2003. The rubbing test was carried out on a CKA Crockmeter fastness tester (Augusto Miro´S.L.,Spain) [117].
Light test
• Vitamin E microcapsules were applied on dyed cotton fabrics and light test was evaluated according to the AATCC test method 16-2004 (option 3) using Xenon Test Chamber (Q-SUN, Xe-1-B, Q-Panel Lab Products, USA) after irradiation for 20 h [112].

3 METHODOLOGY

3.1 Methodology of experiment



*
oil free EC MC = oil-free ethyl cellulose microcapsules
EC-toc MC = ethyl cellulose microcapsules containing α-tocopherol
EC-EOI MC = ethyl cellulose microcapsules containing essential oil immortelle
EC-toc and EOI MC = ethyl cellulose microcapsules containing α-tocopherol and essential oil immortelle

3.2 Materials

Textile materials used in this thesis, for cosmetotextiles, were: cotton, modal, PES/cotton, viscose, silk and liocel (Table 6). Cotton is mostly used because of it's cost and availability, made of natural fibres and has good wearable properties. In general, it was fabric in plain wave; other material was liocel, which was a nonwoven fabric.

Table 6 Technical specification of textile materials

Textile material	Structure	Mass per unit area / gm^{-2}	Warp yarn density / cm^{-1}	Weft yarn density / cm^{-1}
Cotton	Fabric in plain weave	90.9	52	43
PES/cotton		170	27	27
Silk		90	30	23
Modal		143.8	36	30
Liocel	nonwoven	50	/	/

3.3 Chemicals

Chemicals for microcapsules synthesis

Reference of α-tocopherol (Merck 613420 | DL-α-Tocopherol – CAS No.: 10191-41-0 – Calbiochem, purity ≥ 98 %) was used as active ingredient for microcapsules synthesis.

Immortelle (*lat. Helichrysum italicum*) essential oil (EOI*) supplied by Croatian company Irex Aroma d.o.o. was used as active ingredient for microcapsules synthesis. Gass chromatography analyse of this oil is presented in *ANNEX III*.

Ethyl cellulose (EC) was applied as wall constituent during the preparation of microcapsules. It was purchased from Sigma-Aldrich, Austria (viscosity 4 cP, 5 % in toluene/ethanol 80 : 20, extent of labelling: 48 % etoxyl), CAS No.: 9004-57-3.

Other chemicals used for microcapsules synthesis were: ethyl acetate (EA) (used as solvent), CAS No.: 141-78-6, supplied by Prolabo and anionic surfactant - sodium dodecyl sulphate (SDS), CAS No.: 151-21-3 from Fluka.

Chemicals for application of microcapsules on textile

Binders used for this research were Tubicoat WLI and Helizarin Binder TW. Tubicoat WLI (soft acrylate) from Bezema AG, Schwitzerland, was used for fixation of EC microcapsules on selected textile materials by impregnation and exhaustion. Helizarin Binder TW (aqueous acrylic dispersion) from BASF, Germany, was used for fixation of EC microcapsules containing EOI (EC-EOI microcapsules) onto textile materials by impregnation.

Polyethylene oxide (PEO), Acros Organics, Belgium, was used for electrospinning for microcapsule application on textile, CAS No.: 25322-68-3.

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* When analyse was caryed out on EOI it was called „pure EOI“

Chemicals for testing

Chemicals used for High pressure liquid chromatography (HPLC) analyses were methanol and n-Hexane. They were used for isolation of active substances from microcapsules and cosmetotextiles. Methanol, HPLC grade, obtained from Lachner was used for HPLC analyse of α-toc n-Hexane, 95 % p.a., obtained from Kefo was used for HPLC analyse of EOI.

For antioxidant analyses of active substances α-toc, EOI and EC microcapsules 2,2-Diphenyl-1-picrylhydrazyl (DPPH) supplied by Sigma Aldrich were used.

For antioxidant analyses of cosmetotextiles ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), $K_2S_2O_8$ (potassium sulfate), CAS No.: 7727-21-1 and phosphate buffered saline supplied by Sigma Aldrich, were used.

Chemicals used for *Drop test* were: Iron (III) chloride ($FeCl_3$) supplied by Gram-mol d.o.o., Croatia, 2-propanol supplied by VWR chemicals and 2,2'bipiridine supplied by Kemika d.o.o., Croatia.

For washing, the ECE non phosphate reference detergent (A) without optical brightening agent (ECE A) supplied by James Heal was used.

3.4 Methods and devices

Microcapsules synthesis

Two different techniques of mixing: stirring and ultrasound were used for microcapsules synthesis. Used devices were:

- Laboratory stirrer (Schott SLR, GmbH), for synthesis of EC microcapsules with all used active substances: α-toc, EOI and mix of α-toc and EOI. Speed of stirrer was 400 rpm in 10 min period (stirring technique).
- Vibra-CellTM Ultrasonic Liquid Processor VCX 500/VCX 750 controlled with On/Off pulser, used in synthesis of EC-EOI microcapsules.

This ultrasound device enabling safe treatment of the temperature-sensitive samples at high intensity thus providing uniform mixing allowing the sample to settle back under the probe after each burst [118]. On/Off Pulser (2s on/ 1s off) provided amplitude of 60 %. Time of ultrasound treatment was 5 min. Additionally, ice was used to adjust and to stabilize temperature during preparation (ultrasound technique).

Microcapsules application on textile

For microcapsules application on textile three different application techniques were used: impregnation, exhaustion and electrospinning. Used devices were:

- Laboratory Foulard, Konrad Peter Laboratory, Swiss, located at Faculty of Textile Technology (TTF), Zagreb, Department of Textile Chemistry and Ecology, used for EC microcapsules impregnation on textile.
- Polycolor, Mathis, located at TTF, used for application of microcapsules on textile by exhaustion, as well as for washing cycles and dyeing with natural dye Cochineal.
- NanoSpider NS LAB 500 (Elmarco) is a device for microcapsules application on textile materials (modal and cotton) by electrospinning. Voltage of device was set to 60 kV. 5 % solution of PEO (polyethylene oxide), minimum volume of 30 ml

is required for spinning. Device were applied during the CEEPUS mobility in Maribor, Slovenia, Faculty of Mechanical Engineering.

Microcapsules and cosmetotextiles analyses

After synthesis of microcapsules and their application on textiles all samples were analysed using qualitative and quantitative tests, morphology analyse (size and shape of the microcapsules), efficiency (dermatology test, antioxidant analyse and antibacterial activity) and fastness (on washing, rubbing and light). Devices and parameters used in this research are listed below.

SEM

Field Emission Scanning Electron Microscope (SEM) FE-SEM//Mira, Tescan, Czech Republic, was used for surface characterization of EC microcapsules and cosmetotextiles. SEM microscope was operated at 5 - 10 kV and various magnification levels. The samples were placed on the SEM stubs (10 mm) using a two-sided adhesive tape. Prior to the SEM measurements, samples were sputter-coated with palladium/gold /chromium alloy before the scanning in order to increase their electrical conductivity.

HPLC

High Performance Liquid Chromatography (HPLC) Agilent chromatographic system, series 1220 Infinity LC (USA), was applied. The Agilent Open LAB CDS ChemStation Edition software with selection of UV detector was used. Column packed with 4 μm Poroshell 120, EC-C18 (4.6 x 250 mm) in a reverse phase was applied. Response (peak area) was expressed in mili absorption unit per second (mAU*s) and intensity of absorbance in mili absorption unit (mAU). HPLC was used for qualitative and quantitative analyse of active substances α-toc and EOI before and after synthesis in EC microcapsules. Preliminary, it was necessary to optimise the procedure of HPLC analyses for α-toc and for EOI.

UV-VIS spectrophotometer

Ultraviolet-visible (UV-VIS) spectrophotometer, Spectrophotometer Cary 50 Solascreen (Varian Inc, USA), was used for qualitative and quantitative spectrophotometric analysis of EC-toc microcapsules, qualitative and quantitative analyses of EC-EOI microcapsule in ultra violet range (UV).

Device is computer controlled via software packages "Analysis" and "Solascreen". The results are computer recorded namely with numbers if there is specific wavelength and with curve and numbers for concerned range of wavelengths. At solution measuring, optical cuvette line is 1 cm. Also, protective characteristics of the fabrics from ultraviolet irradiation (UPF) at range of 280 - 400 nm were measured [119].

Antioxidant activity of cosmetotextiles using ABTS method was tested using Spectrophotometer Carry 60 (Agilent Technologies) during CEEPUS mobility in Maribor, Faculty of Mechanical Engineering.

FTIR

Fourier transform infrared spectrophotometer (FTIR) Perkin Elmer 100, with ATR (Attenuated Total Reflectance) was applied for qualitative analyse of EC-toc microcapsules measuring α-toc standard, ethyl cellulose (EC) and synthetized EC-toc microcapsules. Measurement conditions included a resolution of 16 cm^{-1}, number of scans was 8 and pressure of ATR head was 125. Technique was applied for the spectroscopic transmission analysis over the wave number range of 4.000 – 400 cm^{-1}. The evaluation and comparison of measured samples was performed by spectrogram curves.

CLSM

Confocal laser scanning microscopy (CLSM), Leica TCS SP5 II equipped with a LAS AF imaging software (Leica Microsystems, Germany), located at Faculty of Medicine University of Maribor was used to analyse EC-EOI microcapsules qualitatively and oil-free EC microcapsules. Prior to the CLSM analysis, EC microcapsules were dyed with the fluorescein isothiocyanate isomer I (≥ 90 %, HPLC grade, Sigma Aldrich, Austria)

with characteristic wavelengths: excitation λ_{max} = 490 nm, emission: λ_{max} = 525 nm. The assumption is that only oil-containing microcapsules can be dyed.

ESR measurements of radical-scavenging activity

Electron spin resonance (ESR) measurements of radical-scavenging activity (antioxidant activity) were performed at room temperature (22 °C) using a Varian E-109 spectrometer equipped with a Bruker ER 041 XG microwave bridge, located at Ruđer Bošković Institute, Zagreb. The spectroscopic parameters were: frequency 9.27 GHz, field sweep 10 mT, microwave power 4.9 mW and modulation amplitude 0.10 mT for all measurements. Spectra were accumulated and analyzed using EW (EPRWare) Scientific Software.

According to the literature α-toc and EOI are great antioxidants [58, 120]. Antioxidant activity of active substances (α-toc and EOI), oil-free EC microcapsules, EC-toc microcapsules, EC-EOI microcapsules and EC-toc and EOI microcapsules were analysed using radical-scavenging activity (ESR method).

Antioxidant activity was monitored by measuring the relative radical concentration of freshly prepared 0.15 mM ethanol solution of free radical 2,2-diphenyl-picrylhydrazyl (DPPH) by electron spin resonance (ESR) for 1-20 min.

DPPH method is an assay for scavenging activity against free radicals. The scavenging activity of natural products can also be monitored using UV-VIS spectrophotometry by measuring the decrease in absorbance at 517 nm of the stable free radical DPPH [121-123]. The purple-coloured free radical reacts with scavenger to yield the yellow product, the reduced form of 2,2-diphenyl-picrylhydrazyl (Figure 8). The advantage of the ESR method is that it allows direct and accurate measurement of the concentration (number) of radicals, regardless of colour, transparency and aggregation state of the sample.

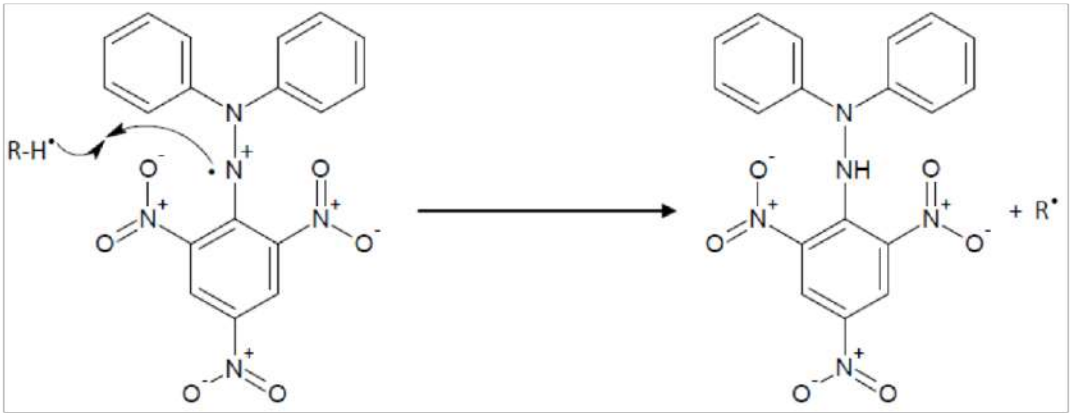


Figure 8 Free radical reaction with scavenger to yield the yellow product of 2,2-diphenyl-picrylhydrazyl [121]

Preparation of DPPH radical

DPPH solution was prepared by dissolving 3 mg of DPPH powder in ethanol (96 %), in a 50 ml volumetric flask. The mixture was then stirred for 15 minutes on a magnetic stirrer until DPPH was completely dissolved.

Sample preparation

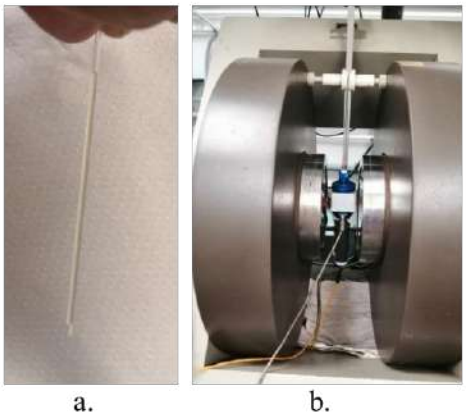
The chosen concentrations of active substances in EC microcapsules were 51.4 %. Therefore, the samples of active substances α-toc and EOI were prepared in lower concentration then samples of microcapsules. More precisely, the amount of the active components inside EC microcapsules was chosen to be equal to the amount of the pure active substances. This fact makes it possible to compare directly the antioxidant potential of both, pure active components and MC containing active components.

The exact composition of investigated samples is given in Table 7.

Table 7 Composition of the samples prepared for ESR measurements

sample	amount of sample	0.15 mM DPPH / ml	γ / mg/ml
active substances α-toc	0.0425 g	5.00	8.50
active substance EOI	0.089 g	5.00	17.80
oil-free EC microcapsules	0.085 g	2.45	34.69
EC-toc microcapsules	0.085 g	2.45	34.69
EC-EOI microcapsules	0.085 g	2.45	34.69
EC-toc and EOI microcapsules	0.085 g	2.45	34.69

Selected concentrations of samples were mixed on Vortex for 3 s before starting the first measurement. Afterward, part of the sample was taken with a capillary (Figure 9 a), the bottom of the capillary was plugged with clay and placed in a quartz glass ESR tube and put into magnetic field of the spectrometer (Figure 9 b).



a. b.

Figure 9 Sample preparation for ESR measurements: a. capillary containing a sample preparared for ESR measurements; b. sample placed in the magetic field of the spectrometer

Sample measurement

Two active substances (standard α-toc and EOI) and four microcapsule samples (oil-free microcapsules, EC-toc microcapsules, EC-EOI microcapsules and microcapsules containing a combination of α-toc and EOI) were measured. Sample solutions were quickly mixed in the test tubes and immediately put into capillaries that were placed in standard ESR tubes. ESR spectra were recorded during 30 min, starting from the first minute after the oil/ EC microcapsules were put in contact with DPPH solution. Recording intervals were 1 min during the first 10 min of the reaction and 2 min during the rest of the measuring process. The signal intensities of DPPH free radical were calculated by the double integration of ESR spectra, using the EW (EPRWare) Scientific Software Service program and expressed in arbitrary units. The signal intensity of the pure 0.15 mM DPPH solution, measured immediately before starting the sample measurement, was taken as the reference signal intensity, i.e. signal intensity of the sample (I_0) for the reaction time zero ($t = 0$ min). The rest of the signal intensity (ΔI) after the reaction time t, expressed in %, was calculated as:

$$\Delta I = 100 - \frac{I_0 - I}{I_0} \cdot 100\,, \qquad (1)$$

where:

I_0 – signal intensity of DPPH at t = 0 min, before the addition of oil / EC microcapsules

I - signal intensity of DPPH in a sample solution measured at time t.

Remission spectrophotometer

Remission spectrophotometer, Datacolor Spectraflash type SF 300 (Datacolor, Switzerland) is software guided. This remission spectrophotometer is used for measurement of colour parameters of textiles, plastic, metal or paper substrates; measurement of whiteness and fluorescent colours.

Due to drying and thermal condensation conditions that can cause yellowing of textile materials, whiteness quality was performed by spectral measurements under the following conditions: the aperture size of 20 mm, the standard illuminate D65, at the wavelength range from 360 to 700 nm with excluded speculum. Whiteness quality of all set of cotton samples was estimated by parameters, whiteness degree (W_{CIE}), basic whiteness (Y), tint value (TV) and tint deviation (TD).

Gravimetric method

The gravimetric method was used to analyse the amount of active substances in the synthesis and to calculate the residual amount, expressed in grams (g). It was calculated as:.

$$m_{(MC)} = m_A - m_B \, , \tag{2}$$

where:

$m_{(MC)}$ - mass of synthesized microcapsules / g

m_A - mass of filter paper/cosmetotextile after synthesis/application on textile / g

m_B - mass of filter paper/cosmetotextile before synthesis/application on textile /g

Crockmeter

Crockmeter for testing textile colourfastness to rubbing, American Association of Textile Chemist & Colorist (AATCC), Lowell Textile Institute, was used for rubbing fasteness test.

Xenotest

Xenotest 440 (SDL Atlas) is used for laboratory simulation of external weather influences on the stability and durability of textile and other materials.

3.5 Application procedures

The microcapsules can be applied by impregnation, exhaustion, electrospinning, spraying, coating, screen-printing techniques etc. or by direct incorporation in the fibre without modifying its touch and colour [9, 100]. A binder is required for all these techniques [57]. In this research, EC microcapsules with active substance (α-toc or EOI) were applied on textile using three different techniques: impregnation, exhaustion and electrospinning.

Impregnation was used for application EC-toc microcapsules on: cotton fabric, PES/cotton, silk and liocel. Also, impregnation was used for application EC-EIO microcapsules on cotton and modal fabrics. Impregnation bath for application EC microcapsules on textile materials contained: EC microcapsules, SDS, binder and deionised water.

Second type of application EC-EOI microcapsules was **exhaustion** on Polycolor, Mathis. Apparatus was used for the application of EC-toc microcapsules on cotton fabric and EC-EOI microcapsules on white and dyed cotton fabric.

Third type of application on textile (used for EC-EOI microcapsules) was **electrospinning** with NanoSpider NS LAB 500 device. Application was on modal fabric. Electrospinning bath was based on PEO, EC microcapsules and water (in same cases).

Impregnation

Application of EC-toc microcapsules on textile by impregnation

EC-toc microcapsules were applied on: cotton fabric, PES/cotton fabric, silk fabric, modal fabric and liocel nonwoven textile (Table 6) by impregnation. Materials was cut on 10x3 size and treated with application bath (Table 8). Treated materials were air dried.

Table 8 Application bath for impregnation

EC-toc microcapsules (E-2) / g	1.898
SDS /g	1.303
Tubicoat WLI /g	0.998
Water /ml	50
pH	7-8

Application of EC-EOI microcapsules on textile by impregnation

For application of EC-EOI microcapsules synthetized by "stirring" (A) and "ultrasound" (B) cotton and modal fabric was used (Table 6). Fabrics was cut on 20 x 20 cm size and treated with two different baths for application.

First were treated with 15 ml of oil-free EC microcapsules (0.0 g) and second were treated with 15 ml of EC-EOI microcapsules (0.2 g). That volume of microcapsules was added in 200 ml of deionized water, together with the 0.3 g of anionic surfactant sodium dodecyl sulphate (SDS) and 1 g of binder (Helizarin Binder TW).

The impregnation (foulard, wet pick-up of approx. 100 %) was used to apply microcapsules onto textiles. Treated samples were finally dried at ambient temperature and prepared for further analysis.

Exhaustion

Exhaustion was used for application of EC-toc microcapsules and EC-EOI microcapsules on cotton fabric (Table 9). EC microcapsules were synthetized in 1:3 amount rations. Three different concentrations of EC microcapsules were applied on cotton fabric: 4, 8 and 12 % on mass of a material. Concentration of SDS was the same like the concentration of EC microcapsules. Concentration of binder Tubicoat WLI was 5.6 % on mass of material [101]. Liquid ratio was 1:50. Temperature during the treatment was 30 °C in 60 min period.

Table 9 Application bath for exhaustion

Application bath	EC microcapsules	c of EC microcapsules / %	c of SDS / %	c of binder / %
ex_e-I	α-toc (e)	4	4	5.6
ex_e-II		8	8	
ex_e-III		12	12	
ex_S-I	EOI (S)	4	4	
ex_S-II		8	8	
ex_S-III		12	12	

Electrospinning

EC-EOI microcapsules were applied on modal fabrics (Table 6) by electrospinning using device NanoSpider NS LAB 500 (Elmarco). Voltage of device was set to 60 kV. Modal fabric, dimensions 56.0 x 49.7 cm, was treated with solution for electrospinning (5 % Polyethylene oxide (PEO) solution and EC-EOI microcapsules). Microcapsules used for this part of research was EC-EOI microcapsules (m_2). Application bath No. I contained only 5 % PEO solution, application bath No. II contained 5 % PEO solution and EC microcapsules in solid state and application bath No. III contained 5 % PEO solution and EC microcapsules in solution (0.15 g EC microcapsules). The values of the composition are presented in Table 10.

Table 10 Application baths for electrospinning

No.	Composition		Material
I	5 % PEO solution	100 ml	modal (M_I)
	EC microcapsules	-	
II	5 % PEO solution	30 ml	modal (M_II)
	EC microcapsules - solid[1]	0.080 g	
III	5 % PEO solution	50 ml	modal (M_III)
	EC microcapsules - solution[2]	50 ml (0.15 g)	

[1] mass of oil in synthesis = A0.2g dry

[2] mass of oil in synthesis = A0.2g

Analyses on bath for electrospinning application were performed: using conductivity method with SevenMulti pH Conductivity Meter (Mettler Toledo) and viscosity method with rotational viscometer (Fungilab) on University of Maribor.

Before analyses of cosmetotextiles it was necessary to analyse application baths used for electrospinning for application EC-EOI microcapsules.

Conductivity and viscosity of bath for electrospinning application were tested and results are presented in Table 11. Application bath No. III contained water in solution what was reflected with high conductivity and low viscosity in comparison with application bath No. II which contained EC microcapsules in solid state and application bath No. I which contained only PEO solution.

Table 11 Analyse of application baths for electrospinning on modal fabric (M)

Application	Sample		Conductivity / μS/cm	Viscosity / mPas	Time of electrospinning / s
I	modal	M_I	63.2	7080.6	240
II		M_II	55.6	8239.2	300
III		M_III	167.4	114.8	15

Time of electrospinning depends on application baths composition. The longer the time of electrospinning is, the procedure is more successful. So, it was obtained that application bath for electrospinning (No. III) which contain water in solution wasn't efficient enough.

3.6 Finishing procedures

Natural dye Cochineal (Naturex, Carmine powder), from Brenntag Croatia d.o.o. was used for selected cotton fabrics dyeing, Table 12.

One part of cotton fabrics (D1 and D3) were dyed with natural dye Cochineal. Concentration of dye used was 10 % on the mass of textile material. Sodium chloride was used as electrolyte in concentration of 40 g/l. Acetic acid (10 %) was used for pH adjustment to (pH 3). Dyeing of fabrics D1 and D3 with natural dye Cochineal was performed in a laboratory type dyeing machine Polycolor, Mahtis, by exhaustion and with material to liquor ratio of 1:30 at 90 °C for 60 min. After dyeing process, the fabrics were thoroughly washed in cold water followed by hot soaping, cold wash and dried on air.

Additionally, fabric D3 was applied with EC-EOI microcapsules with EOI (m_2). Fabric D2 was also treated with EC-EOI microcapsules (m_2) but not dyed before. Treated fabrics (with microcapsules or/and dyed) presented in Table 12 were tested by Patch test.

Table 12 Treated fabrics

Finishing	Cotton fabric		
	D1	D2	D3
dyestuff	+	-	+
microcapsules	-	+	+

EC-EOI microcapsules were applied on fabrics D2 and D3 by exhaustion, described in Methodology part, *page 50*. Concentration of microcapsules and SDS were 8 % on mass of material. Concentration of binder Tubicoat WLI was 5.6 % on mass of material [101]. Liquid ratio was 1:50. Temperature during the treatment was 30 °C and duration 60 min. After the application of EC-EOI microcapsules cotton fabrics were dried on air. Cotton

fabrics D1, D2 and D3 were cut, placed in sterilised plastic bottle and delivered to Clinical Hospital Centre Zagreb (KBC Zagreb) for Patch test. Before testing, all patients previously signed an informed consent approved by the Ethics Commission of KBC Zagreb.

3.7 Cosmetotextiles analyses

Cosmetotextiles were analysed using previously optimized methods on pure substances and synthetized microcapsules: microscopic method (SEM), HPLC analyses, spectrophotometry methods (UV-VIS and Remission), Drop test, ABTS method (antioxidant activity), antibacterial activity and dermatology test (Patch test). Fastness of selected cosmetotextiles was tested using: washing, rubbing and light fastness tests. After fastness tests quantification of active substances were carried out using HPLC (α-toc) and UV spectrophotometer (EOI).

Scanning electron microscopy (SEM) was used for morphological analyse of cosmetotextiles. SEM microscope was operated at 10 kV and various magnification levels due to the need to obtain an SEM image of optimal quality. The samples were placed on the SEM stubs (10 mm) using a two-sided adhesive tape. Prior to the SEM measurements samples were sputter-coated with palladium/gold/chromium alloy before the scanning in order to increase their electrical conductivity.

HPLC

HPLC is a key instrument to quantify α-toc on different materials, so it was used for analyse of active substances in cosmetotextiles in this research. European standard "Foodstuffs – Determination of vitamin E by high performance liquid chromatography – Measurement of α-, ß-, γ- and δ-tocopherol" (EN 12822:2014) was followed for the quantification of α-toc from cosmetotextiles in this research [124, 125]. Before the HPLC analyses it was necessary to isolate active substance from the cosmetotextile. Based on the previous research, insulation of α-toc by mixing on a vortex mixer was chosen as a synergy and balance of the tested insulation methods [126].

Sample analyse

In 5 ml methanol, 0.05 g of **cosmetotextiles with EC-toc microcapsules** (E-2) applied by exhaustion in three different concentration (Table 9) was cut into pieces of approximately 2 x 2 mm and mixed. For isolation Vortex was used (1900 rpm, in period

of 60 s, filtrated with PTFE filter). Prepared samples were analysed using HPLC method for α-toc analysis (***HPLC 1).*** Parameters of HPLC 1 analysis were: injection volume 100 µl, 35 °C ± 0.8 °C, mobile phases ratio v/v = 97/3 ($MeOH/H_2O$) and flow rate 1.8 ml/min. Retention time for α-toc was 9.4 - 9.9 min. UV detector for α-toc was set at 292 nm and the duration of analyses was 15 min.

Using the same HPLC method cosmetotextiles were analysed after light fastness using Xenotest 440.

In 5 ml n-Hexan, 0.05 g of **cosmetotextiles with EC-EOI microcapsules** (m-2) applied by exhaustion in three different concentration (Table 9) were cut into pieces of approximately 2 x 2 mm and mixed. For isolation Vortex mixer was used (1900 rpm, in period of 60 s, filtrated with PTFE filter). Prepared samples were analysed using HPLC method for EOI analysis (***HPLC 2).*** Parameters of HPLC 2 analysis were: injection volume 100 µl, 25 °C ± 0.8 °C, mobile phases ratio v/v = 97/3 ($MeOH/H_2O$) and flow rate 0.5 ml/min. Retention time for α-toc was 15.6 - 15.8 min. UV detector for EOI was set at wavelength of 265 nm and the duration of analyses was 20 min.

UV/VIS Spectrophotometry

Qualitative and quantitative analyse of EOI on cosmetotextiles

Spectrophotometric analysis of cosmetotextiles with EC-EOI microcapsules was with Spectrophotometer Cary 50 Solascreen. Cosmetotextiles with EC-EOI microcapsules was measured at 265 nm using software *Simple reads*.

Before analysis of active substance (EOI) it was extracted from 0.05 g cosmetotextile (cuted into small pieces) using 5 ml appropriate solvent (n-Hexane), mixed on vortex 60 s, 1600 rpm and then measured. After washing, rubbing and light tests fastness cosmetotextiles were analysed by the same procedure.

Antioxidant activity (ABTS method)

Antioxidant activity of cosmetotextiles with α-toc and cosmetotextiles with EOI were analysed on UV-VIS Spectrophotometer Carry 60 (Agilent Technologies) located at Maribor, Faculty of Mechanical Engineering. ABTS method was used for antioxidant activity analyse. Measurement of antioxidancy with the ABTS method is based on spectrophotometric measurements. The ABTS chromophore is blue-green in colour and is formed by the reaction between prepared 7 mM ABTS (38.4 mg) and 2.45 mM $K_2S_2O_8$ (potassium sulphate) (6.6 mg) in 10 ml water. A cationic radical is formed which has a maximum absorption at wavelengths of 645 nm, 734 nm and 815 nm.

Prior to the measurements was necessary to prepare PBS buffer (dissolve 1 tablet in 200 ml of water) which serves as a blank.

Textile samples were prepared in 0.1 g and placed in test tube. 3.9 ml of prepared ABTS solution was added. The addition of antioxidants (samples) to the cationic radical ABTS reduces the radical content and causes a change in the colour of the solution. Absorption was initially measured at 734 nm to determine the initial absorption. Absorbance was measured again after 15 and 60 min. As a result of measurements, the percentage of inhibition (IC) was calculated, which reveals how much free radicals were reduced in contact with the antioxidant:

$$IC = \frac{A_{initial} \times A_{end}}{A_{initial}} \times 100 \qquad (3)$$

IC – percentage of inhibition after 15 / 60 min / %,

$A_{initial}$ – initial absorption at 0 min,

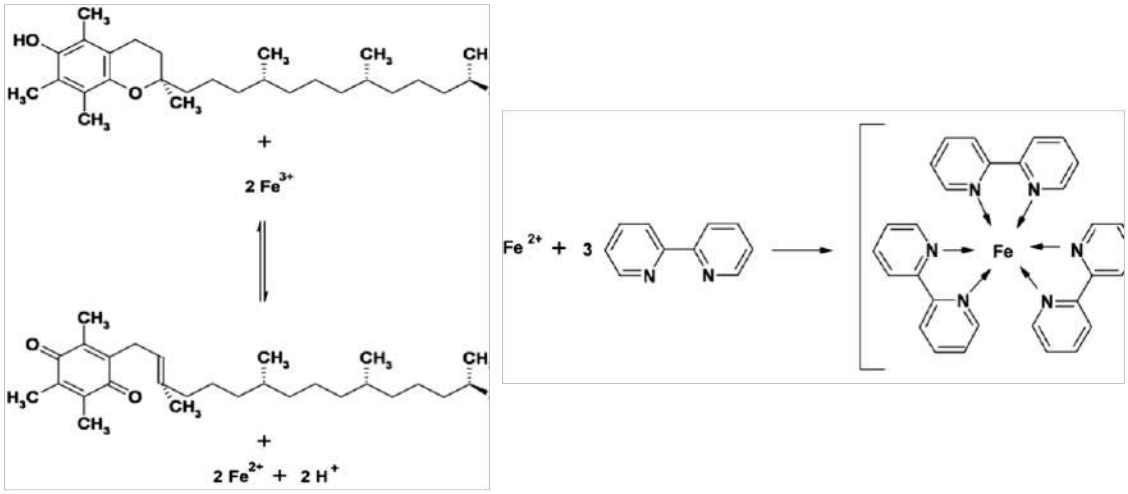
A_{end} – absorption after 15/60 min.

Remission spectrophotometry

Remission spectrophotometry was used for cosmetotextile analyses of whiteness under the conditions described on *page 46*.

Drop test

Drop test was one of the preliminary test methods for fast identification of α-toc [125, 127- 129]. This qualitative test is based on a redox reaction between α-toc and iron (III)-chloride (solution A) reduced to iron (II), whereas α- tocopherol is oxidised to tocoquinone (Figure 10 a). After addition of the dipyridyl solution (solution B), the iron (II) ions form a red coloured metal organic chelate complex with dipyridyl (Figure 10 b) [11, 88, 130]. Identification of α-toc on cosmetotextiles with EC-toc microcapsules was performed by drop test as one of the fast preliminary test methods [62, 125, 128].



a. b.

Figure 10 Reaction mechanism: a. redox and b. complexation [127]

Preparation of solution A:

In 5 ml volumetric flask was added 50 mg $FeCl_3$ and 4.5 ml of 2-propanol and then stirred until the salt was completely dissolved. After dissolution, flask was filled up to the mark with 2-propanol.

Preparation of solution B:

In 5 ml volumetric flask was added 100 mg 2,2´-bipiridine and 4,5 ml 2-propanol and then stirred until the salt was completely dissolved. After dissolution, flask was filled up to the mark with 2-propanol.

For determination of α-toc the fabric was processed in a way that successively, one after the other, at the same place pipette dropped in this order:

1. 2 drops of distilled water
2. 2 drops of solution A
3. 2 drops of solution B.

Within a maximum of 10 seconds (no longer) the colour hue changes from yellow to red if α-toc is present on the textile. The colour hue remains yellow if α-toc is not present. It is important to estimate the colour hue within 10 seconds. It is recommended to perform this analysis on untreated material for the control.

Antibacterial activity

According to the literature [120, 131] EOI possess antibacterial activity and there for cosmetotextiles with EC-EOI microcapsules were tested in this research. Analyses were performed at KBC Zagreb using AATCC Test Method 147-2004 (Antibacterial activity assessment of textile materials: Parallel streak method) [132]. This test method was used for the qualitative evaluation of the antibacterial effect of lubricating antimicrobial agents on treated textile materials with some active substance. In other words, this test method determines the ability of the textile to prevent the growth of microorganisms and to be antibacterial. Three types of bacteria were used for the analyses: *Klebsiella pneumonia,*

gram-negative bacteria (ATCC 700603), *Acinetobacter baumannii,* gram-negative bacteria (hospital strain) and *Staphylococcus aureus,* gram-positive bacteria (ATCC 29213).

Cotton cosmetotextiles with three different concentrations of **EC-EOI microcapsules** applied using **exhaustion** were analysed (ex_S-I, ex_S-II and ex_S-III) (Table 9). All samples were analysed in triplicate.

Dermatology test - Patch test

Patch test was used for the analysis of dermatologic (alergologic) impact of cosmetotextiles with EC-EOI microcapsules (m_2) in concentration of 8 % on mass of material and textile materials dyed with natural dye Cochineal, described in *Methodology, chapter: "3.2. Materials", page 36*.

Procedure of performing of Patch test:

- The test was performed on the skin of the back (e.g. on the upper arm or thighs) where allergen patches are applied;
- Allergens were removed after 2 days;
- Test reading was performed at: second, third or fourth and 7th day;
- The test was read after 48 and 72 hours. Criteria for the reading the Patch test is presented in Table 13;
- In case of a positive reaction to a particular allergen, edema occurs at the site of contact and redness with small bubbles filled with clear liquid occurs.

Table 13 Criteria for assessment the Patch test [133, 134]

Symbol	Description
„-„	no reaction = negative reaction
"? +"	mild erythema = possible reaction
"+"	erythema, infiltration, papules = weakly positive reaction
"++"	erythema, papules, edema, vesicles = very positive reaction
"+++"	erythema, edema, vesicles, blisters, erosions, exudation = extreme positive reaction
IR	irritant reaction

Cotton fabrics D1, D2 and D3 (Table 12) were tested on 50 patients aged 9 to 69 years (37 female - F and 13 male - M) (Table 14), who were referred to the Allergology Clinic of the Clinic for Dermatovenerology of the Clinical Hospital Center Zagreb and the Medical Faculty of the University of Zagreb due to the diagnosis of contact dermatitis. Patch test for the basic series of allergens was indicated.

Table 14 Information about tested patients (gender F/M and year of birth)

No.	Gender	Year of birth	No.	Gender	Year of birth	No.	Gender	Year of birth
1	F	1956.	18	F	1960.	35	F	1988.
2	M	1954.	19	F	1965.	36	M	1963.
3	F	1966.	20	M	1957.	37	F	1952.
4	F	1954.	21	M	1957.	38	M	1959.
5	F	1991.	22	F	1977.	39	F	1960.
6	F	1958.	23	F	1950.	40	F	1974.
7	F	1958.	24	F	1999.	41	M	2009.
8	F	1963.	25	F	1987.	42	M	2002.
9	F	1982.	26	F	1978.	43	F	1979.
10	F	2010.	27	F	1977.	44	F	1957.
11	F	1965.	28	F	1968.	45	F	1956.
12	M	1954.	29	F	1995.	46	M	1979.
13	F	1988.	30	F	1966.	47	F	1988.
14	F	1962.	31	F	1991.	48	F	1988.
15	M	1988.	32	F	1984.	49	F	1989.
16	F	1983.	33	M	1970.	50	F	1976.
17	M	1980.	34	M	1981.			

Washing fastness

The washing fastness of cotton cosmetotextiles (cosmetotextiles with EC-EOI microcapsule) was tested with standard detergent ECE A in the concentration of 2.5 g/l through 10 cycles at 40 °C. The washing bath ratio was 1:50. Textile materials were rinsed four times with distilled water and dried at the ambient temperature.

Rubbing fastness

Rubbing fastness of cotton cosmetotextiles (containing EC microcapsule with EOI) was tested using a standard Crockmeter. Tanned crust beef leather from Psunj d.o.o. was used in this test. Leather was not dyed in posttanning process and didn't have any finish layer on grain side (pigment, varnish, etc.) and represented human skin.

Cosmetotextile was placed on the bottom, and it was fixed on Crockmeter. Leather was placed on mobile part of Crockmeter (Figure 11). 10 dry rubbing cycles on the materials per cosmetotextile were done, on five parallel samples.

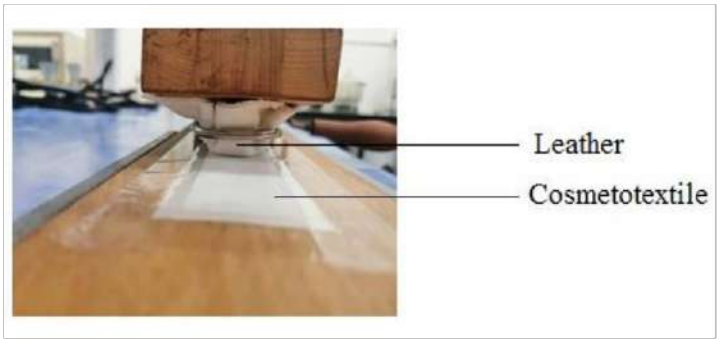


Figure 11 Testing rubbing with Crockmeter

Light fastness

Light fastness was evaluated according to the modified ISO 105-B02 and 13 B04 test methods using Xenotest 440. It was used for analysing cosmetotextiles with different concentration of EC-toc microcapsules and for cosmetotextiles with different concentration of EC-EOI microcapsules. Test conditions simulated in this research were presented in Table 15.

Table 15 Test conditions used for analyse with Xenotest

Condition	Amount
Total light time / h	41:10
Radiant exposure / kJ/m^2	6226
Irradiance control / nm	300 – 400
Filter system	B04
E / W/m^2 (± 2 W/m^2)	42
CHT / °C (± 3 °C)	32
BST / °C (± 8 °C)	47
RH / % (± 8 %)	40
spray	-
fan speed / rpm	2000

Fastness rating for washing, rubbing and light were assessed by a whiteness quality using a remission spectrophotometer. Qualitative and quantitative analyse before and after fastness tests was carried out with HPLC and UV spectrophotometry.

4 RESULTS AND DISCUSSION

A. Results of EC microcapsules analysis

The results of EC microcapsules synthesis and their optimisation are presented in this section.

Synthesis of **EC-toc microcapsules** and synthesis optimisation of this microcapsules are described. Microcapsules were analysed using SEM, FTIR, UV spectrophotometry, HPLC and ESR analyses. Drop test and FTIR was used for qualitative analyse of EC-toc microcapsules. By FTIR technique, can be also compared synthetized microcapsules with other constituents in synthesis. UV spectrophotometry and HPLC was used for qualitative and quantitative analyse of synthetized microcapsules. ESR method was used for antioxidant activity of active substances.

Synthesis of **EC-EOI microcapsules** and synthesis optimisation are described. Microcapsules were analysed using confocal microscope for oil detection in EC microcapsules and for analysing differences in size using different mixing techniques through synthesis. Gravimetric method was used for analyse of EOI amount in synthesis (Stirring technique) and for calculating residual amount. UV spectrophotometry was used for qualitative and quantitative analyse of EC-EOI microcapsules and for utilization of EOI in microcapsules synthesis. Synthetized EC microcapsules with different mass of oil was morphologically analysed using SEM. HPLC was used for qualitative and quantitative analyses of microcapsules and ESR method was used for antioxidant activity.

Synthesis of **EC-toc and EOI microcapsules** are described. Microcapsules were analysed by SEM, HPLC, UV spectroscopy and ESR (antioxidant activity).

4.1 Synthesis and optimisation of parameters of EC microcapsules synthesis

Ethyl cellulose (EC) microcapsules were prepared by the phase separation method, in aqueous and organic phases, according to the procedure as described in patent No.: US 6932984 B1 [50]. Used active substances were α-tocopherol (α-toc), immortelle oil (EOI) and mix of α-toc and immortelle oil.

Preparation of EC microcapsules by steps:

1. Dissolving different amounts of active ingredient together with a wall-forming material (EC, m = 0.6 g) in an organic solvent (Ethyl acetate (EA), V = 15 ml) partially miscible in water, to form an organic solution or dispersion. Mass of active ingredient was optimised for both active substances (α-toc and EOI);
2. Mixing organic solution with an aqueous solution; aqueous solution being saturated with organic solvent (EA, V = 10 ml) dissolved in 100 ml of distilled water and comprising an emulsifier (SDS, m = 1 g), to form an emulsion (pH of the aqueous phase was adjusted with citric acid to pH 3 to prevent the hydrolysis of EA.);
3. By mixing the aqueous phase (stirring/ultrasound), the organic phase was gradually added;
4. Adding an excess amount of water (200 ml) to initiate extraction of the organic solvent from the emulsion;
5. Mixing the emulsion long enough to allow the formation of microcapsules in the mixture (centrifugation at 2000 rpm for 5 minutes);
6. Further removal of the residual amount of organic solvent in formed microcapsules by filtration.

Synthetized microcapsules were air-dried in the dark for 24 h and then stored in the refrigerator until their usage.

Optimisation of EC microcapsules synthesis parameters

Parameters optimised in EC microcapsules synthesis were: a) amount of active substance (α-toc) and speed of stirring, b) mixing technique (electrical stirrer and ultrasonic device).

a) Optimisation of EC microcapsules with active substance amount and speed of stirring

In synthesis of EC-toc microcapsules, amount of α-toc and stirring speed were optimised. Amounts of α-toc were: 0.20 g, 0.30 g, 0.35 g, 0.70 g. Analysed stirring speed was: 250 rpm, 400 rpm and 550 rpm. Parameters of synthesis are presented in Table 16 were analysed by SEM, Figure 12.

Table 16 Specifications of synthesis parameters - optimization by active substance (α-toc) and stirring speed increase

Synthesis	amount of α-toc / g	stirring speed / rpm
E*-1	0.2	250
E-2	0.2	400
E-3	0.3	250
E-4	0.3	400
E-5	0.3	550
E-6	0.7	400

* E = type of EC-toc microcapsules synthesis

b) Optimisation of EC microcapsules with mixing technique

Two mixing techniques were used in preparation of EC microcapsule with EOI stirring (laboratory stirrer) (technique A) and ultrasound (ultrasonic device) (technique B). The mass of EOI in a separate treatment bath was constant 0.2 g (m_2). Additionally, oil-free EC microcapsules (m_0) were also prepared as a reference. The aim of this research was preparation of EC-EOI microcapsules by using different techniques (stirring and ultrasound) in order to study their influence on the size of microcapsules. Characterization of synthesized microcapsules in dispersion was performed by confocal laser scanning microscopy (CLSM).

Type of synthesis, depending on EIO mass is presented in Table 17. Amounts of EOI were: 0.00 g (m_0), 0.15 g (m_1), 0.20 g (m_2), 0.30 g (m_3) and 0.60 g (m_4). Ratio between amounts of oil and EC (1:1, 1:2, 1:3, 1:4) in synthetized microcapsules is also presented in Table 17.

Table 17 Optimisation of EOI amount in synthesis

Type of synthesis m*	m_{SDS} / g	m_{EC} / g	m_{EOI} / g	ratio (oil:EC)	V_{EA} / ml
m_0	1	0.6	0.00	/	25
m_1			0.15	1:4	
m_2			0.20	1:3	
m_3			0.30	1:2	
m_4			0.60	1:1	

* m = type of EC-EOI microcapsules synthesis

Synthesis of EC microcapsules containing α-toc and EOI

EC microcapsules containing α-toc and EOI (EC-toc and EOI microcapsules) were also synthetized by protocol described above. It was used stirring technique (400 rpm), amount of α-toc and EOI were together 0.2 g (0.1 g α-toc and 0.1 g EOI).

4.2 SEM analysis

All synthetized EC microcapsules (oil-free EC microcapsules, EC-toc microcapsules, EC-EOI microcapsules and EC-toc and EOI microcapsules) were analysed by SEM for determing morphology and synthesis optimisation efficiency

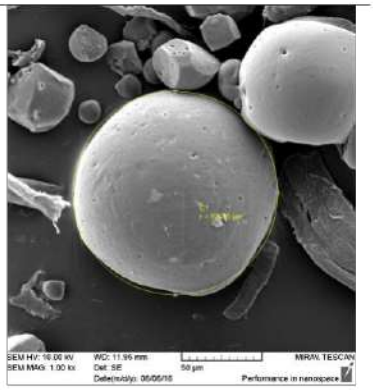
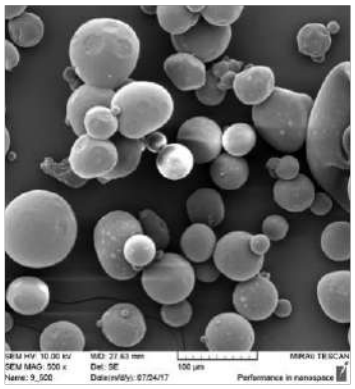
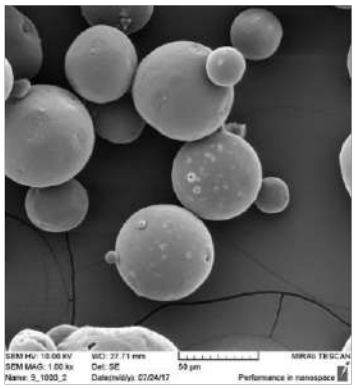
SEM microscope was operated at 5 - 10 kV and various magnification levels. Applied detector was SE detector (SE-secondary electrons).

Depending on the current availability of the evaporating material and device, the samples were evaporated:

- Gold/palladium/carbon-based sputter coater has been used (SC7620-CF Mini, Quorum Technologies),
- Chromium-based evaporator that coats non-conductive samples with electrically conductive particles (Q150T ES Plus, Quorum Technologies).

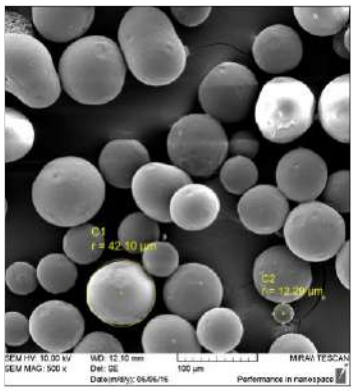
Synthesis optimisation of EC-toc microcapsules

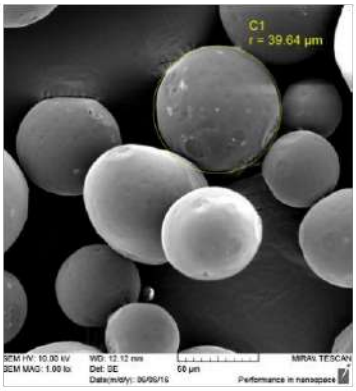
Results of synthesis optimisation using different amounts of α-toc and speed of stirring technique is presented in this part. Detail parameters of optimisation were presented at Table 16. In Figure 12 SEM images of synthesis* E-1 (0.2 g/ 250 rpm), E-2 (0.2 g/ 400 rpm), E-3 (0.3 g/ 250 rpm), E-4 (0.3 g / 400 rpm), E-5 (0.3 g / 550 rpm) and E-6 (0.7 g / 400 rpm) optimisation are presented. Magnification is 500x and 1000x.

Synthesis	Magnification	
	500x	1000x
E-1		
E-2		

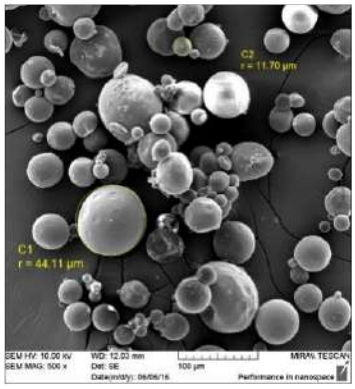
* **EC-toc microcapsules** were evaporated with an alloy of palladium and gold (2 x 180 s).

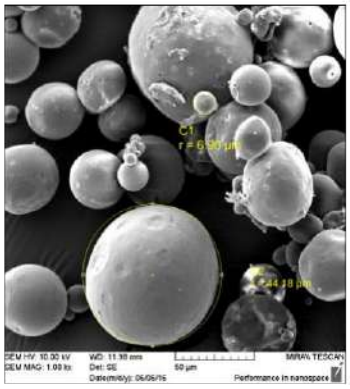
E-3



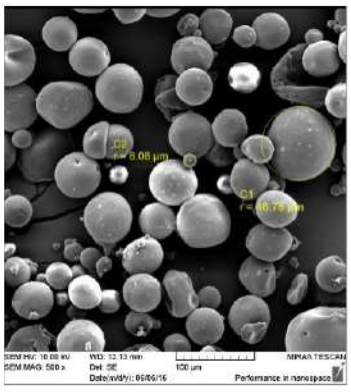


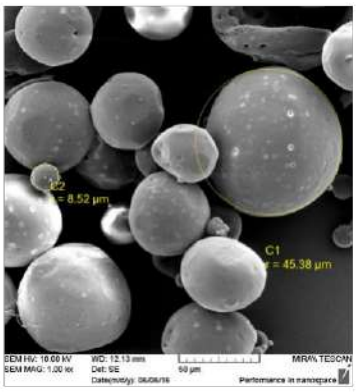
E-4





E-5





E-6

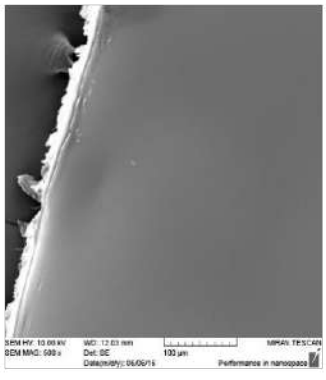


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Figure 12 SEM figures of synthesis optimization, magnification 500x and 1000x; E-1 (0.2 g/ 250 rpm), E-2 (0.2 g/ 400 rpm), E-3 (0.3 g/ 250 rpm), E-4 (0.3 g / 400 rpm), E-5 (0.3 g / 550 rpm) and E-6 (0.7 g / 400 rpm)

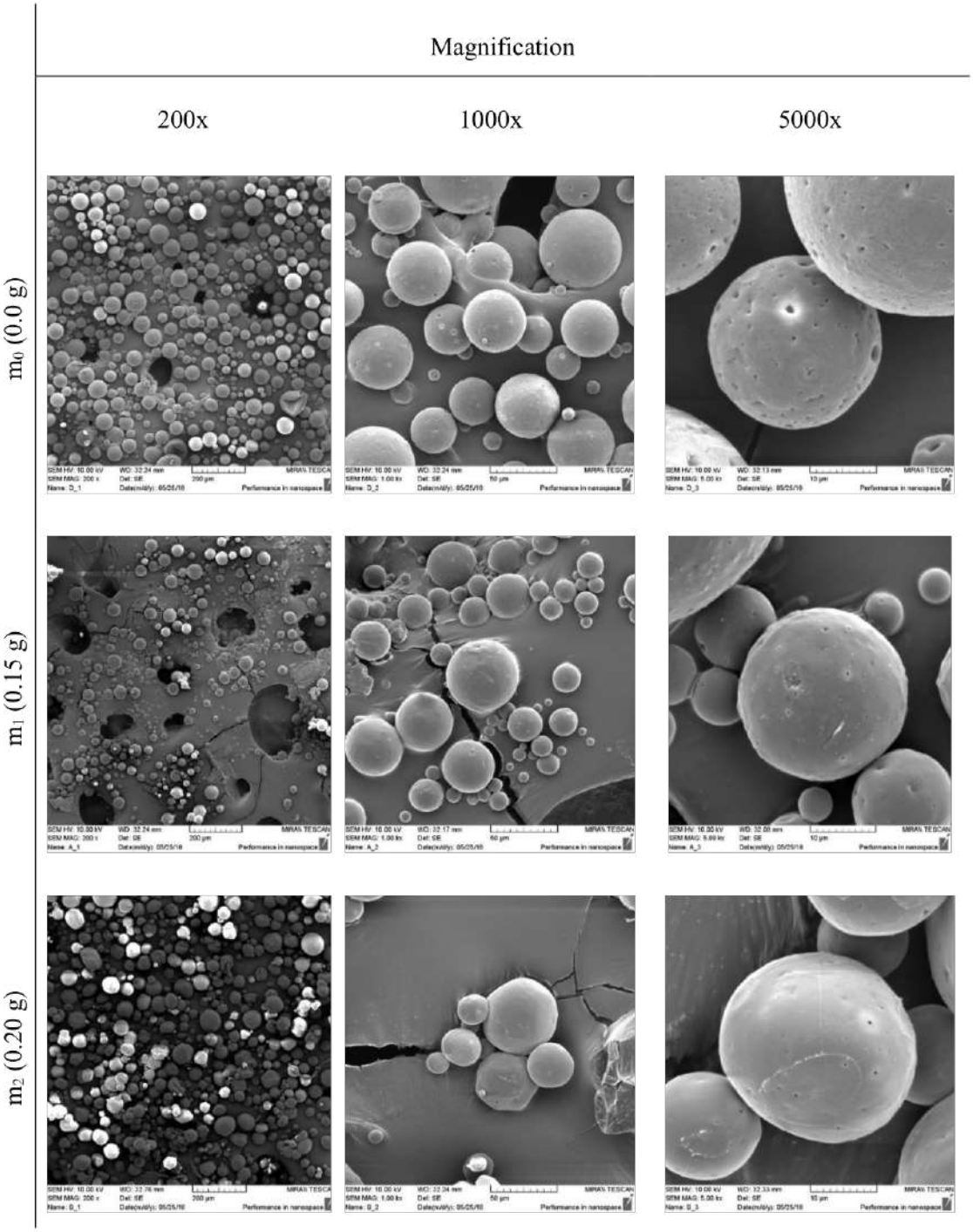
Microcapsules made by synthesis E-1 have spherical shape and their size is between 10 - 65 μm. Very small number of microcapsules was synthetized by this type, so the next synthesis with the same speed of stirring but with greater amount of α-toc was used. Synthesis E-2 also have spherical shape and their diameter is between 10 - 50 μm and with this type of synthesis higher amount of microcapsules were obtained. Next synthesis E-3 - E-5 were made aiming in greater microcapsules amount production and stirring speed optimisation.

By increasing mass of α-toc, microcapsules couldn't be removed from filter paper on the final step of synthesis and speed of stirring doesn't have influence on it. Using 0.70 g of α-toc (E-6), sticky film on filter paper was produced and microcapsules were not visible at SEM analyses. The optimal mass of α-toc used for synthesis of EC-toc microcapsules is 0.2 g. This kind of microcapsules will be applied on cosmetotextiles.

EC-EOI microcapsules

SEM analyse of synthesised EC microcapsules* with four different EOI masses and oil-free EC microcapsules: m_0 (0.0 g), m_1 (0.15 g), m_2 (0.20 g), m_3 (0.30 g) and m_4 (0.60 g) (Figure 13) confirmed quality of synthesized EC microcapsules, noticeable in their regular spherical shape. Magnifications were 200x, 1000x and 5000x. Microcapsules were in the 10 - 50 μm size range with an average diameter of 30 μm.

* **EC-EOI microcapsules** were evaporated with an alloy of chrome (300 s).


Magnification
200x
1000x
5000x
m_0 (0.0 g)
m_1 (0.15 g)
m_2 (0.20 g)

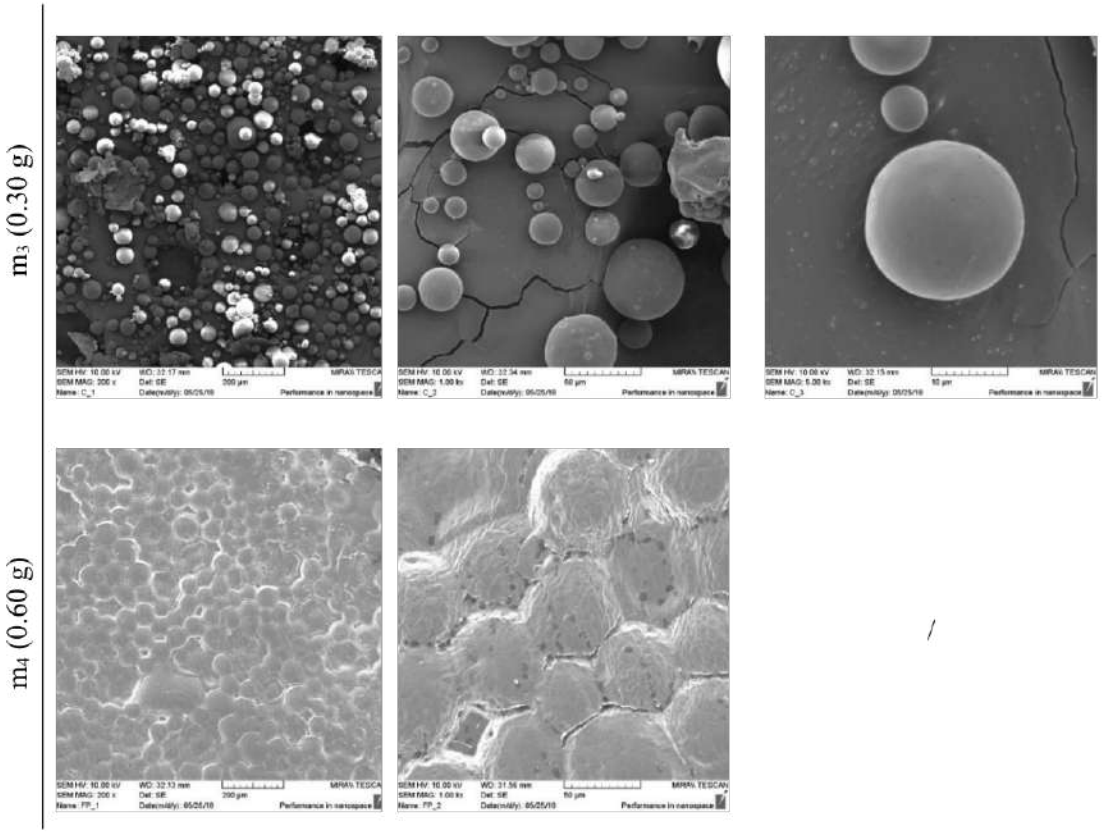


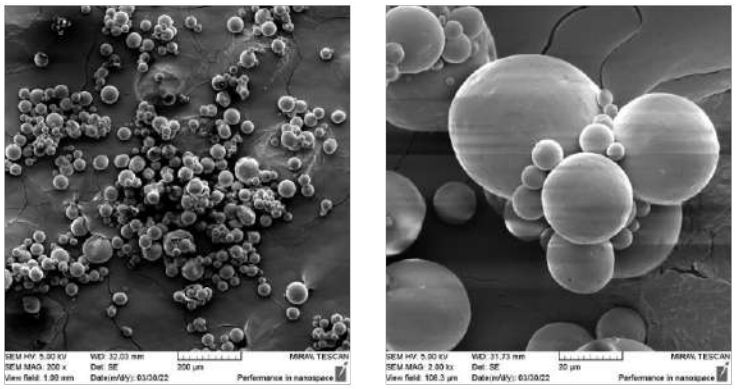
Figure 13 SEM images of EC microcapsules with four different EOI masses and oil-free EC microcapsules: m_0 (0.0 g), m_1 (0.15 g), m_2 (0.20 g), m_3 (0.30 g) and m_4 (0.60 g); magnification 200x, 1000x and 5000x

The synthesized microcapsules, regardless of the mass of EOI, were regular oval shape and micro size. Only during the synthesis of EC microcapsules with 0.60 g of EOI, agglomerates were formed, due to the too large amount of oil, and deformation of the microcapsules occurred.

Generally, synthesized microcapsules m_2 (0.2 g) showed the best results, taking into the consideration the whole synthesis procedure. Synthetized microcapsules have good gravimetric results (0.389 g of synthesized microcapsules) and microcapsules were easily removed from the filter paper.

EC-toc and EOI microcapsules

The results of SEM analyse of synthesised EC-toc and EOI microcapsules* are presented at Figure 14. Amount of α-toc and EOI were together 0.2 g (0.1 g α-toc and 0.1 g EOI). Magnifications were 200x and 2000x.



a. b.

Figure 14 SEM images of EC-toc and EOI microcapsules (0.20 g), magnification: a. 200x and b. 2000x

The quality of the synthesized EC microcapsules is noticeable in their regular spherical shape. Dimensioning of the diameter has been carried out during the measurement and microcapsules were in the 3 - 60 μm size range with an average diameter of 30 μm.

* **EC-α-toc and EOI microcapsules** were evaporated with an alloy of chrome (120 s).

4.3 Results of qualitative and quantitative analysis of α-toc and EOI with HPLC

Due to specify of HPLC and conditions that could impact the results, some parameters (injection volume, detection wavelength and time of analysis) were tested on standard solution of α-tocopherol.

HPLC was used for qualitative and quantitative analysis of α-toc standard, pure EOI and for all synthetized EC microcapsules (oil-free EC microcapsules, EC-toc microcapsules, EC-EOI microcapsules and EC-toc and EOI microcapsules).

Optimisation of HPLC analysis for α-toc

In order to be able to begin the HPLC analysis it was necessary to isolate α-toc and filtrate the samples with methanol [124]. Selected concentration of α-toc standard was diluted in 10 ml methanol. More specifically, 0.84 ml of stock solution prepared by the dissolution of 30 mg of the α-toc standard in 100 ml of methanol was diluted. For HPLC analyses, 0.1 g of microcapsules was diluted in 10 ml methanol. All solutions were mixed in the flask using Vortex mixer at 1900 rpm, in period of 60 s, filtrated with PTFE filter and analysed with HPLC. α-toc standard and EC-toc microcapsules were analysed by HPLC following the standard EN 12822:2014 [124].

First step was using UV-VIS spectrophotometry for analysis of the α-toc stock solution purity. The measurement showed an absorbance peak of α-toc at wavelength of 292 nm, which was in accordance with the standard protocol. The next step of the analysis was the optimisation of HPLC parameters through five protocols. It was important to find out proper conditions for this application through variation of the injection volume, detection wavelength and time. Optimisation was carried out through five protocols and preliminary results of the analysis are presented in Table 18.

Table 18 Parameters for HPLC analysis of α-toc in solutions

Protocol	Injection volume / µl	Detection wavelength / nm	Time / min	Response / mAU*s	Retention time / min
1	1.8	284	20	20.7	10.16
2	20.0	284	20	261.5	10.30
3	20.0	290	20	297.6	9.86
4	20.0	292	15	300.7	9.92
5	100.0	292	15	2120.0	9.97

It can be noticed that the variation in the injection volume was proportional to the response. The results confirmed that peak responses for α-toc were recorded in the interval 284 - 292 nm. Protocol 5 was selected as the most suitable for further cosmetotextiles analysis. Optimised parameters were: injection volume of 100 µl, retention time of 9.97 min, analyse time 15 min and detection wavelength at 292 nm (Protocol 5*).

*Selected HPLC method for α-toc analysis was **HPLC 1** with parameters:*

The injection volume (100 µl) of all solutions was filtered through a PTFE filter prior to injection. The measurement was performed at 35 °C ± 0.8 °C in duration of 15 min, with mobile phases ratio v/v = 97/3 (MeOH/H_2O) and flow rate of 1.8 ml/min. Retention time for α-toc was 9.4 - 9.9 minutes. UV detector for α-tocopherol was set at wavelength of 292 nm and the duration of analyses was 15 min.

* In futher text: *Protocol 5* is called *HPLC 1 method*

Calibration diagram for α-toc

The reference of α-toc was a standard substance diluted in methanol, HPLC grade for calibration diagram. Stock solution prepared by the dissolution of 30 mg of the α-toc in 100 ml of methanol was stored at 5 °C and protected from light. Concentration and purity tests of the solution prepared were checked by UV-VIS Spectrophotometer, Carry 50 at 292 nm according to the European standard (EN 12822:2014) [124]. After the checkpoint of the solution, ten different volumes of the stock solution were pipetted into a 10 ml volumetric flask and diluted to the mark with methanol and HPLC calibration curve of α-toc was made. Measurement was performed following conditional parameters for method *HPLC 1*. The HPLC calibration curve of α-toc, presented in Figure 15, shows linear relationship (y = 22.964x - 84.9) between the response and concentration range from 24 to 225 μg/ml with the regression and correlation coefficient $R^2 = 0.999$.

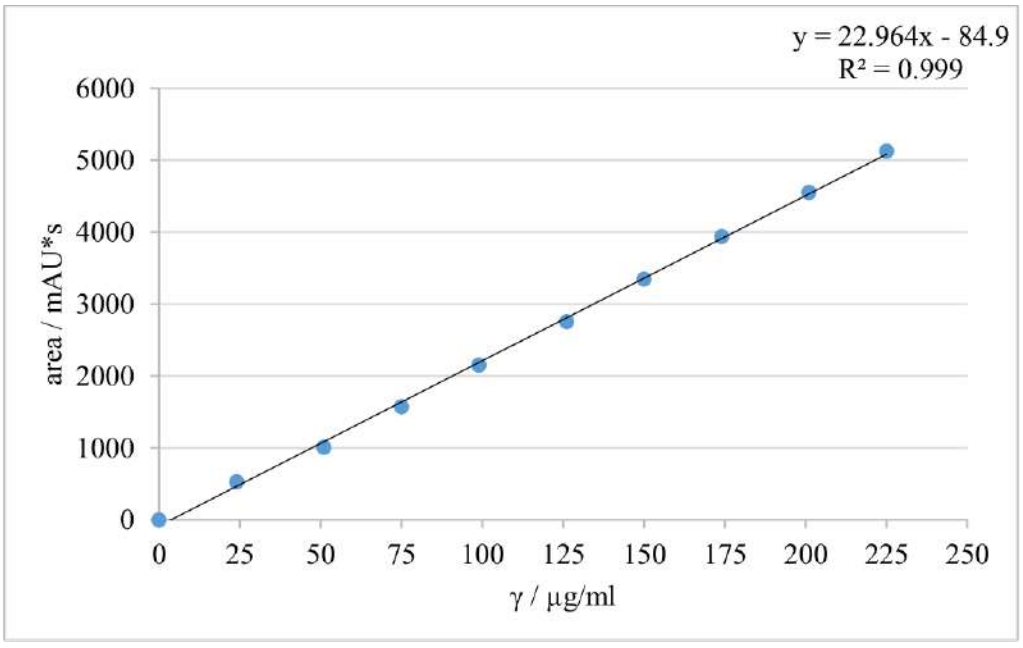


Figure 15 Calibration diagram of α-toc

α-toc standard

α-toc standard (0.84 ml of stock solution) was analysed by HPLC procedure. The sample solutions were mixed in the flask using Vortex mixer 1900 rpm, in period 60 s, filtrated with PTFE filter and analysed with HPLC. HPLC chromatogram of α-toc standard is presented in Figure 16.

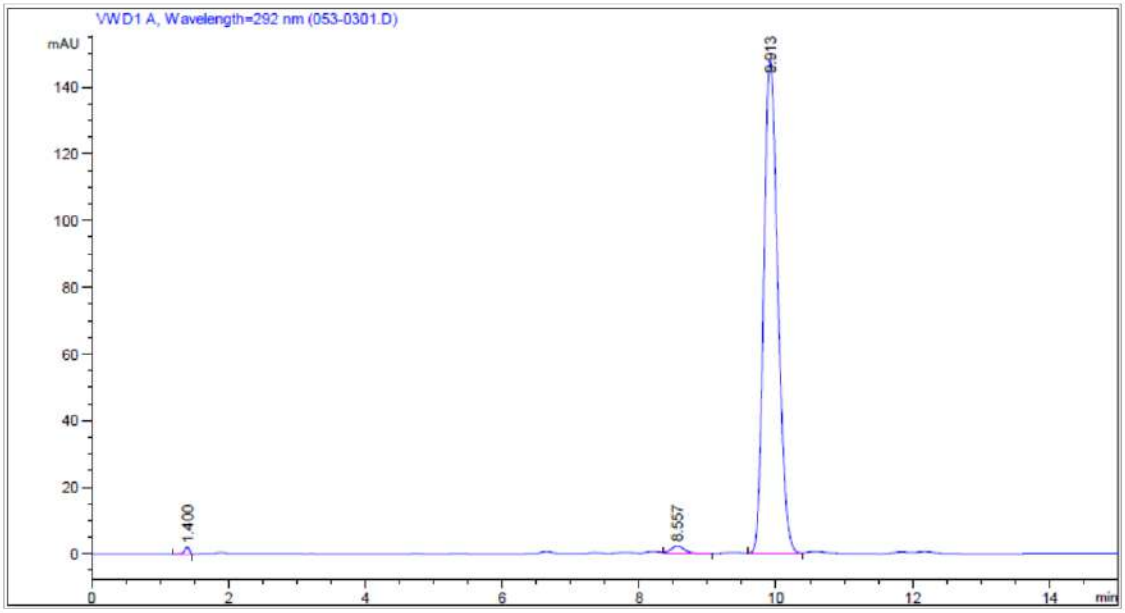


Figure 16 HPLC chromatogram of α-toc standard

The measurement showed maximum absorbance peak of α-toc at wavelength of 292 nm, and retention time 9.913 min which was expected, and in accordance with the standard protocol. Value of the peak area was 2120.60010 mAU*s and the hight of the peak was 148.03633 mAU. Using the Calibration curve of α-toc (Figure 15) the concentration of analysed α-toc standard was calculated as 88.65 µg/ml.

EC-toc microcapsules

EC-toc microcapsules were also analysed by HPLC. Prior to HPLC analysis it was necessary to isolate and filtrate the samples. α-toc was isolated from EC microcapsules by extraction with methanol. Microcapsules (0.01 g) were diluted with 10 ml methanol. The sample solution was mixed in the flask using Vortex mixer: 1900 rpm, in period 60 s, filtrated with PTFE filter and analysed with HPLC. HPLC chromatogram of EC-toc microcapsules (E-2) is presented in Figure 17.

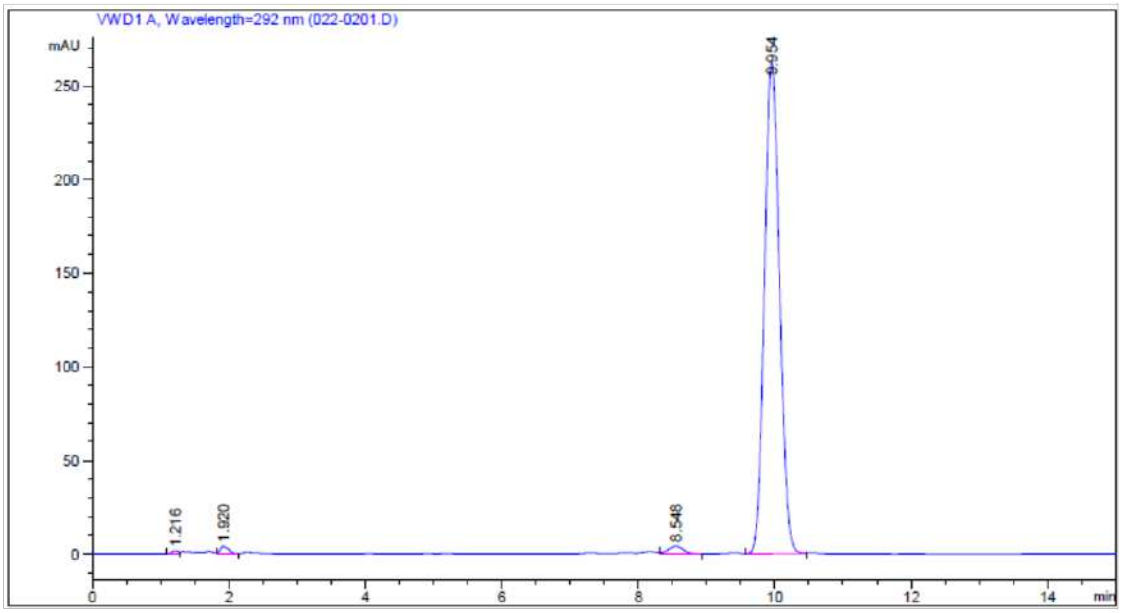


Figure 17 HPLC chromatogram of EC-toc microcapsules

The measurement showed an absorbance peak of α-toc at wavelength of 292 nm, in retention time 9.954 min which was in accordance with the standard protocol. Peak was expected at the specified wavelength because microcapsules are containing α-toc. Value of area below the peak was 4024.16626 mAU*s and the hight of the peak was 262.89807 mAU. Using the Calibration curve of α-toc (Figure 15) the concentration of analysed α-toc in EC microcapsules was calculated as 178.94 μg/ml. The peak height depends on the α-toc concentration in the microcapsules.

Optimisation of HPLC analysis for EOI

Calibration diagram for EOI

Stock solution of EOI for calibration diagram was prepared by the dissolution of 33.47 mg of the EOI in 10 ml of n-Hexane. Maximum absorption was analysed by UV Spectrophotometar Carry 50 at 265 nm. Five different volumes of the stock solution were pipetted into a 10 ml volumetric flask and diluted to the mark with n-Hexane and HPLC calibration curve of EOI was made. After preliminary analysis, selected HPLC method for EOI was *HPLC 2*.

Selected HPLC method for EOI analysis was ***HPLC 2*** *with parameters:*

Injection volume of 100 ml and filtering of all solutions through a PTFE filter prior to injection. The measurement was performed at 25 °C ± 0.8 °C in duration of 20 min, with mobile phases ratio: v/v = 97/3 ($MeOH/H_2O$) and flow rate of 0.5 ml/min. Retention time for EOI was 15.6 - 15.8 minutes. UV detector for EOI was set at wavelength of 265 nm.

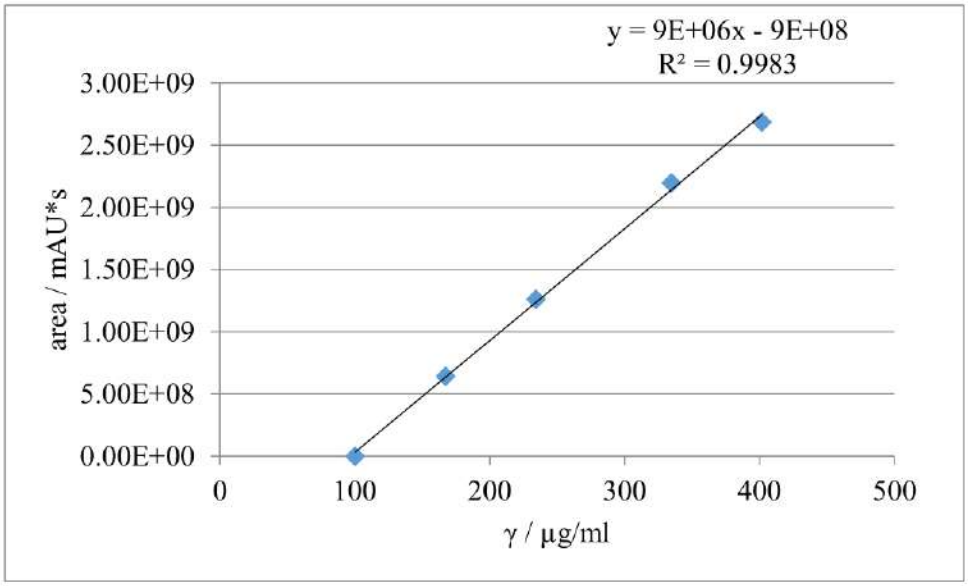


Figure 18 HPLC calibration diagram of EOI

The HPLC calibration curve of EOI, presented in Figure 18, showed linear relationship (y = 9E+09x - 9E+08) between the response and concentration range from 100.41 to 401.64 µg/ml with the regression and correlation coefficient $R^2 = 0.9983$.

Pure EOI

EOI was prepared in 10 ml n-Hexane, i.e. 0.70 ml of stock solution of EOI (33.47 mg of the EOI in 10 ml of n-Hexane) was diluted in 10 ml n-Hexane. The sample solution was mixed in the flask using Vortex mixer: 1900 rpm, in period 60 s, filtrated with PTFE filter and analysed with HPLC. HPLC chromatogram of pure EOI is presented at Figure 19.

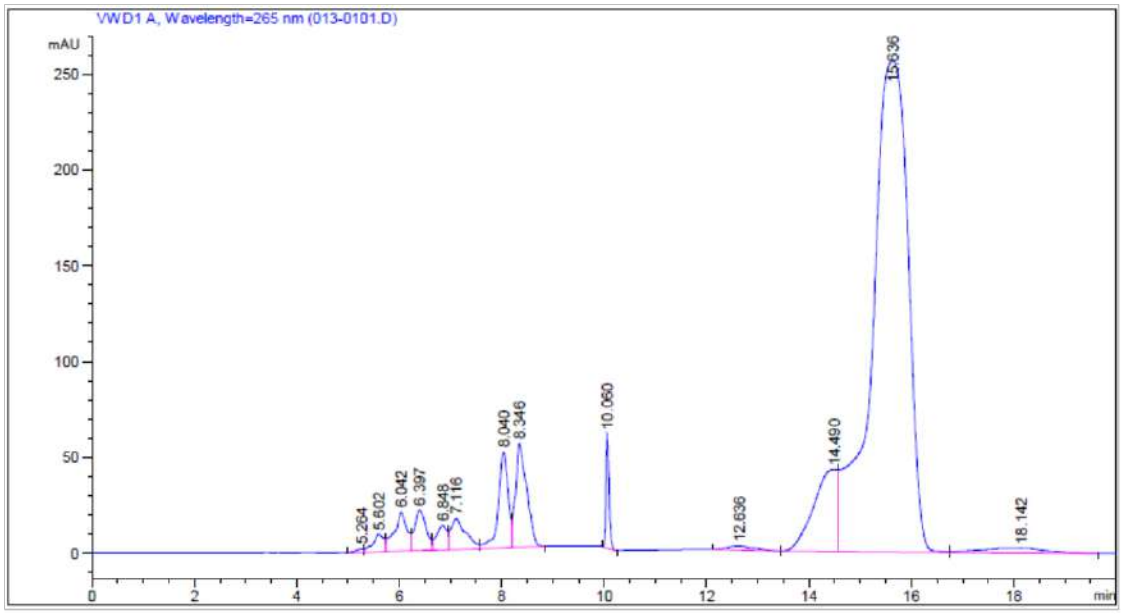


Figure 19 HPLC chromatogram of EOI

The measurement showed an absorbance peak of EOI at wavelength of 265 nm, in retention time 15.636 min. Peak was expected at that specified wavelength according to UV-spectroscopy results presented in the next chapter. Value of area below the peak was 1.26E+09 mAU*s and the hight of the peak was 256.64325 mAU. Using the Calibration

curve of EOI (Figure 18) the concentration of analysed EOI was calculated as 240.00 μg/ml.

EC-EOI microcapsules

EC-EOI microcapsules were also analysed by HPLC. Prior HPLC analysis it is necessary to isolate and filtrate samples. EOI was isolated from EC microcapsules by extraction with n-Hexane. Microcapsules (0.01 g) were diluted with 10 ml n-Hexane. The sample solutions were mixed in the flask using Vortex mixer 1900 rpm, in period 60 s, filtrated with PTFE filter and analysed with *HPLC 2* method. HPLC chromatogram of EC-EOI microcapsules synthetized by synthesis m_2 are presented in Figure 20.

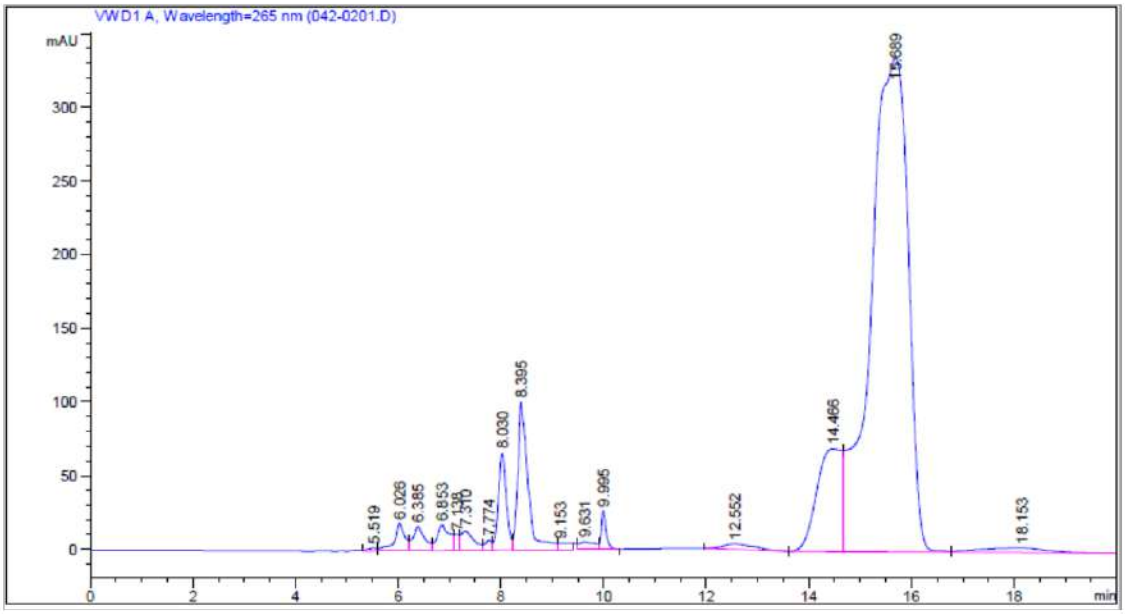


Figure 20 HPLC chromatogram of EC-EOI microcapsules

The results showed an absorbance peak of EOI at wavelength of 265 nm, in retention time 15.689 min which was in accordance with the standard protocol. Peak was expected at

the specified wavelength because microcapsules contained EOI. Value of area below the peak was 1.70E+09 mAU*s and the height of the peak was 335.35217 mAU. Using the Calibration curve of EOI (Figure 18) the concentration of analysed EOI in EC microcapsules was calculated as 289.26 μg/ml. The height of the peak depends on the EOI concentration in the microcapsules.

EC-toc and EOI microcapsules

EC-toc and EOI microcapsules (0.01 g) were diluted with 10 ml n-Hexane and also analysed by HPLC. All sample solutions were quickly mixed in the flask using Vortex mixer 1900 rpm, in period 60 s, filtrated with PTFE filter and analysed with HPLC. Because of combination of two different active substances in microcapsules, it was analysed by two different methods. In fist case method parameters for analyse were by protocol for α-toc (*HPLC 1*), in second by protocol for EOI (*HPLC 2*). Preparation of the samples was the same as for microcapsules with one active substance. In first case, α-toc was isolated from EC microcapsules by extraction with methanol, and in second case EOI was isolated from EC microcapsules by extraction with n-Hexane. In both case 0.01 g of microcapsules was diluted with 10 ml selected solvent. The sample solutions were mixed in the flask using Vortex mixer 1900 rpm, in period 60 s, filtrated with PTFE filter and analysed with HPLC. In the first case, parameters for the analyses were determined by protocol for α-toc (*HPLC 1*), in second by protocol for EOI (*HPLC 2*).

HPLC chromatogram of EC-toc and EOI microcapsules with method *HPLC 1* are presented in Figure 21 and with method *HPLC 2* are presented in Figure 22.

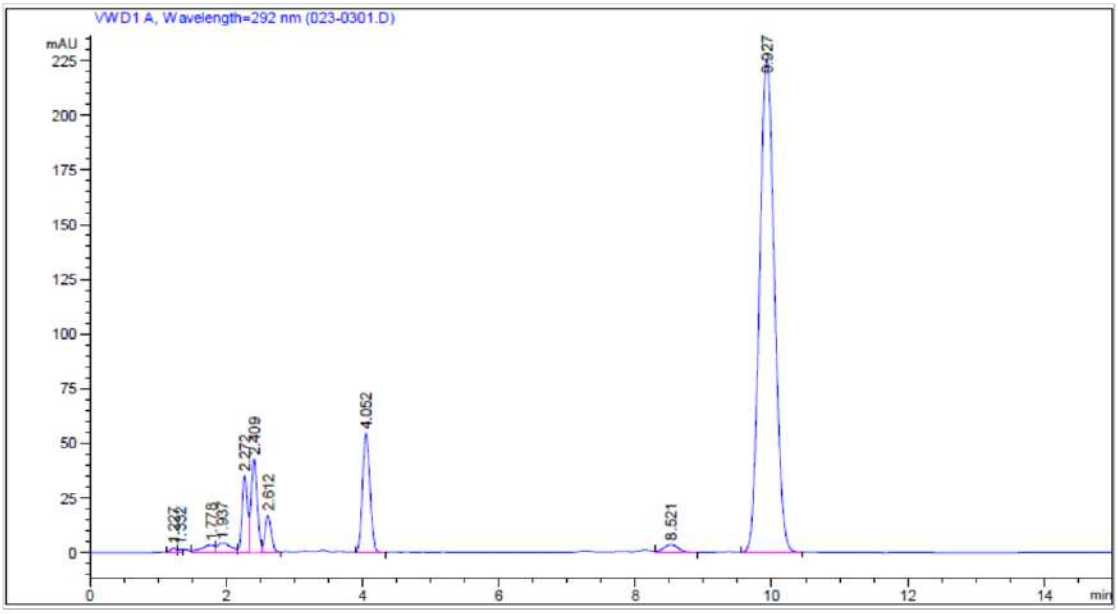


Figure 21 HPLC chromatogram of EC-toc and EOI microcapsules by method for α-toc *HPLC 1*

The HPLC measurement by *HPLC 1* method for α-toc showed an absorbance peak of α-toc at wavelength of 292 nm in retention time 9.927 min. Peak was expected at this specified wavelength because microcapsules were containing α-toc. Value of area below the peak was 3437.45557 mAU*s and the height of the peak was 225.21692 mAU. Using the Calibration curve of α-toc (Figure 15) the concentration of analysed α-toc in EC microcapsules was calculated as 153.39 μg/ml. The height of the peak depends on the α-toc concentration in the microcapsules.

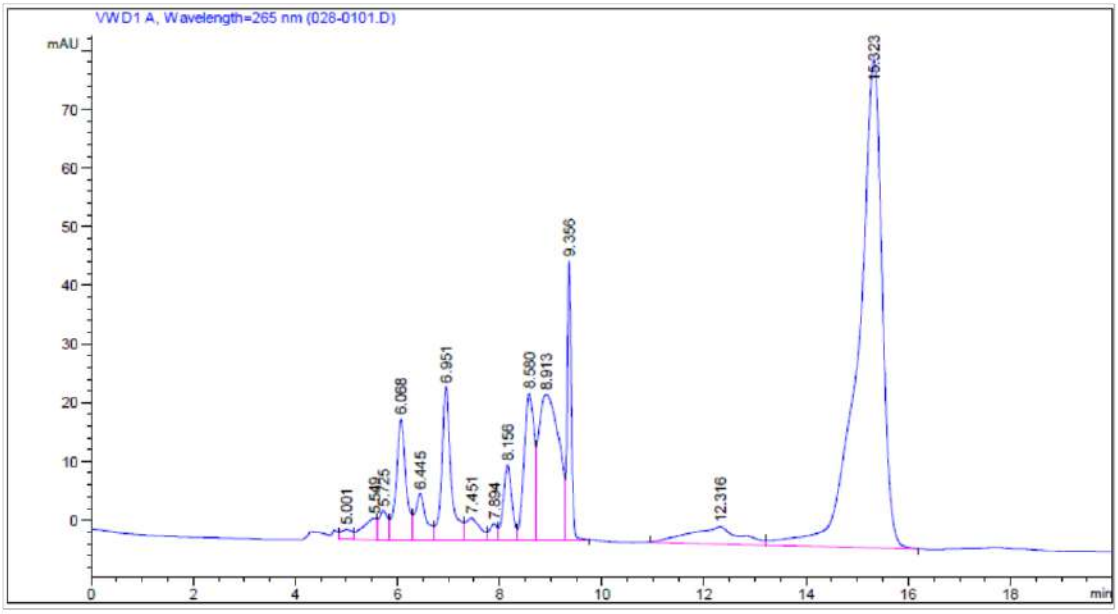


Figure 22 HPLC chromatogram of EC-toc and EOI microcapsules by method for EOI *HPLC 2*

In the second case, *HPLC 2* method for EOI showed an absorbance peak of EOI at wavelength of 265 nm, in retention time 15.323 min. Peak was expected at this specified wavelength because microcapsules were containing EOI. Value of area below the peak was 2707.57959 mAU*s and the height of the peak was 83.17848 mAU. Using the Calibration curve of EOI (Figure 18) the concentration of analysed EOI in EC microcapsules was calculated as 100.00 µg/ml. The height of the peak depends on the EOI concentration in the microcapsules.

Oil-free EC microcapsules

Oil-free EC microcapsules, without active substances (m_0) were also analysed by HPLC. Microcapsules were analysed by the same principle like microcapsules with active substance adhering to the chosen method. In first case method parameters for analyse were by protocol for α-toc (*HPLC 1*), and in second by protocol for EOI (*HPLC 2*).

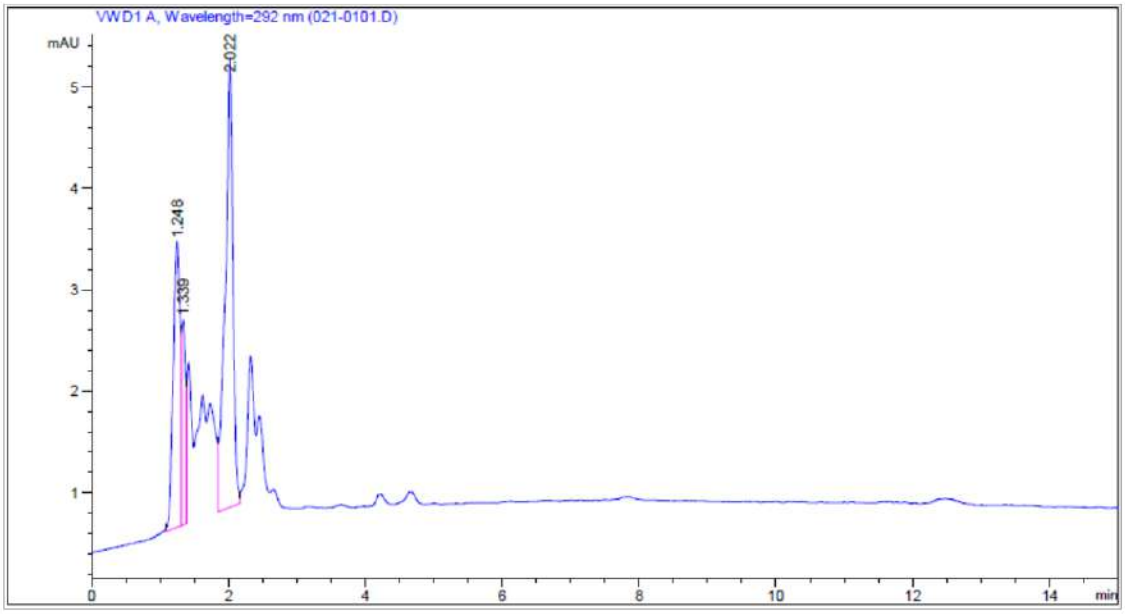


Figure 23 HPLC chromatogram of oil-free EC microcapsules (m_0) by method for α-toc *HPLC 1*

In both cases microcapsules 0.01 g was diluted with 10 ml selected solvent. The sample solutions were mixed in the flask using Vortex mixer 1900 rpm, in period 60 s, filtrated with PTFE filter and analysed with HPLC. HPLC chromatogram of EC microcapsules with method *HPLC 1* is presented in Figure 23 and with method *HPLC 2* are presented is Figure 24.

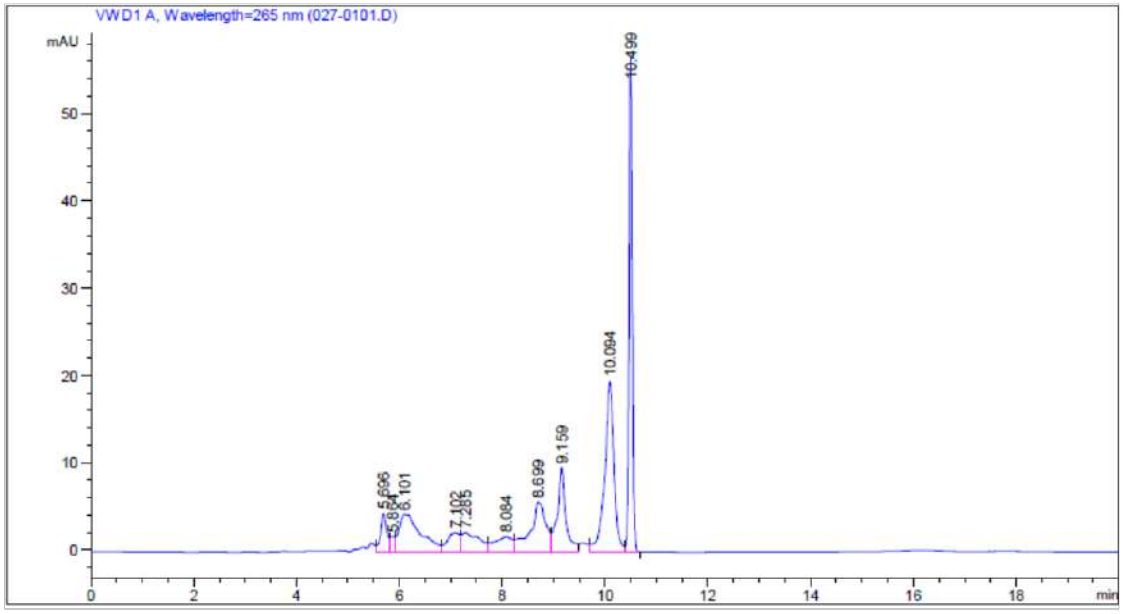


Figure 24 HPLC chromatogram of oil-free EC microcapsules by method for EOI *HPLC 2*

The HPLC measurement by method for α-toc (*HPLC 1*) didn't show an absorbance peak of α-toc at wavelength of 292 nm in expected retention time ~ 9.0 min. In the second case, also HPLC measurement by method for EOI (*HPLC 2*) didn't show an absorbance peak of α-toc at wavelength of 265 nm in expected retention time ~ 15.0 min. Those results confirmed absence of active substances in oil-free EC microcapsules, which was expected.

HPLC analysis summary

In Table 19 the results of HPLC analysis of oil-free EC microcapsules (oil-free EC MC), EC-toc microcapsules (EC-toc MC), EC-EOI microcapsules (EC-EOI MC) and EC-α.-toc and EOI microcapsules (EC-toc + EOI MC), by two HPLC methods are presented.

Table 19 Results of HPLC analysis of EC microcapsules

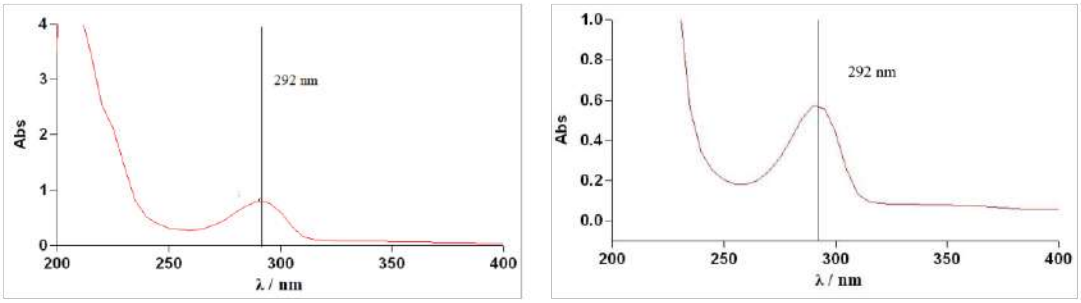
sample	method	time /min	area / mAU*s	hight / mAU	γ / µg/ml
oil-free EC MC	*HPLC 1*	~ 9.0	/	/	/
	HPLC 2	~ 15.0	/	/	/
EC-toc MC	*HPLC 1*	9.954	4024.16626	262.89807	178.94
EC-EOI MC	*HPLC 2*	15.689	1.70E+09	335.35217	289.26
EC-toc + EOI MC	*HPLC 1*	9.927	3437.45557	225.21692	153.39
	HPLC 2	15.323	2707.57959	83.17848	100.00

From the obtained results, it can be seen that oil-free EC microcapsules don't contain active substances (neither α-toc nor EOI) which was expected. Comparing the concentration of active substances (α-toc and EOI) in synthetized microcapsules (EC-toc MC and EC-EOI MC) of the same mass (0.01 g), results showed that EC-EOI MC contain 38.14 % more active substance than MC-toc using the same procedure in synthesis. HPLC results of microcapsules with combination of active substances (EC-toc + EOI MC) showed that both active substances were present in microcapsules. For quantitative analyse this type of identification isn't applicable and indicates the need for further research.

4.4 Results of UV spectrophotometry

EC-toc microcapsules

UV spectrophotometry was used for qualitative analyse of EC-toc microcapsules masses 0.001 g (E-2) (Table 8). It were dissolved in 5 ml methanol mixed on Vortex 1900 rpm, 60 s and analysed with Cary 50 Solascreen.



a. b.

Figure 25 UV spectrum of α-toc: a. standard α-toc, b. EC-toc microcapsules

Characteristic peak at 292 nm is present measuring α-toc (Figure 25 a). Comparing with HPLC measurements (Figure 17) the peak is confirmed. From UV spectrum of EC microcapsules it can be seen that the same absorption peak is present at 292 nm which confirms the presence of α-toc (Figure 25 b).

Pure EOI

Calibration diagram for EOI

Before EC-EOI microcapsules were analysed, the solution of 19 mg EOI was dissolved in 100 ml of n-Hexane. Concentration and purity tests of the prepared solution were measured by UV Spectrophotometer at 265 nm (Figure 26).

Five different concentrations of the stock solution were prepared and UV calibration curve of EOI was made [135].

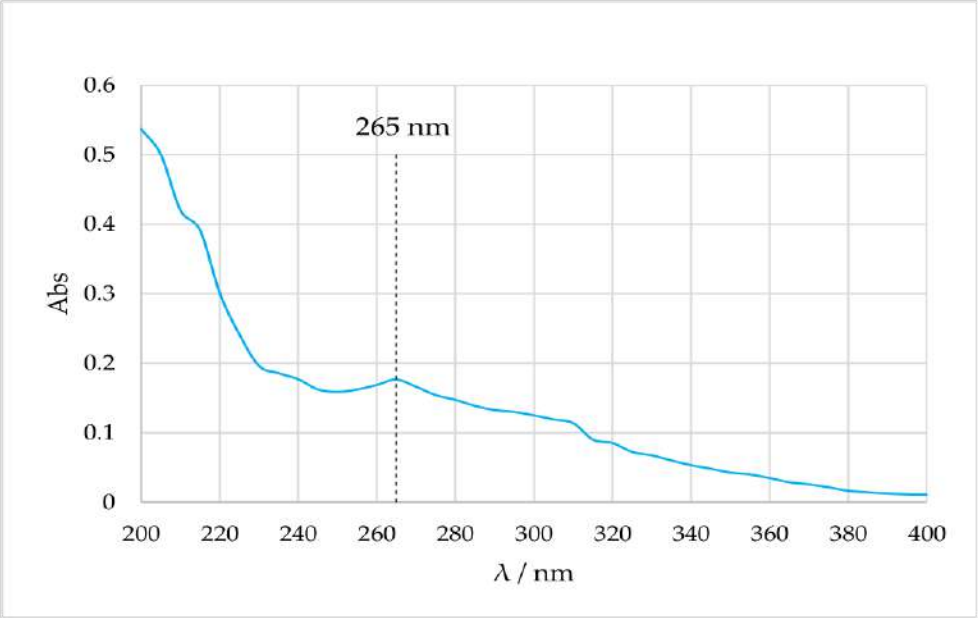


Figure 26 UV spectrum of EOI [135]

Absorbance of various concentrations of EOI at wavelengths with the UV range 200 - 400 nm were measured. Maximum absorbance of these solutions was at wavelength of 265 nm, shown in Figure 27 (Table 20) [135].

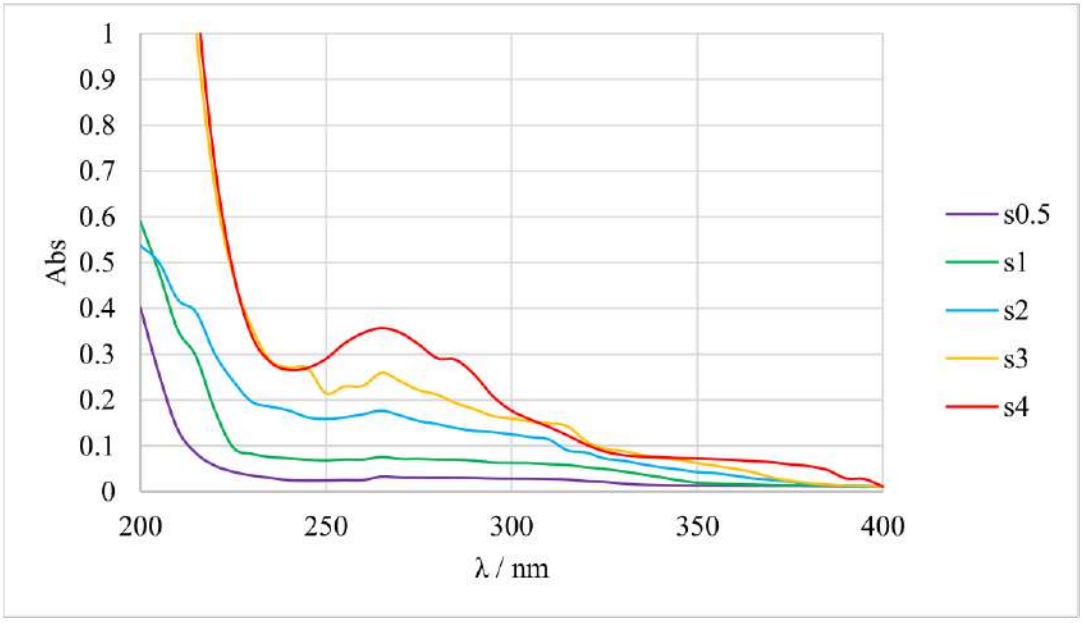


Figure 27 UV spectrum of various concentrations of EOI (Table 20) at UV range (200-400 nm) [135]

Table 20 Concentrations and the measured absorbance of 5 different solutions of EOI [135]

Symbol	V (solutions of EOI) / ml	Absorbance	γ / mg/ml
s0.5	0.5	0.0324	0.0095
s1	1	0.0752	0.0190
s2	2	0.1764	0.0379
s3	3	0.2589	0.0569
s4	4	0.3569	0.0758

In Table 20 absorbance and concentrations of baths of calibration diagram are presented.

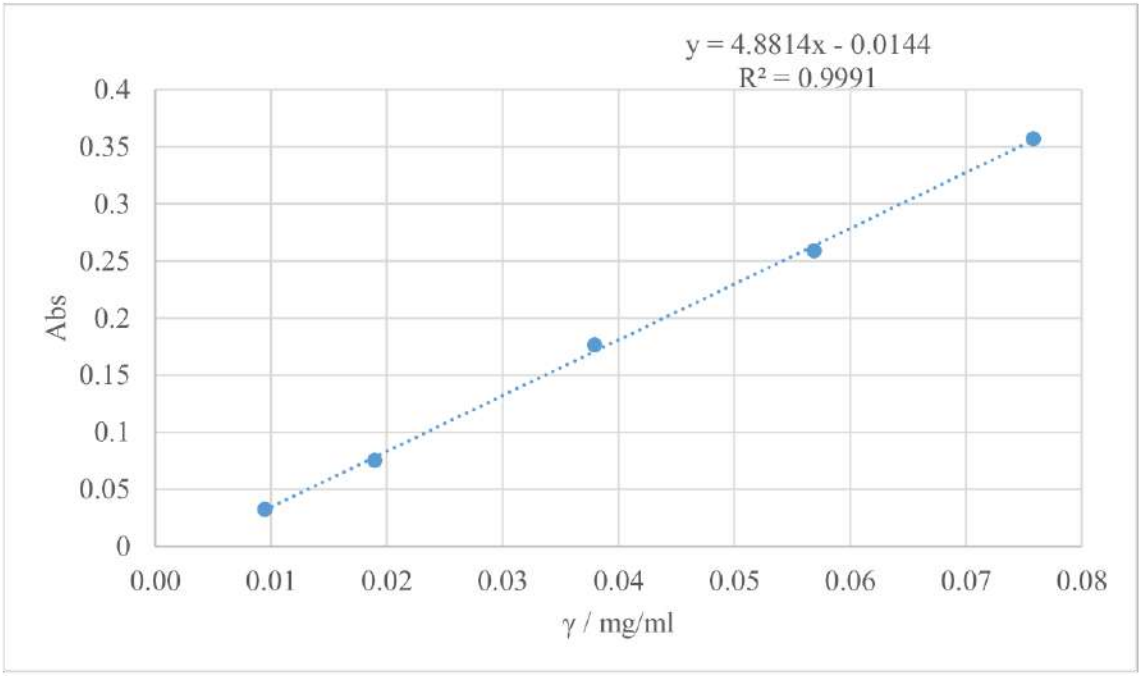


Figure 28 The calibration diagram of EOI [135]

Using the calculated concentrations and the measured absorbance of 5 different solutions of EOI, a calibration diagram was created (Figure 28). The equation of a spectrophotometric calibration curve corresponded to equation y = 4.8814x - 0.0144, where $R^2 = 0.9991$ illustrates high linear correlation between the EOI concentration and the peak area at 265 nm [135].

EC-EOI microcapsules

During the microcapsules synthesis procedure, a specified amount of oil (Table 17) was used but the real amount of oil in synthesised microcapsules was unknown. With the aim of identifying the unknown concentration of EOI in synthesised microcapsules further analysis was performed. 0.001 g of synthesized microcapsules (with varying amount of EOI (m_1 = 0.15 g, m_2 = 0.20 g, m_3 = 0.30 g)) were dissolved in 10 ml of n-hexane

(5 minutes of stirring). Absorbance of isolated EOI was measured with UV spectrophotometer [135].

Based on the qualitative analysis of EOI in microcapsules and the calibration diagram, a quantitative analysis of EOI in EC microcapsules was also performed.

Calibration diagram for EOI

EOI supplied from Irex Aroma d.o.o. was diluted in n-Hexane, HPLC grade, obtained from Lachner. The solution of 0.019 g EOI was dissolved in 100 ml of n-Hexane. Concentration and purity tests of the prepared solution were measured by UV Spectrophotometer, Carry 50 at 265 nm. After the checkpoint of the solution, five different volumes of the stock solution were pipetted into a 10 ml volumetric flask and diluted to the mark with n-Hexane and HPLC calibration curve of EOI was made.

It can be observed that all three types of microcapsules synthesis (m_1, m_2, m_3) have a maximum absorbance at 265 nm (Figure 29) which is characteristic for EOI. Empty microcapsules (m_0) don't present such a behaviour.

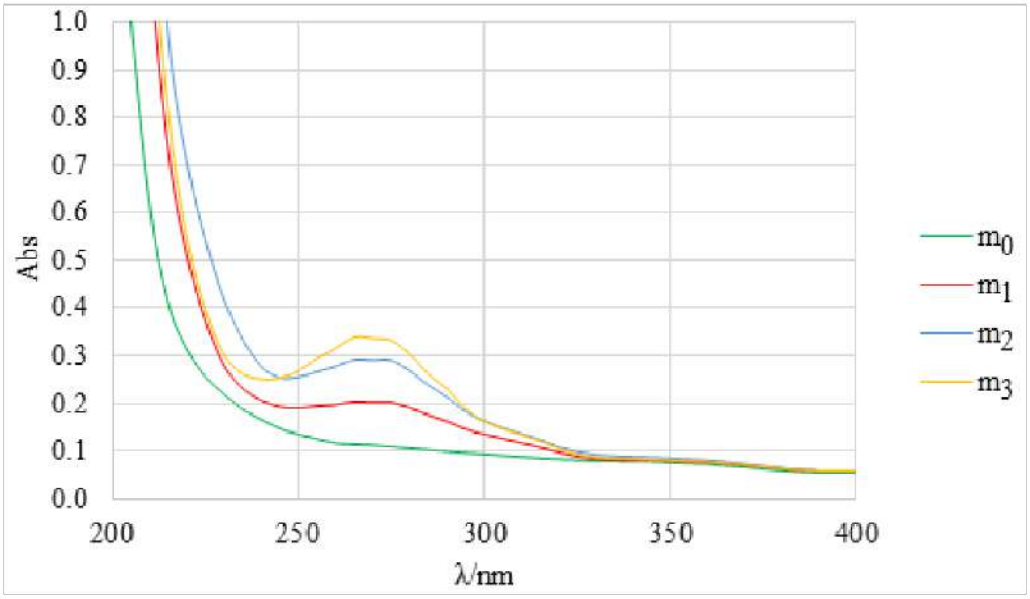


Figure 29 UV spectrum of microcapsules, varying amount of EOI (Table 21) [135]

The absorbance was measured and concentration of synthetized microcapsules were calculated using equation from calibration diagram (Figure 28), their concentrations were presented in Table 21.

Table 21 Measured absorbance and concentration of EOI in microcapsules [135]

Synthesis	m / g (of EOI in MC)	Absorbance	γ / μg/ml
m_1	0.15	0.20226	44.4
m_2	0.20	0.29145	62.7
m_3	0.30	0.33757	72.1

Table 22 shows the amount of utilization of EOI in the synthesis of microcapsules ($m_{2\text{-}2}$). The absorbance was measured at a wavelength of 265 nm on an absorption spectrophotometer. The unknown concentration of EOI, which was not fully synthesized into a microcapsule, was taken after centrifuge and calculated using a calibration diagram as well [135].

Table 22 Utilization of EOI in microcapsules synthesis ($m_{2\text{-}2}$) [135]

EOI	A	γ / μg/ml	Utilization
In microcapsules ($m_{2\text{-}2}$)	0.29145	62.7	82.83 %
In residual solution	0.04902	13.0	

From the concentration of microcapsules $m_{2\text{-}2}$ (γ = 62.7 μg/ml) and the concentration of the residual solution (γ = 13.0 μg/ml) (Table 22), the utilisation of EOI was calculated. The results revealed that 17.17 % of EOI has not been synthesized and stayed in residual solution.

EC-toc and EOI microcapsules

UV spectrophotometry was used for qualitative and quantitative analyse of EC-toc and EOI microcapsules. Synthesized EC-toc and EOI microcapsules of mass 1 mg were dissolved in 5 ml of methanol (γ = 200 μg/ml), mixed on Vortex using 1900 rpm for 60 s and analysed by Spectrophotometer Cary 50 Solascreen afterwards. Quantitative analysis of EOI in EC-toc and EOI microcapsules was also performed using calibration diagram.

Figure 30 presents UV spectrum of EC-toc and EOI microcapsules. The peak is expanded within the area of 250 and 300 nm, which was expected considering the spectra of active substances (Figure 25 a and Figure 26).

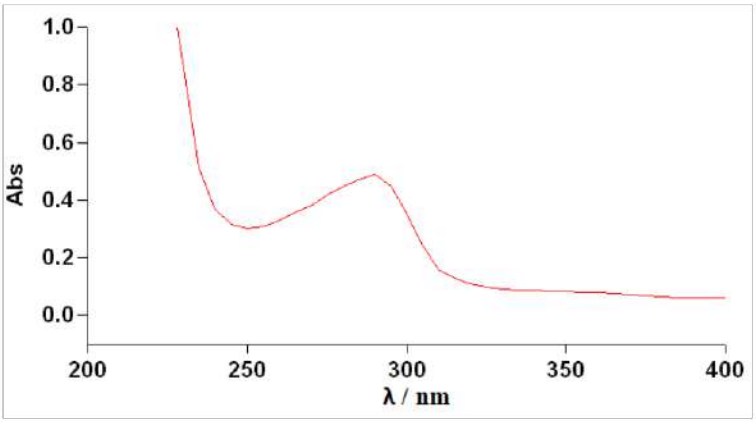


Figure 30 UV spectrum of EC-toc and EOI microcapsules

Detailed quantitative analyse of α-toc in EC-toc and EOI microcapsules was presented with HPLC analyse. Quantitative analyse of EOI in EC-toc and EOI microcapsules was performed using calibration diagram for EOI (Figure 28). Absorption at 265 nm is 0.488

so concentration of EOI in 1 mg EC-toc and EOI microcapsules is 103 μg/ml (concentration was calculated using calibration diagram of EOI (Figure 28)).

UV spectrophotometry analysis summary

In Table 23 are presented UV spectroscopy analysis results of EC-toc microcapsules (EC-toc MC), EC-EOI microcapsules (EC-EOI MC) with varying amount of EOI (m_1, m_2, m_3) (Table 21) and EC-toc and EOI microcapsules (EC-toc + EOI MC).

Table 23 Summary of UV spectroscopy analyses of EC microcapsules

sample		λ_{max} / nm	Absorbance	γ / μg/ml
EC-toc MC*		292	0.57100	/
EC-EOI MC	m_1	265	0.20226	44.4
	m_2	265	0.29145	62.7
	m_3	265	0.33757	72.1
EC-toc + EOI MC		265	0.48800	103

From the obtained results, it can be seen that UV spectroscopy analyse was suitable for qualitative analyse of both analysed active substances (α-toc and EOI). Comparing the concentration of active substances (EOI) in synthetized microcapsules (m_1, m_2, m_3) of the same mass (1 mg), results showed that by increasing the mass of EOI in the synthesis, the EOI concentration in the microcapsules increased as expected. UV spectroscopy results

* Quantitative analyse of EC-α-toc microcapsules will be detailed analysed using HPLC method which is recommended by the norm [50]

of microcapsules with combination of active substances (EC-toc + EOI MC) showed that EOI were present in microcapsules.

4.5 Results of gravimetric method

Detail optimisation of "Stirring technique" in **EC-EOI microcapsules** synthesis was analysed under the protocol described in *chapter: "4.1 Synthesis and optimisation of parameters of EC microcapsules synthesis", page 67*. After the synthesis of microcapsules, the most effective was the m_2 synthesis of microcapsules with 0.20 g of EOI. Microcapsule losses were lower than others after gravimetric analyse, microcapsules were easily removed from the filter paper (Table 24).

Table 24 Synthesis of microcapsules with EOI

Type of synthesis	$m_{(SDS)}$ / g	$m_{(EC)}$ / g	$m_{(EOI)}$ / g	$V_{(EA)}$ / ml	$\mathbf{m_{(MC)}}$ / g	$\mathbf{m_{(MC\ residue\ on\ filter\ paper)}}$ / g
m_0	1.000	0.600	/	25	0.130	0.015
m_1	1.000	0.600	0.15		0.429	0.042
m_2	**1.001**	**0.601**	**0.20**		**0.389**	**0.040**
m_3	1.000	0.600	0.30		0.464	0.081
m_4	1.003	0.602	0.60		/ (film)	/

Synthesis m_4 formed a film on the filter paper and the microcapsules could not be removed from it. It can be concluded that the applied mass of 0.60 g of EOI (m_4) is too high for this microcapsule synthesis process.

4.6 Results of qualitative analysis of EC-toc microcapsules by FTIR

For qualitative analyses of **EC-toc microcapsules** FTIR analyse was used. In FTIR spectrum of EC-toc microcapsules (a), ethyl cellulose (EC) (b) and standard α-toc (c) are shown at Figure 31.

Absorption bands of α-toc (c) were at following wavelengths: 3358 cm^{-1} for -OH, 2925 cm^{-1} and 2866 cm^{-1} for asymmetric and symmetric stretching vibrations of the CH_2 and CH_3, 1642 cm^{-1} for C-C bands formation, 1455 cm^{-1} for phenyl skeletal (1450 cm^{-1}) and methyl asymmetric bending (1460 cm^{-1}), 1376 cm^{-1} for methyl symmetric bending, 1253 cm^{-1} for CH_2, 1082 cm^{-1} for plane bending of phenyl and 915 cm^{-1} for trans CH_2 stretching [124, 125].

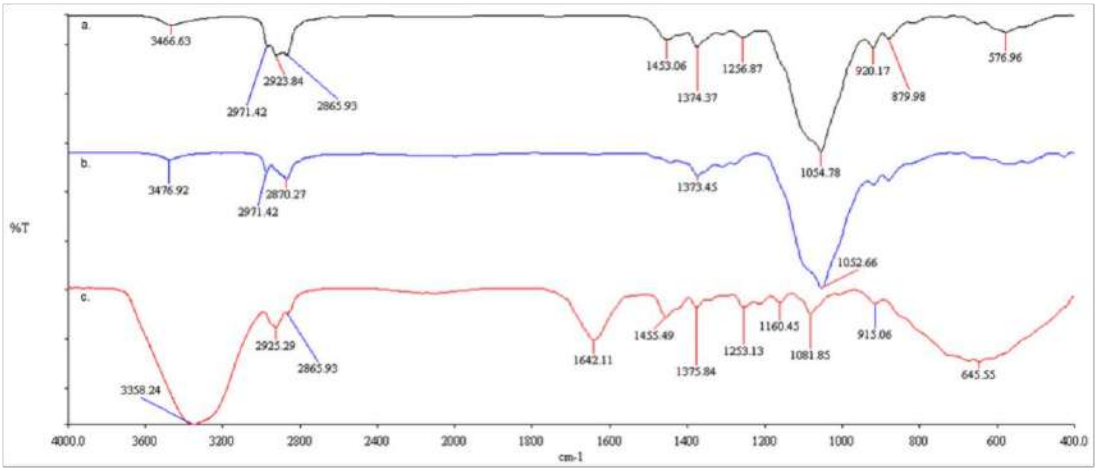


Figure 31 FTIR spectrum of: a. EC-toc microcapsules; b. EC and c. standard α-toc

Comparing FTIR spectrum it can be noticed that majority peaks of EC-toc microcapsules corresponded to peaks of ethyl cellulose, confirming the presence of EC in microcapsules membrane but not the characteristic peak for α-toc. That was not unusual because ATR measuring the microcapsule surface of so it's difficult to see spectrum of tocopherol located in microcapsule core. Considering obtained results, FTIR analyses was not performed on other synthetized microcapsules neither on cosmetotextiles.

During previous research, cosmetotextiles were also analysed by FTIR and spectrograms have not confirmed a presence of active substance on cosmetotextiles [130].

4.7 Results of characterization of EC-EOI microcapsules with CLSM

Synthetized **EC-EOI microcapsules** using two different mixing techniques during synthesis: stirring (A) and ultrasound (B), were analysed using CLSM.

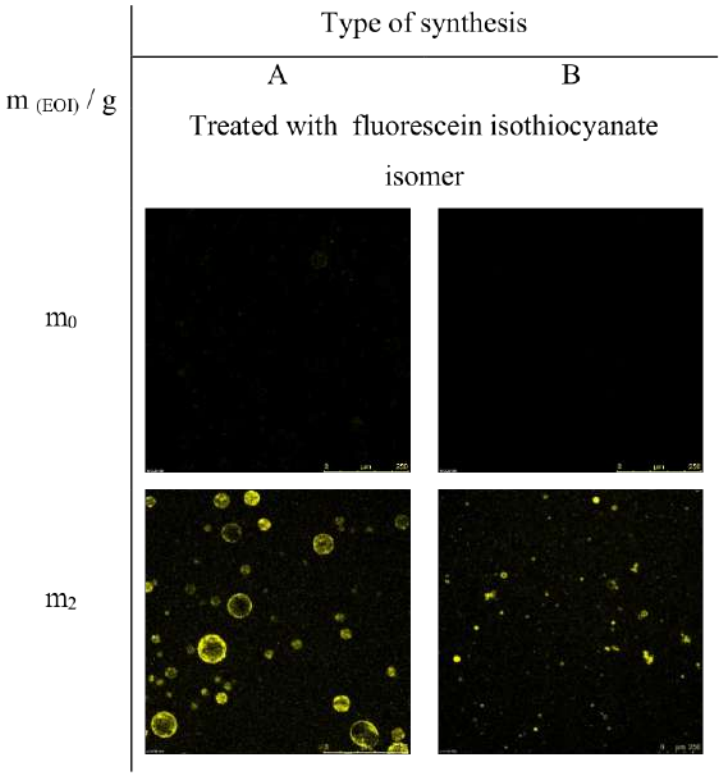


Figure 32 Confocal microscope scans (magnification 250x) of microcapsules with EOI (m_2) and without EOI (m_0), by using two different techniques of synthesis: A (stirring) and B (ultrasonic)

Synthesized microcapsules were coloured with the Fluorescein isothiocyanate isomer (FII) (Sigma Aldrich). Coloured microcapsules, either oil-free (m_0) or with the EOI (m_2)

(mass of oil in synthesis is 0.2 g) were analysed with confocal laser scanning microscope (CLSM), (Figure 32).

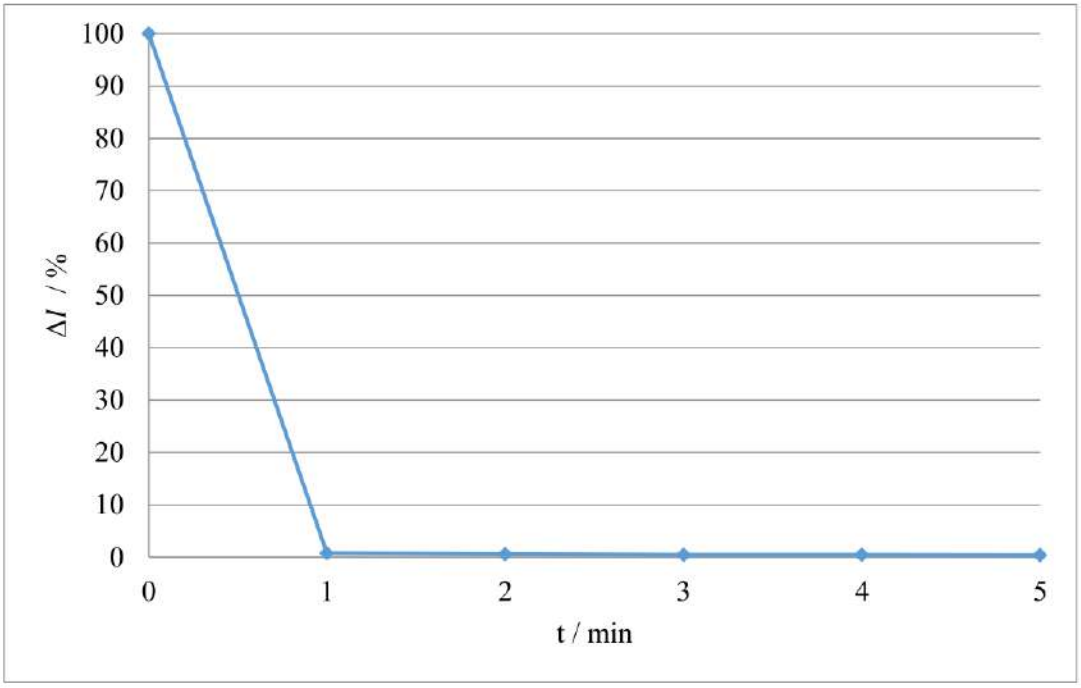
Obtained results confirmed the influence of synthesis technique on microcapsule size. Microcapsules synthetized with the technique A (stirring technique) were in the size range of 10 - 100 μm and microcapsules synthetized with the technique B (ultrasound technique) in the size range of 1 - 40 μm. Microcapsules synthesised with ultrasound (under the technique B) had 10 times smaller size when compared to microcapsules synthesised with stirring (under the technique A) [136]. Mixing with ultrasound resulted in faster mixing and the formation of smaller particles. EC-EOI microcapsules (A m_2 and B m_2) scanned with fluorescence had yellow dyed parts, on the other hand microcapsules without EOI (A m_0 and B m_0) had undyed parts due to the fact that the EOI was not part of this kind of microcapsules. CLSM combined with fluorescence dyeing pre-treatment confirmed the presence of EOI within microcapsules.

4.8 Results of antioxidant activity ESR (Antioxidant activity)

Antioxidant activity of **active substances** (α-toc and EOI), **oil-free EC microcapsules**, **EC-toc microcapsules, EC-EOI microcapsules** and **EC-toc and EOI microcapsules** were investigated using radical-scavenging activity (ESR method) described in *Methodology part, chapter ESR (Antioxidant activity)", pages 42 - 45.*

In Figure 33 results of antioxidant activity of active substances α-toc (Figure 33 a) and EOI (Figure 33 b) are presented. Figure 34 shows results of oil-free EC microcapsules, and Figure 35 antioxidant activity of EC-toc microcapsules, EC-EOI microcapsules and EC-toc and EOI microcapsules.

a.



b.

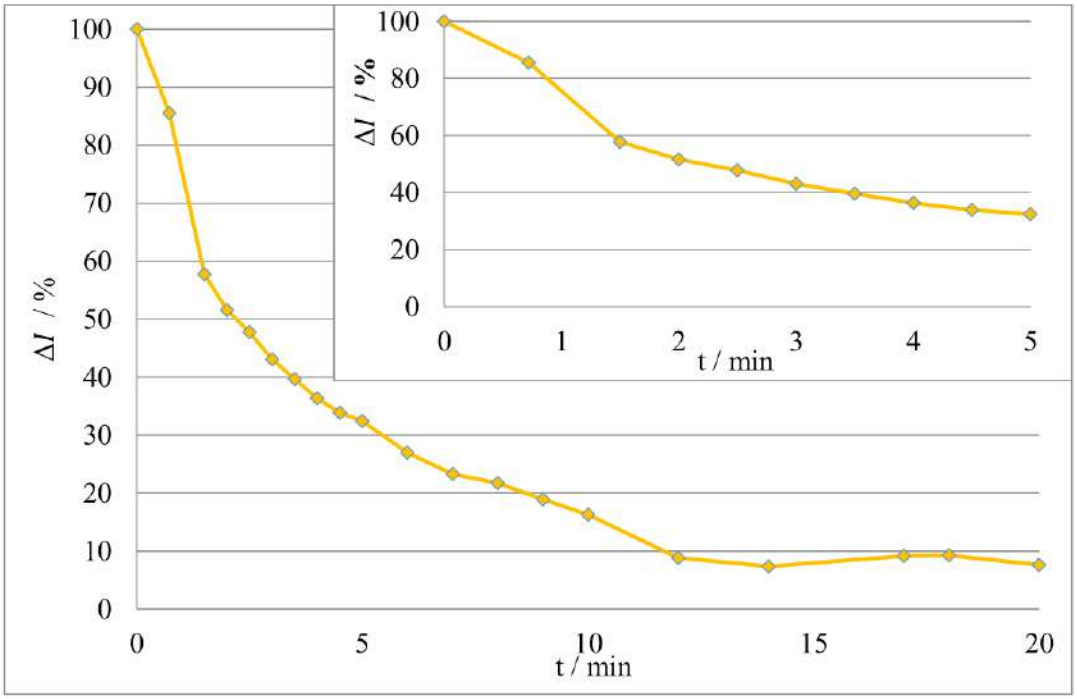


Figure 33 The relative signal intensity of the DPPH as a function of the reaction time t for active substance: a. α-toc and b. EOI

From the Figure 33 it is evident that α-toc had very strong antioxidant activity. Already after only 1 minute DPPH signal was completely lost, indicating that all radical molecules

were scavenged. EOI as an active substance also showed an important antioxidant potential. Relative signal intensity measured 12 minutes after contact of the active substance with DPPH solution was about 8 % and it remained more or less constant until the end of measurement.

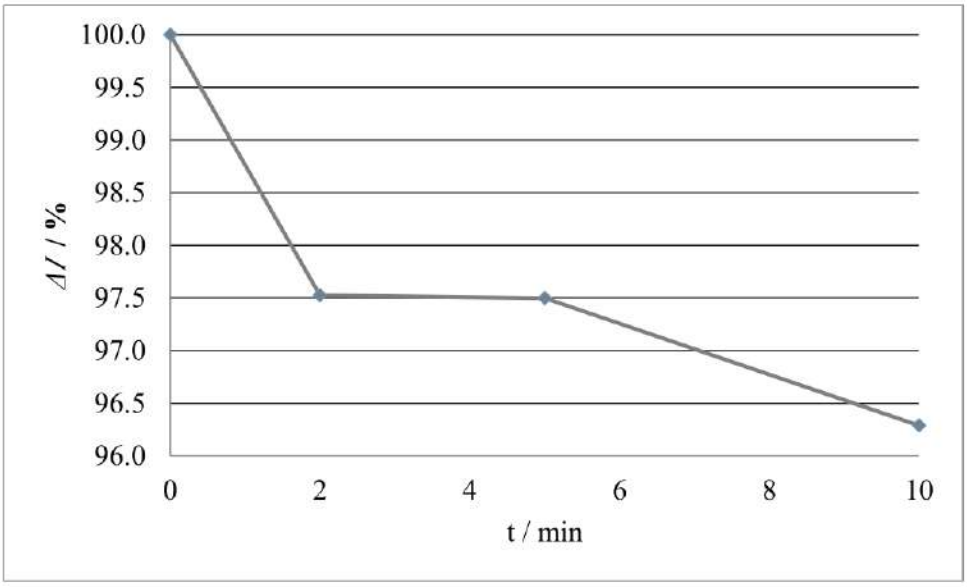


Figure 34 The rest of the DPPH signal as a function of the reaction time t for oil-free EC microcapsules

Oil-free EC microcapsules showed an extremely low (practically insignificant) antioxidant activity (Figure 34), as expected, because they did not contain any active substance. Consequently, it can be assumed that the contribution of the EC microcapsules themselves to the overall antioxidant power of the investigated samples could be negligible.

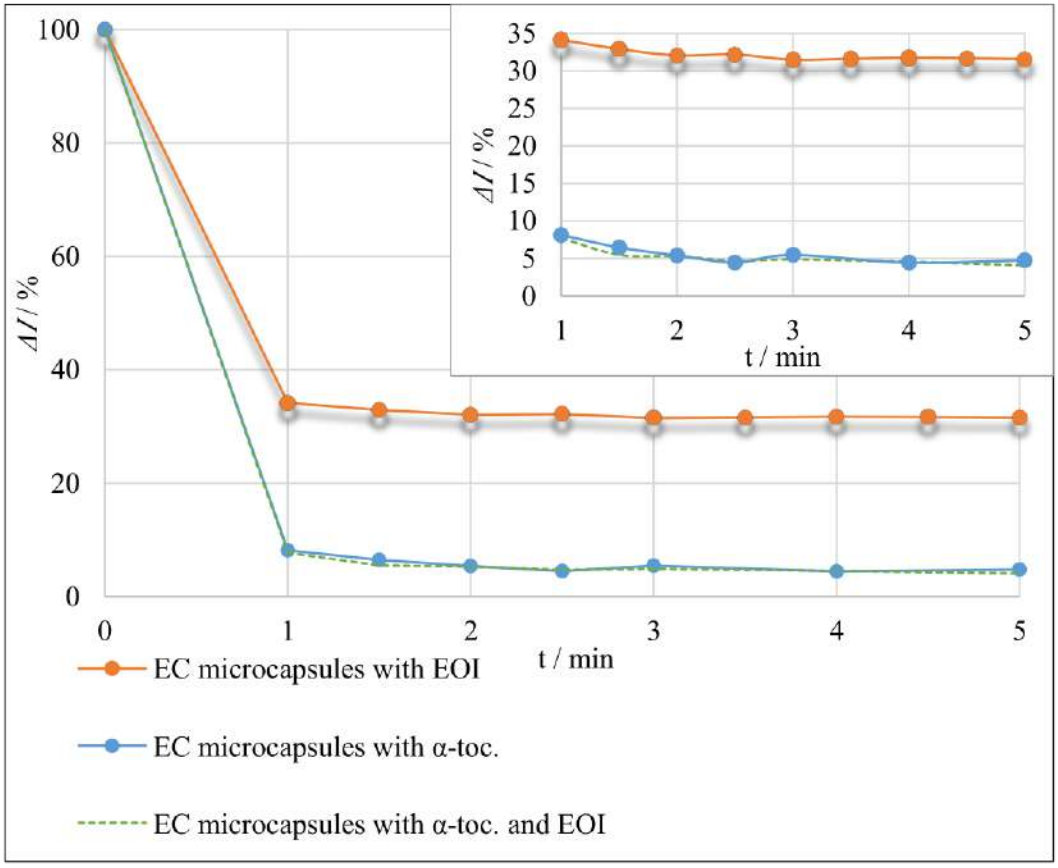


Figure 35 The relative signal intensity of the DPPH as a function of the reaction time t for EC-toc microcapsules, EC-EOI microcapsules and EC-toc and EOI microcapsules

From the Figure 35 it is noticeable that EC-toc microcapsules and EC-toc and EOI microcapsules reacted almost identically and faster than EC microcapsules with EOI only. This was evident during the first minute of the reaction of DPPH with active components. The antioxidant activity of all samples after the first minute seemed to be negligibly small. However, a significantly lower value of the relative intensity ratio for EC-toc microcapsules and EC-toc and EOI microcapsules (about 5 %), compared to that of EC microcapsules with EOI only, suggested their higher antioxidant capacity. Moreover, on the base of practically the same scavenging behavior of EC-toc microcapsules and EC microcapsules with α-toc together with EOI it was possible to assume that some type of synergy might occur between these two active components. In fact, one might think that the presence of α-toc improved the antioxidant power of EOI.

It should be noticed here that the amount of the pure α-toc and α-toc coupled with EOI in investigated microcapsules was the same. For more precise differentiation of the antioxidant power of these two samples it might be useful to perform ESR measurements with the samples containing smaller concentration of EC microcapsules.

Recently, a new method for measuring antioxidant power (AP) has been applied in the Ruđer Bošković Institute. This method is based on the determination of antioxidant power by measuring antioxidant activity and antioxidant capacity of samples using ESR spectroscopy. Results of AP presented absolute values expressed in AU units, where 1 AU corresponds to AP of the solution of vitamin C having a concentration of 1.00 ppm [137, 138]. It would be interesting to apply this method to the samples used in this work. Particularly, as research of special importance for practical application, it was planned to investigate AP of microcapsules embedded in textile fibers (cosmetotextiles).

B. Results of analysed cosmetotextiles

Selected EC microcapsules were applied on textile materials using different application technique. **EC-toc microcapsules** (synthesis E-2) were applied by impregnation and exhaustion. **EC-EOI microcapsules** (synthesis m_2) were applied on textiles using impregnation, exhaustion and electrospinning. All process were described in *chapter: "Application procedures" pages 48 - 52.*

Impregnation was used for application of **EC-toc microcapsules** on cotton fabric, PES/cotton, silk fabric and nonwoven textile liocel (Table 6). Conducted analyses on this kind of cosmetotextiles were: SEM, drop test and whiteness.

Exhaustion was used for application of **EC-toc microcapsules** on cotton fabric in three concentrations (Table 9). That kind of cosmetotextiles were analysed by SEM, HPLC (before and after light fastness), antioxidant activity (ABTS method) and whiteness.

Analyse of **EC-EOI microcapsules** synthetized by "stirring" (A) and "ultrasound" (B) impregnated on cotton and modal fabrics (Table 6) was done using SEM.

EC-EOI microcapsules were applied on cotton fabric using exhaustion. That kind of cosmetotextiles were analysed by SEM, HPLC, UV/VIS spectrophotometry (before and after fastness tests on washing, rubbing and light) and antioxidant activity (ABTS method), whiteness, antibacterial test and dermatology test (Patch test).

Electrospinning was used for application of **EC-EOI microcapsules** on modal fabric. Types of baths and their specification were described in *chapter: "Electrospinning", pages 51 and 52.* Analyses of cosmetotextiles with EC-EOI microcapsules applied by electrospinning was SEM.

4.9 SEM analyses

SEM microscope was operated at 5 - 10 kV and various magnification levels. Detector used for this research was SE detector (detector for secondary electrons). The samples were placed on the SEM stubs (10 mm) using a two-sided adhesive tape. Prior to the SEM measurements all samples were sputter-coated for better electron conductivity.

Depending on the current availability of the evaporating material and evaporating device the samples were evaporated:

- Gold/palladium/carbon-based sputter coater was used (SC7620-CF Mini, Quorum Technologies),
- Chromium-based evaporator that coats non-conductive samples with electrically conductive particles (Q150T ES Plus, Quorum Technologies).

Presence of EC microcapsules, their distribution, uniformity, shape and size on cosmetotextiles were investigated by the SEM analysis under different magnifications (100x - 5000x).

SEM pictures of non-treated textiles and impregnated cosmetotextiles with **EC-toc microcapsules*** are presented at Figure 36. Analysed textiles were: cotton, PES/cotton, silk and liocel. Microcapsules were present on all impregnated textiles. They all had spherical shape and a diameter of 10 - 60 μm. It can be seen that the largest amount of microcapsules was present on liocel, which was to be expected due to the nonwoven structure of textile.

* Cosmetotextiles impregnated with **EC-α-toc microcapsules** were evaporated with an alloy of palladium and gold (2 x 180 s).

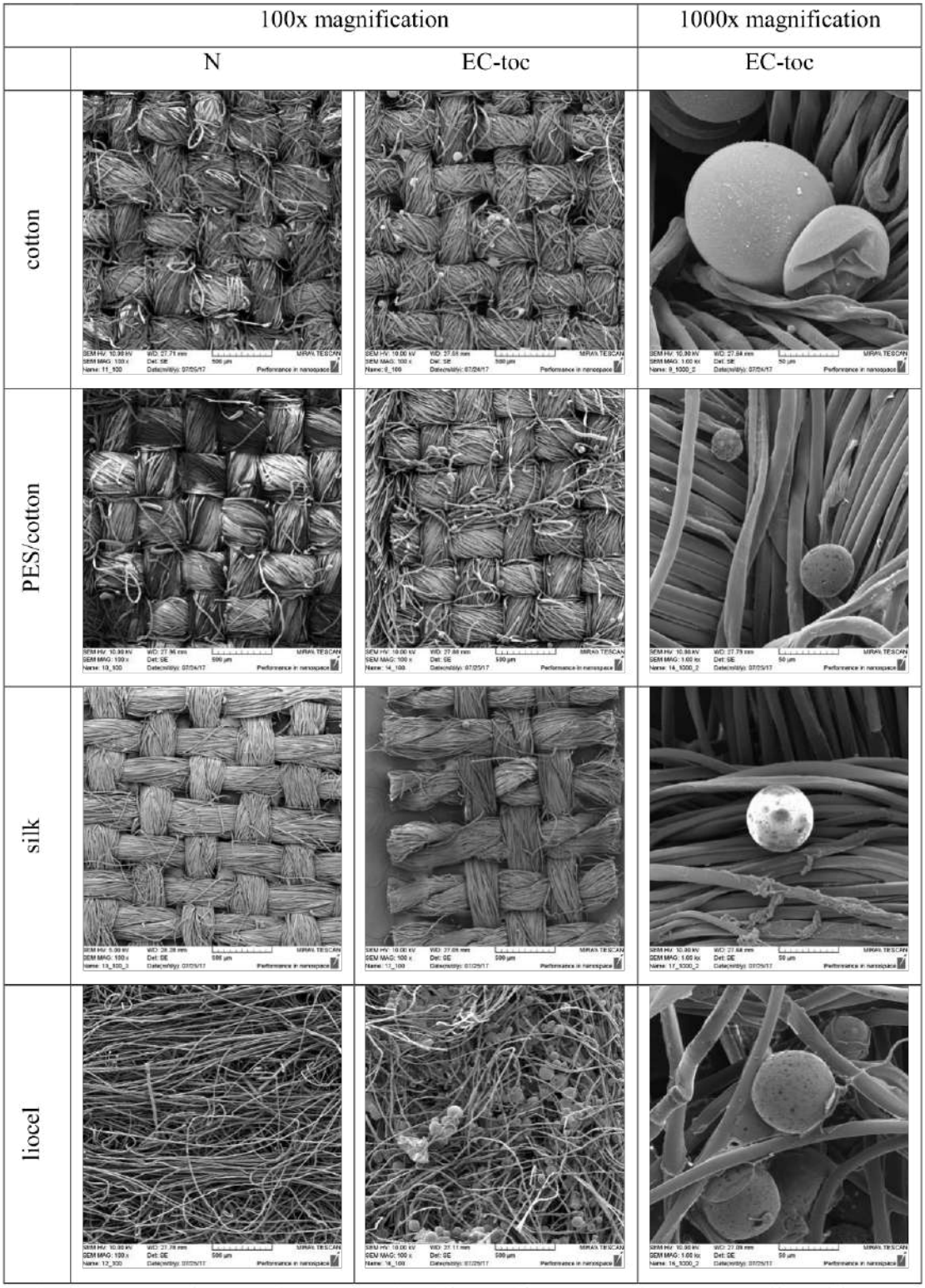


Figure 36 SEM pictures of non-treated textiles and impregnated cosmetotextiles with EC-toc microcapsules: cotton; PES/cotton; silk and liocel, magnification 100x and 1000x

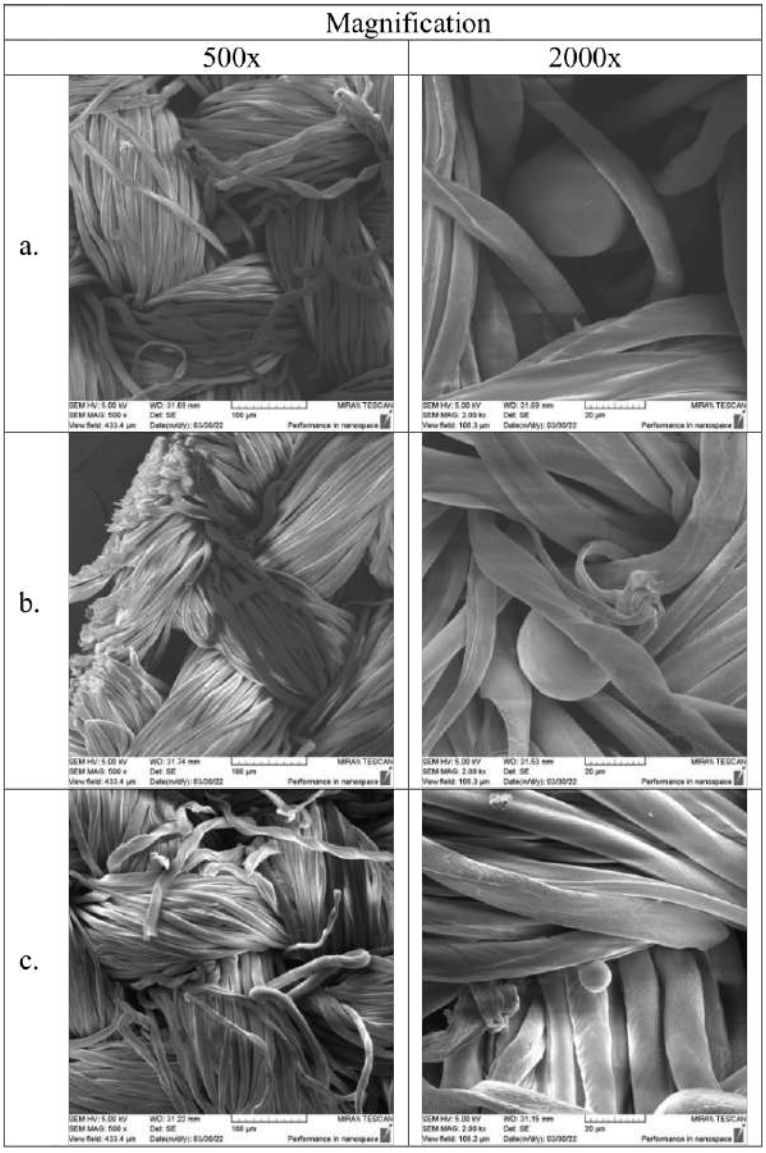


Figure 37 SEM pictures of cotton cosmetotextiles applied with EC-toc microcapsules using exhaustion with three different concentration of MC: a. ex_e-I (4 %), b. ex_e-II (8 %) and c. ex_e-III (12 %), magnification 500x and 2000x

Cotton cosmetotextiles applied (using exhaustion) with three different concentrations of **EC-toc microcapsules** ex_e-I (4 %), ex_e-II (8 %) and ex_e-III (12 %) (Table 9) are presented at Figure 37.*

Bonded microcapsules had spherical shape and exhaustion didn't have any influence on their morphological structure. It can be seen that increasing of concentration didn't affect the amount of microcapsules on cosmetotextiles.

Cotton and modal cosmetotextiles impregnated with **EC-EOI microcapsules** synthetized by "stirring" (A) and "ultrasound" (B) techniques in 1000x and 5000x magnification* are presented at Figure 38 and Figure 39.

* Cosmetotextiles applied with **EC-α-toc microcapsules** using exhaustion, were evaporated with an alloy of chrome (120 s).

* Cosmetotextiles impregnated with **EC-EOI microcapsules** were evaporated with an alloy with palladium and gold (2 x 180 s).

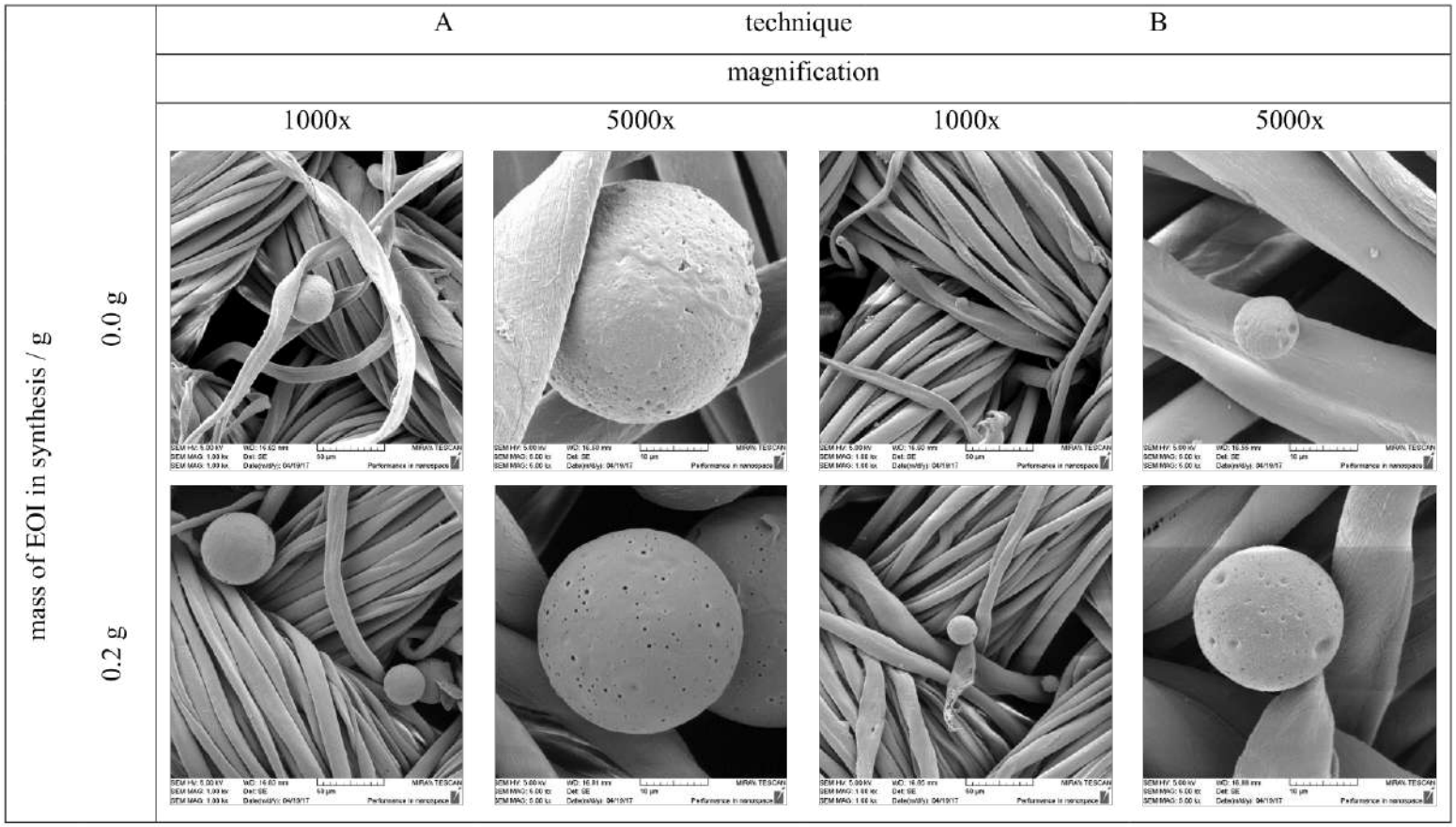


Figure 38 SEM pictures of cotton cosmetotextiles impregnated with EC-EOI microcapsules synthetized by stirring technique (A) and with ultrasonic technique (B) with different mass of EOI: 0.0 g and 0.2 g

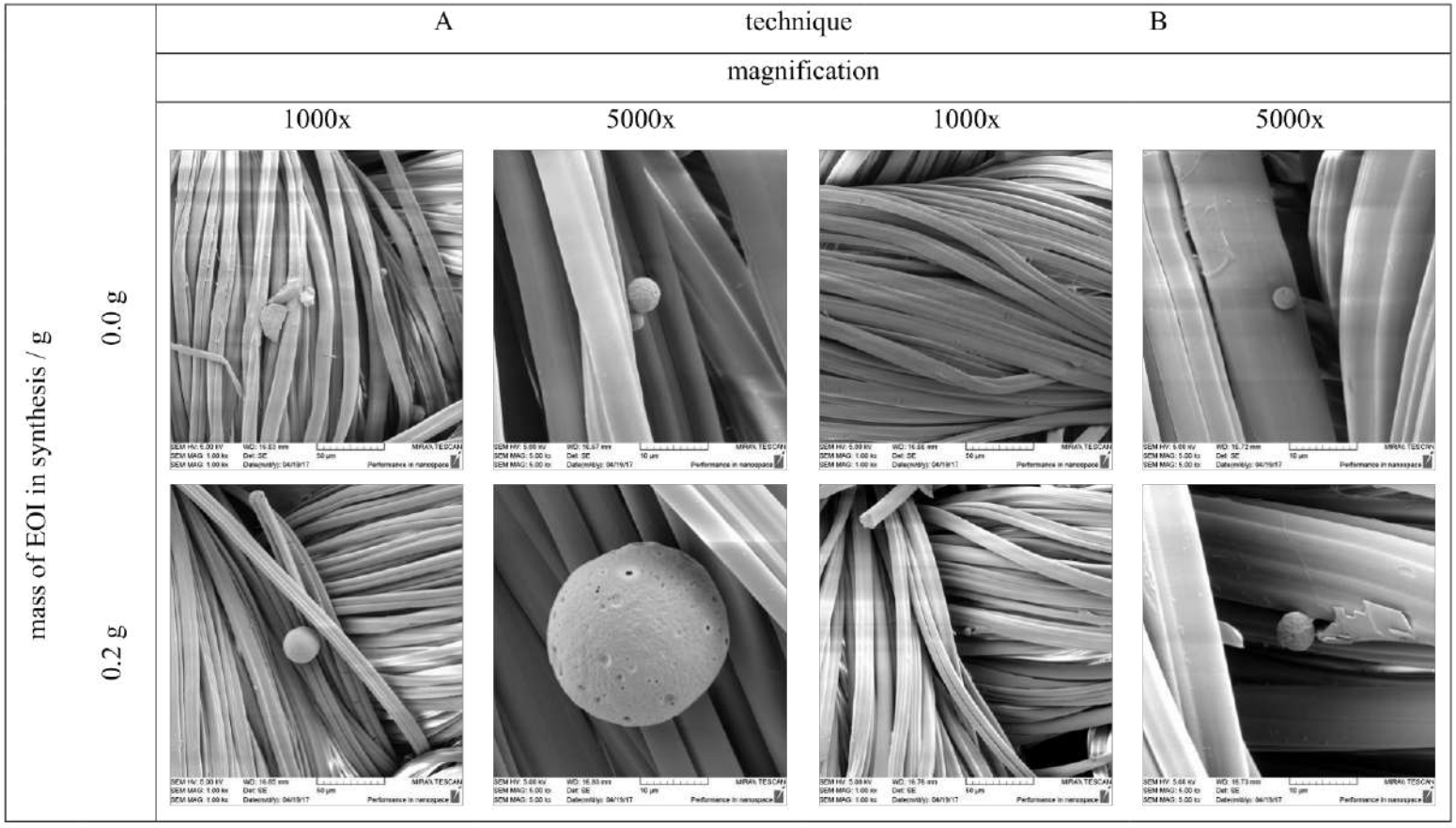


Figure 39 SEM pictures of modal cosmetotextiles impregnated with EC-EOI microcapsules synthetized by stirring technique (A) and with ultrasonic technique (B) with different mass of EOI: 0.0 g and 0.2 g, magnification 500x and 1000x

SEM pictures of cotton and modal cosmetotextiles confirmed the presence of EC-EOI microcapsules on the surface of textiles. Microcapsules synthetized using stirring technique (A) were in the size range of 10 - 60 μm with an average diameter of 45 μm. Microcapsules synthetized using ultrasonic device (B) were in the size range of 10-20 μm with an average diameter of 15 μm. SEM proved to be efficient tool to determine the size of applied microcapsules and therefore to differentiate between two synthesis used within this research. According to the literature, the smaller capsules are better because the greater the covering of the product is (longer the active substance lasts) and microcapsules that are washed out of the fibres in the first washing cycles are the larger microcapsules [67, 139]. It can thus be concluded that the ultrasound technique is more suitable than the stirring technique if smaller microcapsules are required.

SEM pictures of cotton cosmetotextiles applied with three different concentrations of **EC-EOI microcapsules** ex_S-I (4 %), ex_S-II (8 %) ex_S-III (12 %) (Table 9) using exhaustion* are presented at Figure 40.

Bonded microcapsules had spherical shape, confirming that exhaustion didn't have any influence on the morphological structure. It can be seen that increase of the concentration didn't affect the amount of microcapsules on cosmetotextiles.

* Cosmetotextiles applied with **EC-EOI microcapsules** using exhaustion, were evaporated with an alloy of chrome (120 s).

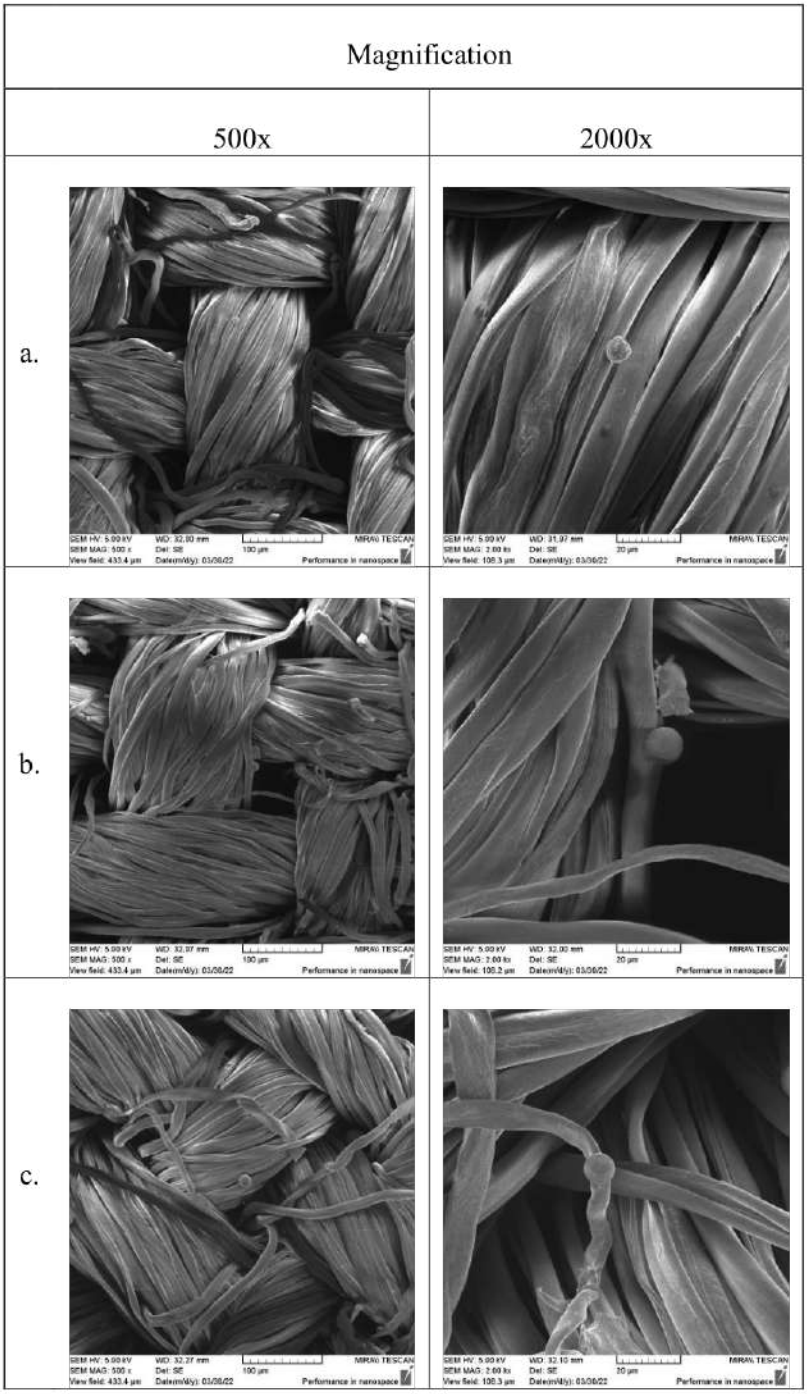


Figure 40 SEM pictures of cotton cosmetotextiles applied with EC-EOI microcapsules using exhaustion with three different concentration of MC: a. ex_S-I (4 %), b. ex_S-II (8 %) and c. ex_S-III (12 %), magnification 500x and 2000x

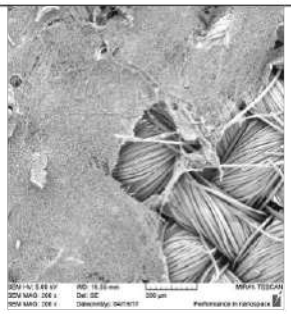
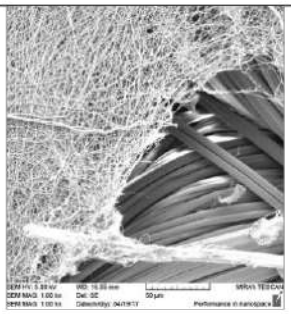
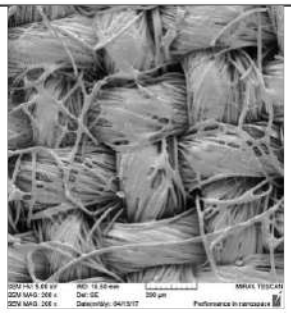
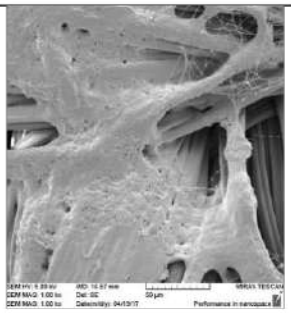
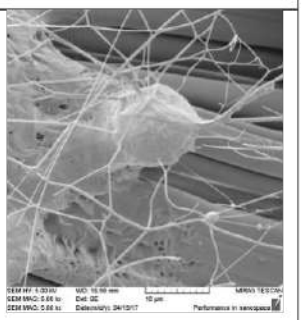
Textiles	Magnification		
	200x	1000x	5000x
a. M_I			
b. M_II			
c. M_III			

Figure 41 SEM figures of modal cosmetotextiles with EC-EOI microcapsules by electrospinning: a. bath with PEO (M_I), b. bath with PEO and EC microcapsules in solid state (M_II), c. bath with PEO and EC microcapsules in solution (M_III), magnification 200x, 1000x and 5000x

Surface of modal cosmetotextiles applied by **electrospinning with EC-EOI microcapsules (m_2)** were investigated by the SEM analysis at the different magnification (200x, 1000x and 5000x)*. A presence of EC-EOI microcapsules, their distribution, uniformity, shape and size was analysed on treated modal fabrics after electrospinning (Figure 41).

Figures of scanned samples after electrospinning showed that PEO fibres are more densely on samples treated solely with PEO (M_I) (Figure 41 a). Value of fibre diameter was within the range 0.5 - 1 μm. Samples treated with PEO and EC microcapsules in solution showed thin layer (Figure 41 c), which was also to be expected regarding the shortest time of electrospinning treatment and presence of water in solution for electrospinning. Samples treated with PEO and EC microcapsules in solid state contained acceptable layer of fibres with presence EC microcapsules (Figure 41 b).

SEM analyses after performed fastness tests (washing and rubbing) are presented at Figure 42*. Cotton cosmetotextiles applied with three different concentrations of **EC-EOI microcapsules** ex_S-I (4 %), ex_S-II (8 %) and ex_S-III (12 %) (Table 9) using exhaustion were analysed by SEM after 1, 2, 3 and 5 cycles of washing (Figure 40). Cotton cosmetotextile with **EC-EOI microcapsules** in optimal concentration ex_S-II (8 %) was also analysed by SEM after rubbing test (R).

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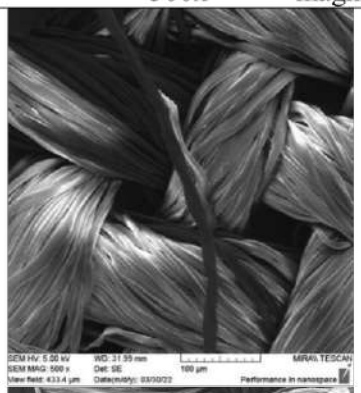
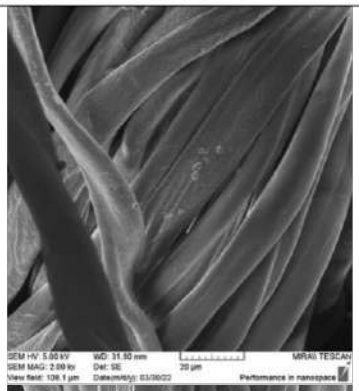
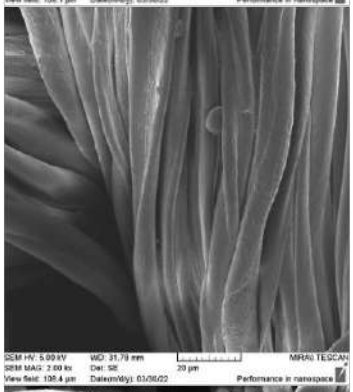
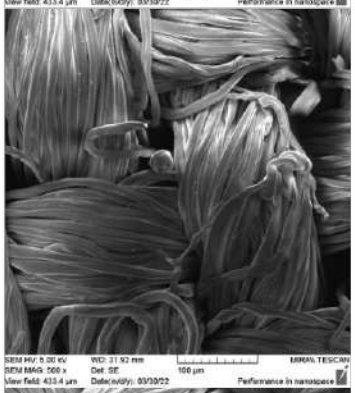
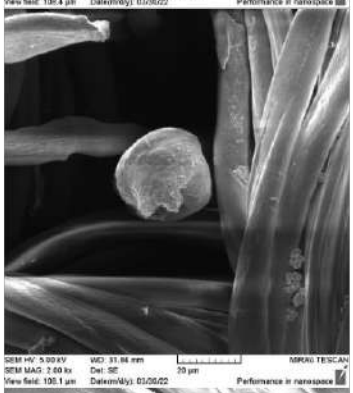
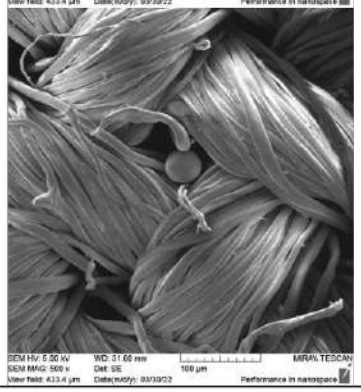
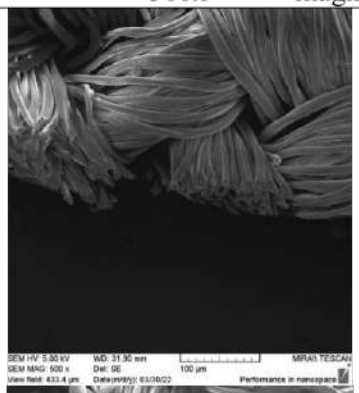
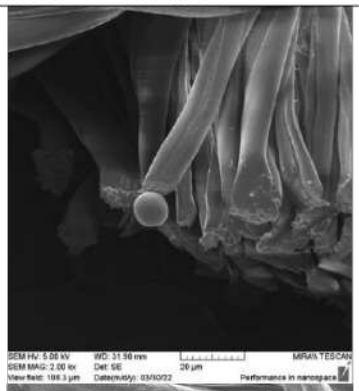
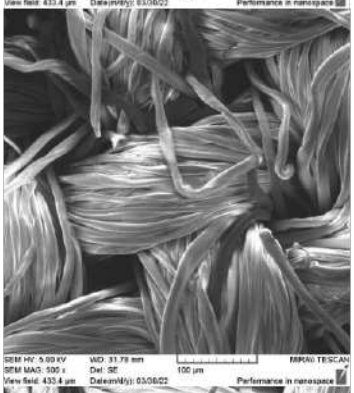
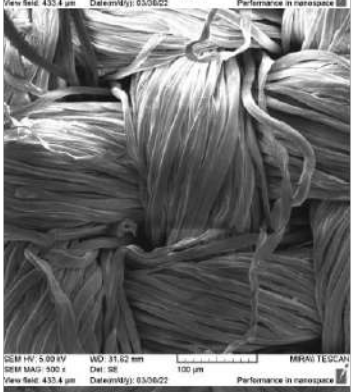
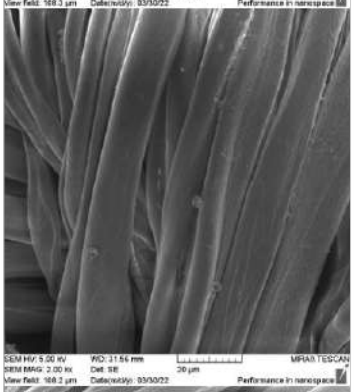
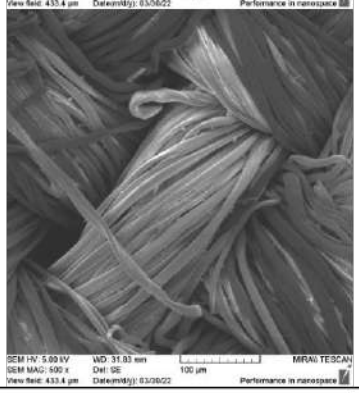
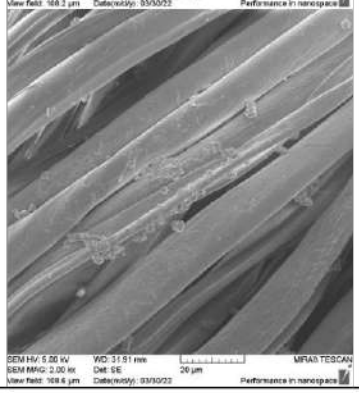
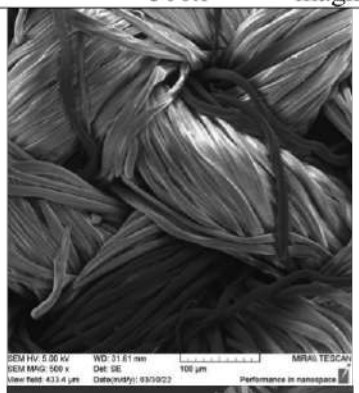
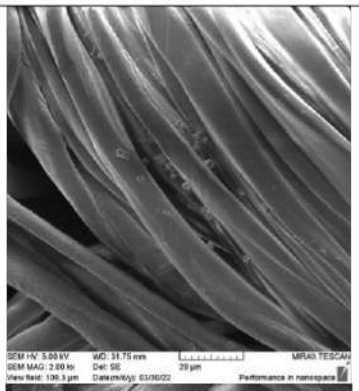
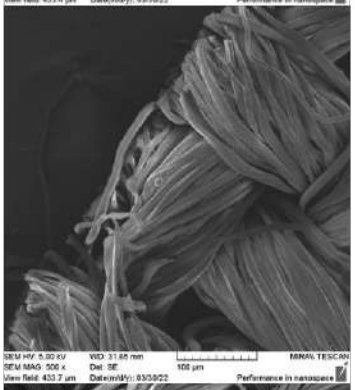
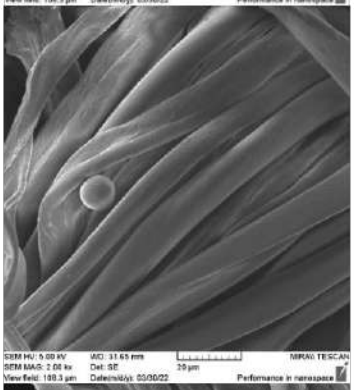
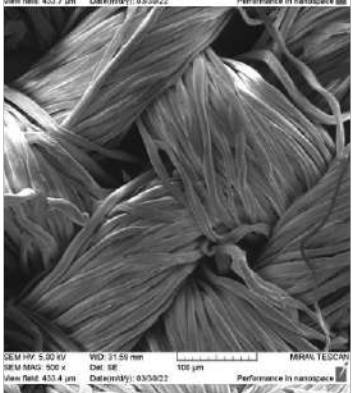
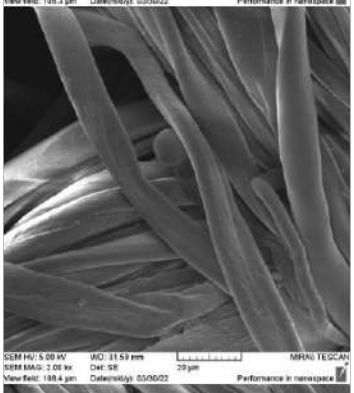
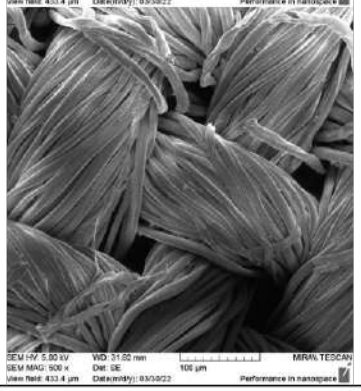
* Cosmetotextiles applied with **EC-EOI microcapsules** using electrospinning were evaporated with an alloy with palladium and gold (2 x 180 s).

* All cosmetotextiles analysed after fastness tests were evaporated with an alloy of chrome (120 s).

sample		500x magnification 2000x	
a. ex_S-I	1x		
	2x		
	3x		
	5x		

sample		500x magnification	2000x
b. ex_S-II	1x		
	2x		
	3x		
	5x		

sample		500x magnification	2000x
c. ex_S-III	1x		
	2x		
	3x		
	5x		

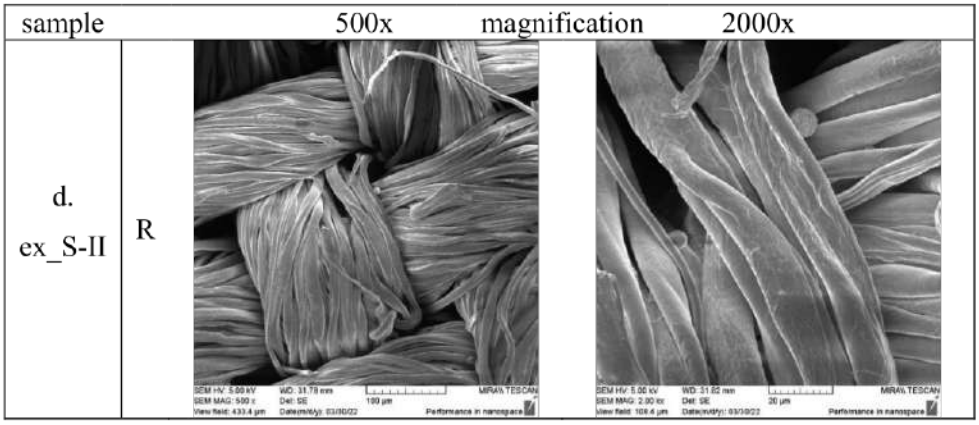


Figure 42 SEM pictures of cotton cosmetotextiles applied with EC-EOI microcapsules with three different concentration of MC: a. ex_S-I (4 %), b. ex_S-II (8 %) and c. ex_S-III (12 %), after 1, 2, 3 and 5 cycles of washing and d. after rubbing test on ex_S-II (8 %), magnification 500x and 2000x

Comparing Figure 40 (before the fastness tests) and Figure 42 (after the fastness tests) it can be concluded that cosmetotextiles still contained microcapsules after performed washing cycles. Concentration of microcapsules didn't have significant influence on fastness properties so it can be concluded that the optimal concentration of microcapsules is 8 %. Rubbing fastness (Figure 42 d) gave satisfactory results because the microcapsules were still present on the textile and, on the other hand, the active ingredients were released. Additional analyses will determine whether there had been a decrease in the EOI concentration after the rubbing test which indicated controlled release of microcapsules.

4.10 Results of HPLC analysis of cosmetotextiles with EC-toc microcapsules

Cotton cosmetotextiles with EC-toc microcapsules (synthesis E-2) applied by exhaustion in three different concentrations (Table 9) were analysed by HPLC method. HPLC chromatograms of cosmetotextiles with EC-toc microcapsules in three different concentrations are presented in Figures 43, 44 and 45.

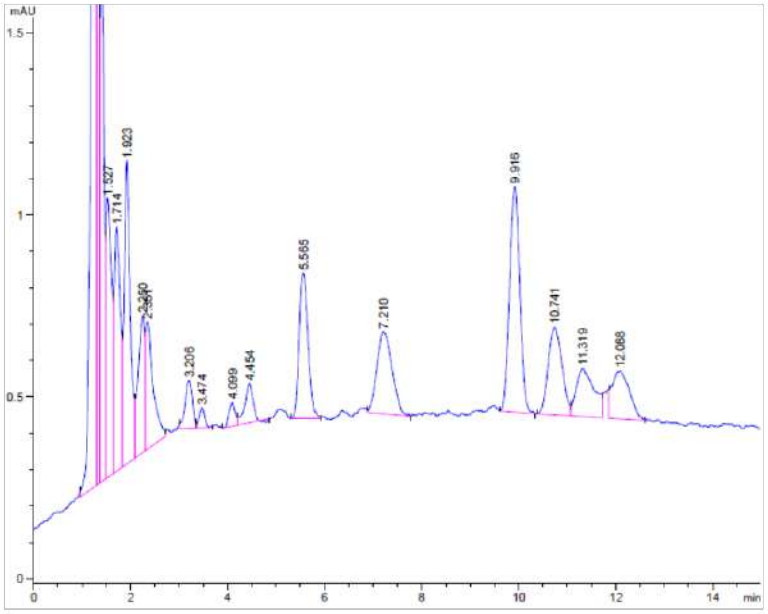


Figure 43 HPLC chromatogram of cosmetotextile with EC-toc microcapsules in concentration 4 % ex_e-I (Table 9)

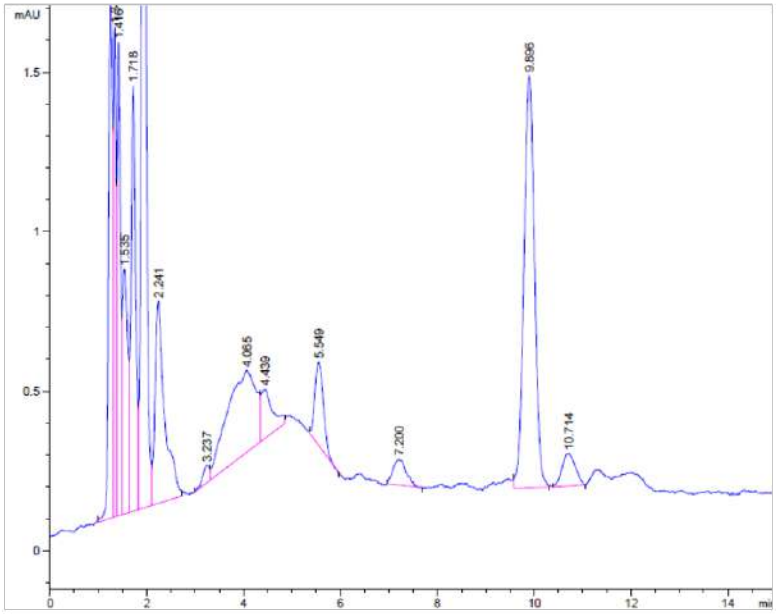


Figure 44 HPLC chromatogram of cosmetotextiles with EC-toc microcapsules in concentration 8 % ex_e-II (Table 9)

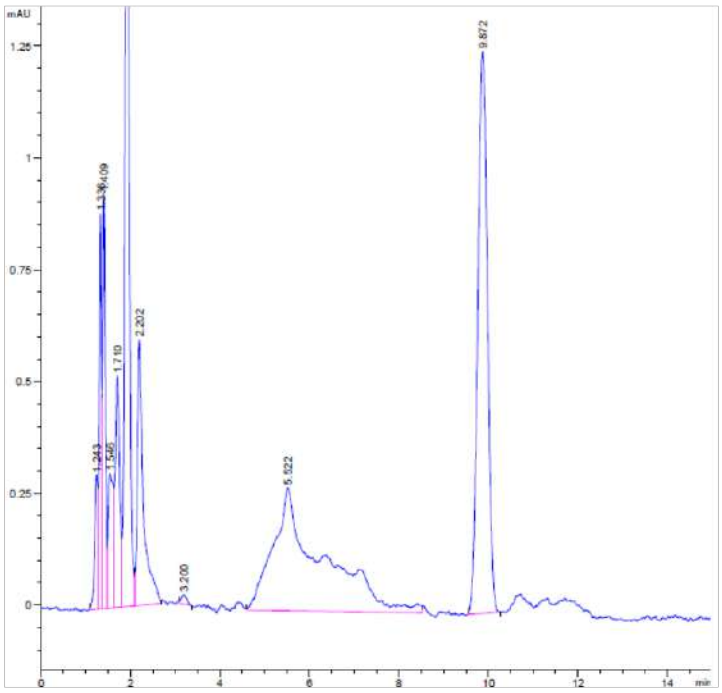


Figure 45 HPLC chromatogram of cosmetotextile with EC-toc microcapsules in concentration 12 % ex_e-III (Table 9)

The measurements of all analysed samples showed absorbance peaks of α-toc at wavelength of 292 nm, in retention time 9.872 – 9.916 min which was in accordance with the standard protocol for α-toc, confirming the presence of α-toc on cosmetotextiles. Value of area below the peak of cosmetotextiles with lowest concentration (ex_e-I) was 9.1 mAU*s (Figure 43), value of cosmetotextiles with higher concentration (ex_e-II) was 19.7 mAU*s (Figure 44) and value of cosmetotextiles with the highest analysed concentration (ex_e-III) was 18.8 mAU*s (Figure 45). Hight of the peak from the lowest to the highest concentration was 0.62 mAu, 1.3 mAu and 1.3 mAu, respectively. Using the Calibration curve of α-toc (Figure 15) the concentration of analysed α-toc was determined: 4.09 μg/ml, 4.55 μg/ml and 4.52 μg/ml, respectively (Table 24). There wasn't significant differences in results between applied concentrations.

Light fastness of cosmetotextiles with EC-toc microcapsules was analysed by HPLC and chromatograms are presented in Figures 46, 47 and 48. For comparison, Figure 50

presents chromatogram of non-treated cotton fabric analysed by HPLC using method *HPLC 1* for α-toc.

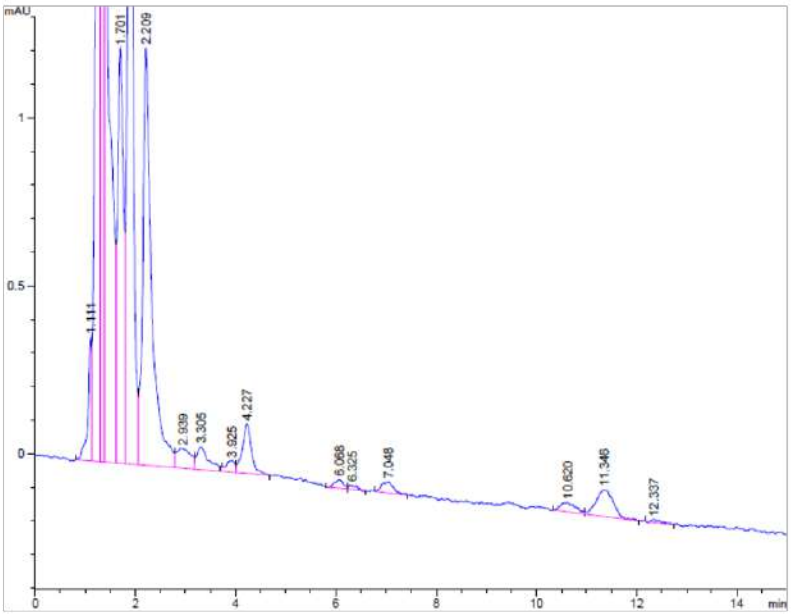


Figure 46 HPLC chromatogram of cosmetotextile with EC-toc microcapsules in concentration 4 % (Table 9) after light test ex_e-I_X

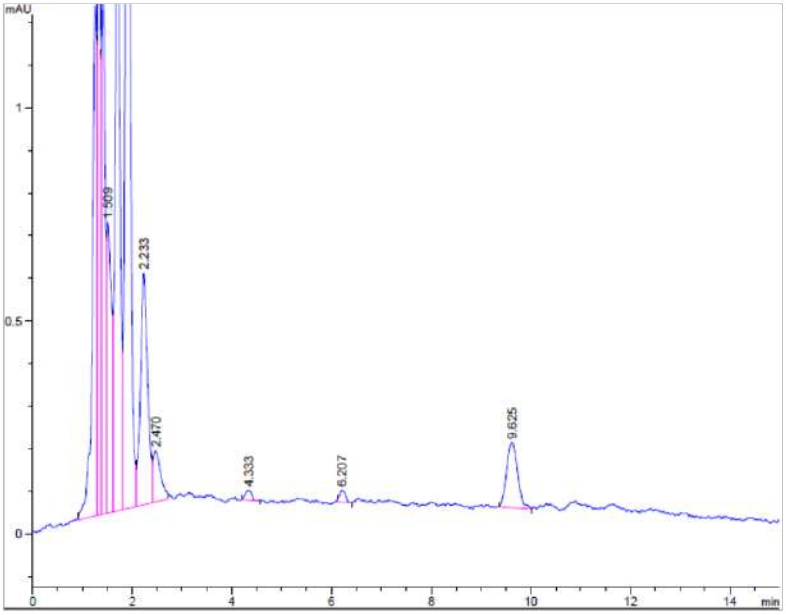


Figure 47 HPLC chromatogram of cosmetotextile with EC-toc microcapsules in concentration 8 % (Table 9) after light test ex_e-II_X

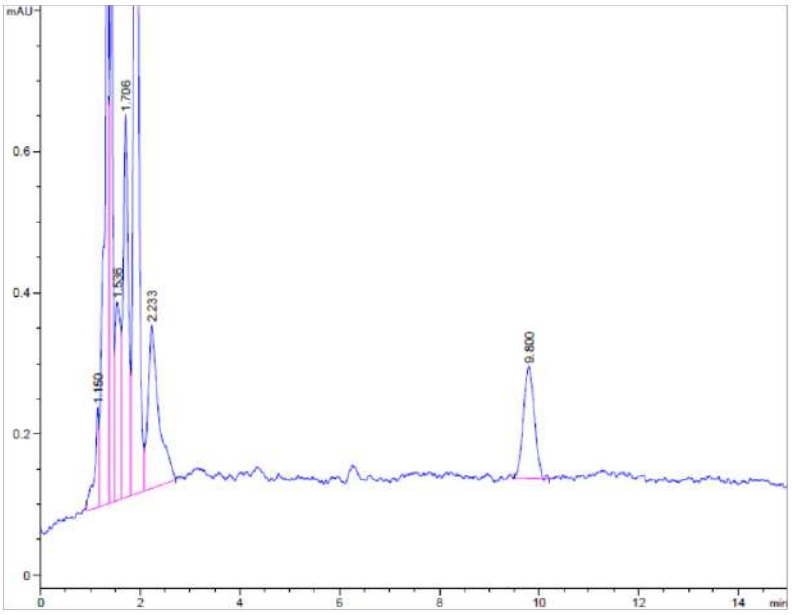


Figure 48 HPLC chromatogram of cosmetotextile with EC-toc microcapsules in concentration 12 % (Table 9) after light test ex_e-III_X

The measurements of samples after light fastness for lowest concentration (Figure 46) didn't show absorbance peaks of α-toc at wavelength of 292 nm, at ~ 9 min retention time. Other two higher concentrations showed absorbance peaks of α-toc at wavelength of 292 nm in retention time 9.800 – 9.896 min (Figures 47 and 48) which was in accordance with the standard protocol for α-toc so α-toc was confirmed in that two type of cosmetotextiles. Value of area below the peak of cosmetotextiles with higher concentration (ex_e-II_X) was 2.3 mAU*s and value of cosmetotextiles with the highest analysed concentration (ex_e-III_X) was 2.4 mAU*s. Hight of the peaks were 0.15 mAu and 0.16 mAu, respectively. Using the Calibration curve of α-toc (Figure 15) concentration of analysed α-toc in analysed cosmetotextiles after light fastness was calculated as: 3.79 μg/ml and 3.80 μg/ml, respectively (Table 24).

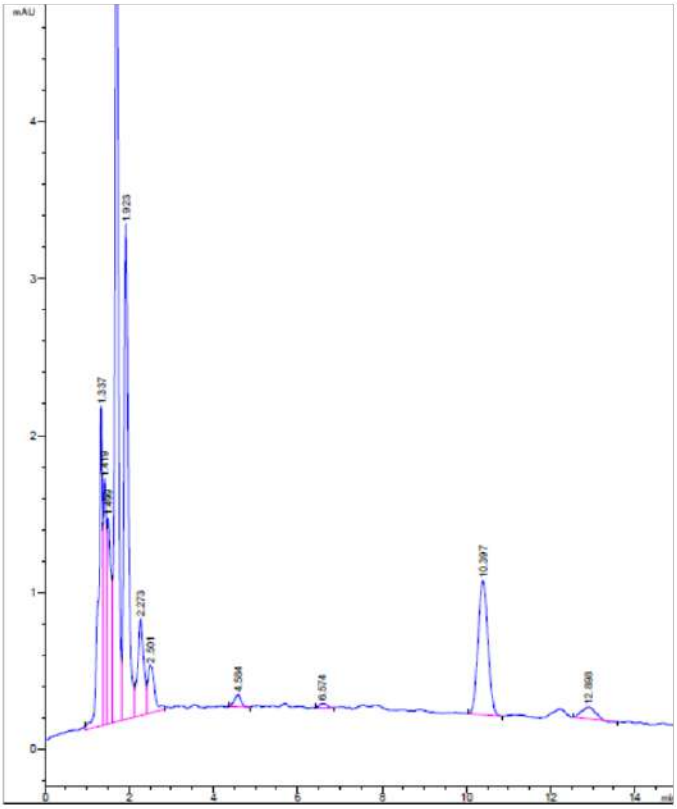


Figure 49 HPLC chromatograms of cosmetotextiles with EC-toc microcapsules in selected concentration (ex_e-II-R) after test on rubbing

Cosmetotextiles with EC-toc microcapsules in optimal concentration (ex_e-II) (Table 9) were tested on rubbing fastness (ex_e-II-R) using Crockmeter and the HPLC chromatograms are presented in Figure 49.

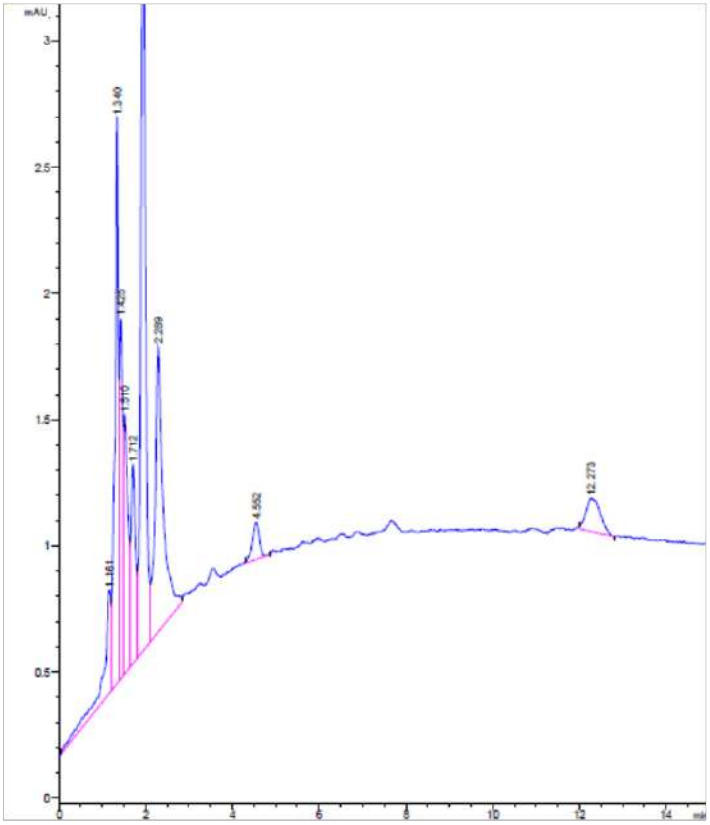


Figure 50 HPLC chromatograms of non-treated cotton fabric at 292 nm

The measurement of analysed sample after performed rubbing fastness test for selected concentration showed absorbance peaks of α-toc at wavelength of 292 nm, at 10.397 min retention time which was in accordance with the standard protocol for α-toc which confirmed the presence of α-toc in the cosmetotextile. Value of area below the peak of cosmetotextiles (ex_e-II_R) was 13.9 mAU*s and hight of the peak were 0.85 mAu. Using the Calibration curve of α-toc (Figure 15) concentration of α-toc in analysed cosmetotextiles after the rubbing fastness was calculated: 4.30 µg/ml (Table 24).

Figure 48 presents chromatogram of non-treated cotton fabric analysed by HPLC using method *HPLC 1* for α-toc where the peak was not present at retention time expected for α-toc. It can be concluded that this method is suitable for analyses of α-toc on cosmetotextiles with EC-toc microcapsules.

HPLC analyse summary of cosmetotextiles with α-toc

Table 25 summarizes results of all analysed cosmetotextiles with three different concentrations (4, 8 and 12 %) of EC-toc microcapsules before (ex_e-I, ex_e-II and ex_e-III) and after the performed light test (ex_e-I_X, ex_e-II_X and ex_e-III_X) and rubbing tests (ex_e-II_X).

Table 25 Results of HPLC analysis of cosmetotextile with EC-toc microcapsules before and after performed light fastness and rubbing fastness tests

cosmetotextiles	time /min	area / mAU*s	hight / mAU	γ / µg/ml	w / %
ex_e-I	9.916	9.1	0.62	4.0934	100
ex_e-I_X	/	/	/	/	/
ex_e-II	9.896	19.7	1.30	4.5549	100
ex_e-II_X	9.625	2.3	0.15	3.7972	83.37
ex_e-II_R	10.397	13.9	0.85	4.3024	94.46
ex_e-III	9.872	18.8	1.30	4.5158	100
ex_e-III_X	9.800	2.4	0.16	3.8016	84.19

HPLC results revealed cosmetotextiles with **EC-toc microcapsules** before and after fastness test that all treated cosmetotextiles (except sample ex_e-I_X) contained EOI. The lowest concentration of EC microcapsule on cosmetotextiles (ex_e-I) showed lowest concentration of α-toc (4.0934 µg/ml). There was not significant difference in results of concentration of α-toc for other measured concentrations of EC microcapsule on cosmetotextiles (4.5549 µg/ml for ex_e-II and 4.5158 µg/ml ex_e-III). As previously mentioned, cosmetotextile with the lowest concentration of EC-toc microcapsules after

light fastness test (ex_e-I_X) could not be measured because of the absence of the peak. Other analysed cosmetotextiles after light fastness test gave results of 83 % and 84 % of α-toc bounded to the cosmetotextiles which were acceptable results because α-toc remained protected from external influences. After rubbing fastness test (ex_e-II_R) results revealed that 94 % of α-toc stayed bounded on cosmetotextiles in comparison with ex_e-II. This result required further analysis, e.g. a test involving both rubbing and heating, with the aim of promoting the release of the active substance and creating conditions as similar as possible to those encountered when wearing cosmetotextiles.

Cosmetotextiles with EC-EOI microcapsules

Cotton cosmetotextiles with EC-EOI microcapsules (synthesis m_2) applied by exhaustion in three different concentration (Table 9) were analysed by HPLC method. Figures 51, 52 and 53 presents HPLC chromatograms of cosmetotextiles with EC-EOI microcapsules in three different concentrations: 4 %, 8 % and 12 %.

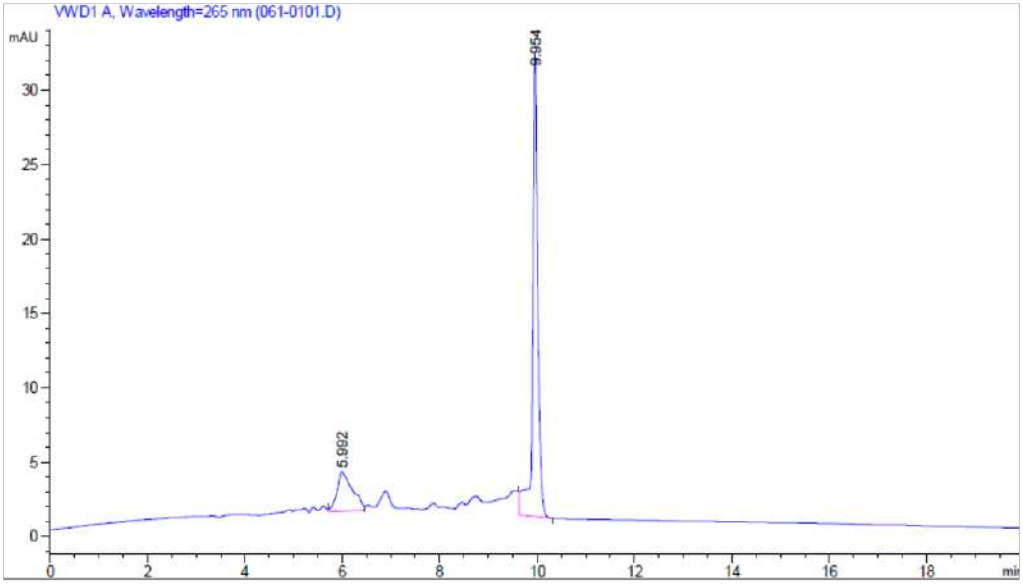


Figure 51 HPLC chromatogram of cosmetotextile with EC-EOI microcapsules in concentration of 4 %, ex_S-I (Table 9)

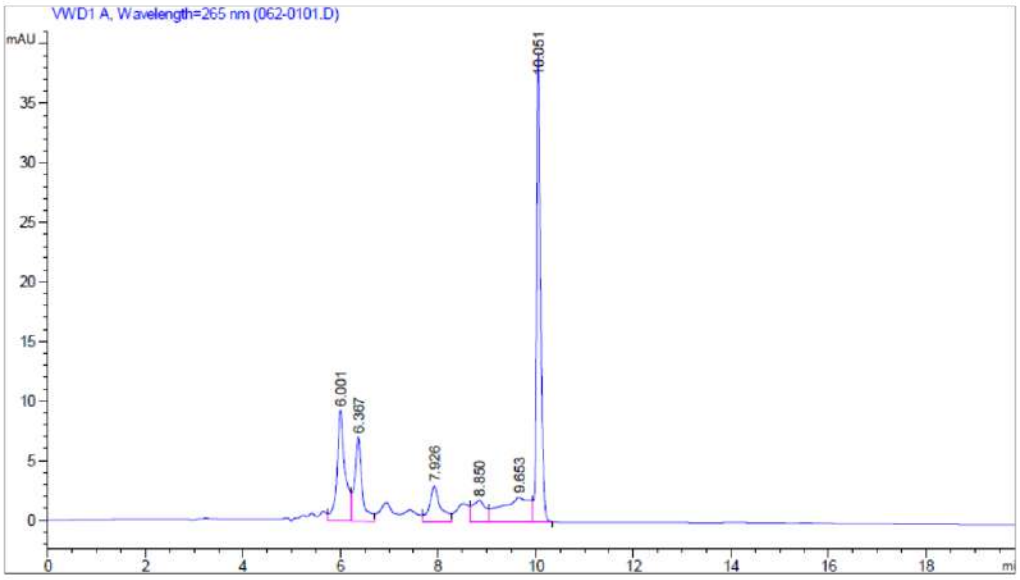


Figure 52 HPLC chromatogram of cosmetotextile with EC-EOI microcapsules in concentration of 8 %, ex_S-II (Table 9)

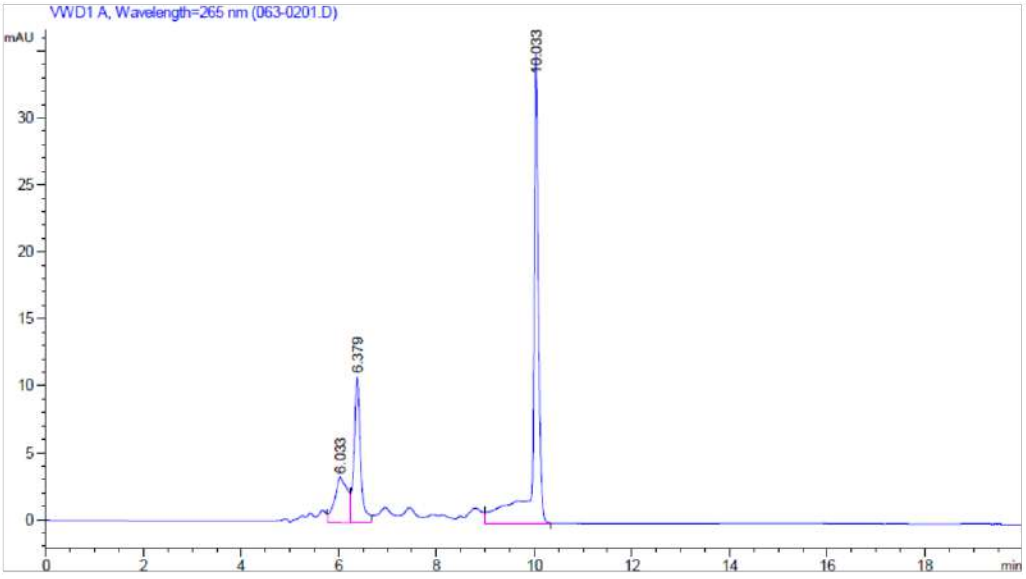


Figure 53 HPLC chromatogram of cosmetotextile with EC-EOI microcapsules in concentration 12 %, ex_S-III (Table 9)

HPLC chromatograms of cosmetotextiles with EC-EOI microcapsules in three different concentrations didn't showed presence of peak at retention time ~15 min which was in accordance with the standard protocol for EOI, so further HPLC analyses are stopped.

For the comparison, Figure 54 presents chromatogram of non-treated cotton fabric analysed by HPLC using method *HPLC 2* for EOI, where the peak was also not present at retention time expected for EOI. It could be concluded that this method wasn't suitable for analyse EOI on cosmetotextiles with EC-EOI microcapsules.

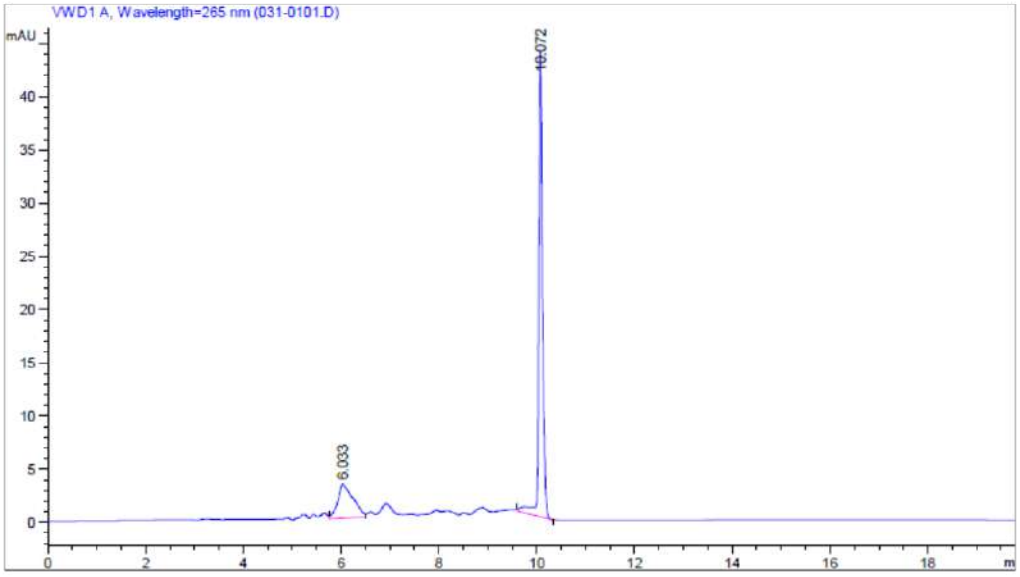


Figure 54 HPLC chromatograms of non-treated cotton fabric at 265nm

4.11 Results of quantitative and qualitative analysis of cosmetotextiles extracts with UV spectrophotometry

Quantitative and qualitative analyse of **EOI on *cosmetotextiles with EC-EOI microcapsules***

Cotton **cosmetotextiles with EC-EOI microcapsules** (synthesis m_2) applied by exhaustion in three different concentration (Table 9) were analysed by UV spectrophotometry. After EOI analysis and literature review chosen wavelength for further research was 265 nm [37, 40].

The measured absorbance and calculated concentration using calibration diagram (Figure 28) of EOI on cosmetotextiles before and after 1 (ex_S-I_1x), 2 (ex_S-I_2x), 3 (ex_S-I_3x), 5 (ex_S-I_5x) and 10 (ex_S-I_10x) cycles of washing, after rubbing (ex_S-II_R) and after light test (ex_S-I_X, ex_S-II_X and ex_S-III_X) are presented in Table 26.

Results of UV spectroscopy of cosmetotextile with EC-EOI microcapsules extracts before and after fastness tests revealed that all treated cosmetotextiles contained EOI. Concentration of EC microcapsules applied on cotton fabric didn't have significant influence. After the first washing cycle, approximately 35 % of EOI still bounded on cosmetotextiles, for all concentrations of EC microcapsules on cosmetotextiles. Further washing cycles showed decrease of EOI concentration on cosmetotextiles. After ten washing cycles the measurements was stopped because the measured absorbance reached minimum level. A light test can assess the durability of the microcapsules during storage and exposure to other external influences. After light fastness test, approximately 55 % of EOI was still bounded on cosmetotextiles, for all concentrations of EC microcapsules on cosmetotextiles. Rubbing test were selected to predict the behaviour of the microcapsules during wearing cosmetotextiles, which can favour the rupture of microcapsules and thus oil release. After rubbing test, performed at ex_S-II, approximately 55 % of EOI was still bounded on cosmetotextile. The quantitative results corresponded to those of SEM (although the microcapsules of SEM were only analysed qualitatively). It was obvious that some of the microcapsules remained on the textile, but

not in the quantity as before the rubbing test. If microcapsules were protected by adjacent fibres or remained in a cavity in the fibre, the rubbing do not touch the microcapsules, according to the literature [116]. It can be concluded that the obtained microcapsules after rubbing test were acceptable.

Table 26 Measured absorbance and concentration of EOI on cotton cosmetotextile extracts before and after cycles of washing, rubbing and light test

cosmetotextiles	Absorbance	γ /μg/ml	w / %
ex_S-I	0.1041	24.2749	100
ex_S-I_1x	0.0219	7.4364	30.45
ex_S-I_2x	0.0073	4.4454	18.11
ex_S-I_3x	0.0018	3.3187	13.58
ex_S-I_5x	0.0008	3.1139	12.76
ex_S-I_10x	0.0001	2.9705	11.93
ex_S-I_X	0.0474	12.6603	52.26
ex_S-II	0.1046	24.3827	100
ex_S-II_1x	0.0225	7.5593	31.15
ex_S-II_2x	0.0075	4.4864	18.44
ex_S-II_3x	0.0020	3.3597	13.93
ex_S-II_5x	0.0011	3.1753	13.11
ex_S-II_10x	0.0002	2.9909	11.89
ex_S-II_R	0.0152	6.0638	26.23
ex_S-II_X	0.0521	13.6231	55.74
ex_S-III	0.1053	24.5269	100
ex_S-III_1x	0.0303	9.1572	37.55
ex_S-III_2x	0.0095	4.8961	20.00
ex_S-III_3x	0.0031	3.5850	14.69
ex_S-III_5x	0.0012	3.1958	13.06
ex_S-III_10x	0.0002	2.9909	11.84
ex_S-III_X	0.0546	14.1353	57.55

Results of antioxidant activity of cosmetotextiles (ABTS method)

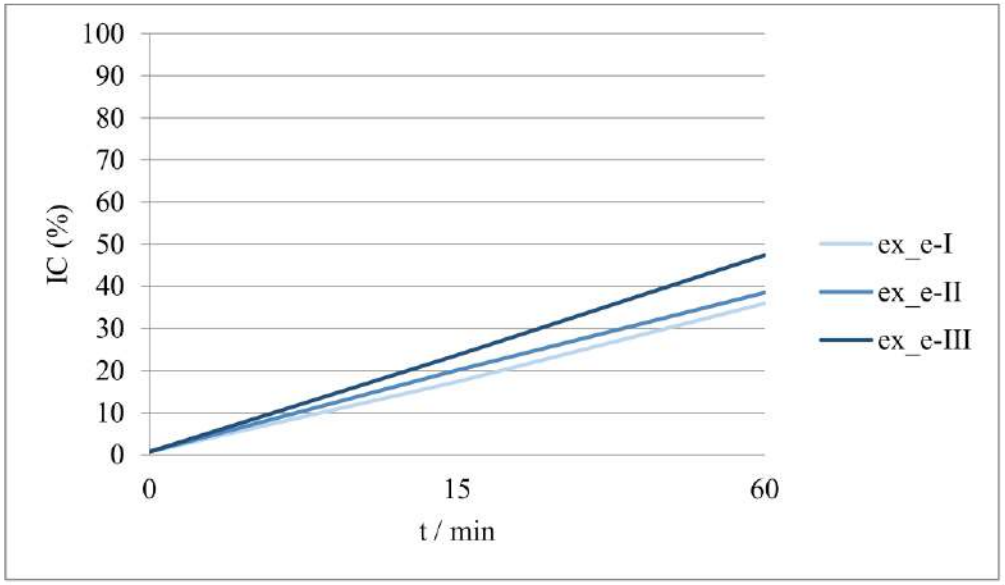
Antioxidant activity of **cosmetotextiles with EC-toc microcapsules** and **cosmetotextiles with EC-EOI microcapsules** was analysed on UV-VIS Spectrophotometer Carry 60. It was measured using ABTS method.

Absorbance was measured: at the beginning, after 15 minutes and after 60 minutes. As a result of the measurement (Table 27), the percentage of inhibition (IC / %) after 15 minutes and 60 minutes was calculated. It indicated theta the amount of free radicals was reduced in contact with the antioxidant in selected time. Figure 55 a. presents graph of antioxidant activity of **cosmetotextiles with EC-toc microcapsules** and Figure 55 b. presents graph of antioxidant activity of **cosmetotextiles with EC-EOI microcapsules**.

Table 27 Results of antioxidant activity of cosmetotextile with EC-toc microcapsules and cotton cosmetotextile with EC-EOI microcapsules using ABTS method

Sample	No.	A_{0min} (0 min)	A_{end} (15 min)	A_{end} (60 min)	IC_{15min} / %	Mean value IC_{15min} / %	IC_{60min} / %	Mean value IC_{60min} / %
ex_e-I	1	0.7187	0.5925	0.4482	17.5595	17.30	37.6374	35.93
	2	0.7187	0.5962	0.4727	17.0447		34.2285	
ex_e-II	1	0.7187	0.5616	0.4544	21.8589	20.03	36.7747	38.49
	2	0.7187	0.5879	0.4298	18.1995		40.1976	
ex_e-III	1	0.7187	0.5402	0.3940	24.8365	23.60	45.1788	47.38
	2	0.7187	0.5580	0.3623	22.3598		49.5895	
ex_S-I	1	0.7126	0.5851	0.4484	17.8922	17.63	37.0755	38.16
	2	0.7126	0.5889	0.4329	17.3590		39.2506	
ex_S-II	1	0.7126	0.5664	0.4438	20.5164	23.05	37.7210	39.85
	2	0.7126	0.5303	0.4135	25.5824		41.9731	
ex_S-III	1	0.7126	0.5837	0.3666	18.0887	15.89	48.5546	45.68
	2	0.7126	0.6151	0.4076	13.6823		42.8010	

a.



b.

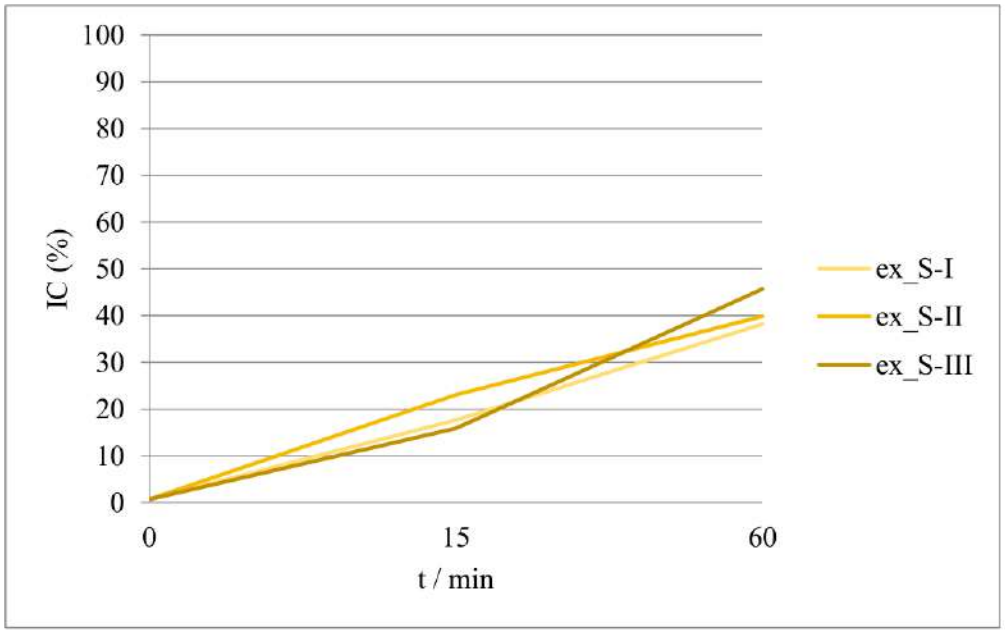


Figure 55 Precentage of inhibition (IC) in time 15 and 60 min of: a. cosmetotextiles with EC-toc microcapsules; b. cosmetotextiles with EC-EOI microcapsules

Results of antioxidant activity confirmed that all analysed cosmetotextiles had antioxidant activity. **Cosmetotextiles with EC-toc microcapsules** from lowest through highest concentration had percentage inhibition after 15 minutes: 17.30 %, 20.03 % and 23.60 %, respectively. After 60 minutes, percentage inhibition was: 35.93 %, 38.49 % and 47.38 %, respectively. It was found that for this type of cosmetotextile, the percentage of inhibition

increased after 15 minutes and after 60 minutes with the concentration of EC microcapsules applied to the textile. **Cosmetotextiles with EC-EOI microcapsules** from lowest through highest concentration had percentage inhibition after 15 minutes: 17.63 %, 23.05 % and 15.89 %, respectively. After 60 minutes, percentage inhibition was: 38.16 %, 39.85 % and 45.68 %, respectively. In this case percentage of inhibition after 15 min decreased for highest concentration of EC microcapsules applied on textile, in comparison with lower concentration. After 60 minutes, the percentage of inhibition increased with increasing concentration of EC microcapsules applied to textiles.

4.12 Results of drop test

Qualitative analyse of cosmetotextiles impregnated with **EC-toc microcapsules** (synthesis E-2) was performed by simple Drop test. Different intensity of red colour on textiles after the Drop test analyse is presented at Figure 56 b, untreated textile materials was tested for the comparison Figure 56 a.

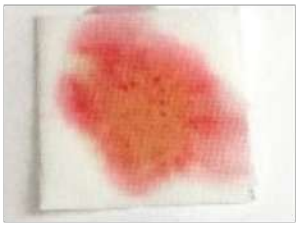
textile material	a.	b.
cotton		
PES/cotton		

Figure 56 Drop test results of: a. cosmetotextiles, b. untreated textiles

All materials showed a difference compared to the untreated ones. The most intense colour was observed on liocel. The nonwoven fabric liocel adsorbed the largest amount of active substance α-toc. Liocel had a high degree of hydrophilicity, which was further enhanced by the nonwoven structure that affected the binding rate of the microcapsules to the material. The silk fabric had partially hydrophobic properties, which certainly slowed down the binding of the microcapsules.

4.13 Whiteness of cosmetotextiles

Whiteness of cosmetotextiles was compared to untreated textile materials, and investigated before and after fastness tests.

Cosmetotextiles with EC-toc microcapsules

Whiteness degree (W_{CIE}) of cosmetotextiles impregnated and applied by exhaustion with EC-toc microcapsules is presented at Table 28. Also cosmetotextiles applied by exhaustion with different concentrations of microcapsules (Table 9) was analysed after

light fastness of cosmetotextiles (ex_e-I_X, ex_e-II_X and ex_e-III_X). Additionally, the W_{CIE} were measured of light exposure.

Table 28 Whiteness of textiles before and after application of EC-toc microcapsules before and after exposure to light

Samples				W_{CIE}	TV	TD	Y
cotton	untreated			62.5	-0.6	R1	69.6
	cosmetotextiles (impregnation)			63.6	-0.6	R1	69.8
	cosmetotextiles (exhaustion)		ex_e-I	63.4	-0.6	R1	69.9
			ex_e-I_X	64.0	-0.6	R1	65.5
			ex_e-II	63.8	-0.6	R1	70.2
			ex_e-II_X	63.8	-0.6	R1	69.3
			ex_e-III	63.4	-0.6	R1	69.8
			ex_e-III_X	64.3	-0.6	R1	69.7
PES/cotton	impregnation →	untreated		74.3	-0.6	R1	84.1
		cosmetotextiles		74.8	-0.7	R1	84.3
silk		untreated		55.0	-1.3	R1	80.6
	→	cosmetotextiles		53.1	-1.4	R1	80.6
liocel		untreated		68.5	-0.9	R1	81.3
	→	cosmetotextiles		65.0	-1.2	R1	81.4

Cosmetotextiles with EC-EOI microcapsules

Whiteness degree of cosmetotextiles (exhaustion) with three concentrations of EC-EOI microcapsules before and after fastness test is presented at Table 29.

Table 29 Whiteness of untreated cotton and cosmetotextile with EC-EOI microcapsules before and after fastness tests

Samples			W_{CIE}	TV	TD	Y
untreated cotton fabric			62.5	-0.6	R1	69.6
cosmetotextiles	ex_S-I		64.2	-0.6	R1	70.5
	ex_S-I_X		64.7	-0.6	R1	70.3
	washing cycles	ex_S-I-1x	64.2	-0.7	R1	70.4
		ex_S-I-2x	64.4	-0.6	R1	70.4
		ex_S-I-3x	64.3	-0.6	R1	70.2
		ex_S-I-5x	64.4	-0.6	R1	70.1
		ex_S-I-10x	64.4	-0.6	R1	70.1
	ex_S-II		63.5	-0.7	R1	69.8
	ex_S-II_X		65.0	-0.6	R1	70.5
	x_S-II_R		64.3	-0.6	R1	70.5
	washing cycles	ex_S-II-1x	64.4	-0.6	R1	70.4
		ex_S-II-2x	64.5	-0.6	R1	70.5
		ex_S-II-3x	64.9	-0.6	R1	70.7
		ex_S-II-5x	65.5	-0.6	R1	71.1
		ex_S-II-10x	65.3	-0.6	R1	71.0
	ex_S-III		64.1	-0.6	R1	70.5
	ex_S-III_X		64.9	-0.6	R1	70.4
	washing cycles	ex_S-III-1x	63.9	-0.8	R1	70.3
		ex_S-III-2x	64.3	-0.6	R1	70.4
		ex_S-III-3x	64.3	-0.6	R1	70.2
		ex_S-III-5x	64.3	-0.6	R1	70.0
		ex_S-III-10x	64.2	-0.6	R1	70.1

Fastness tests were performed on textiles washed with 1 (ex_S-I_1x), 2 (ex_S-I_2x), 3 (ex_S-I_3x), 5 (ex_S-I_5x) and 10 (ex_S-I_10x) washing cycles, after rubbing (ex_S-II_R) and after light fastness tests (ex_S-I_X, ex_S-II_X and ex_S-III_X).

After treatments with EC-EOI microcapsules, there was a slight increase in whiteness, which can be considered as benefit in processing. Application of EC-EOI microcapsules didn't have a significant influence on whiteness degree of cotton cosmetotextiles. Whiteness degree of analysed cosmetotextiles before and after exposure on light and rubbing is insignificant.

4.14 Cosmetotextiles antibacterial activity

Cosmetotextiles with EC-EOI microcapsules (m_2) were applied on textile in three different concentrations 4, 8 and 12 % (Table 9) by exhaustion and analysed by AATCC Test Method 147-2004 (Antibacterial activity assessment of textile materials: Parallel streak method) [132]. Untreated samples of cotton fabric were also analysed. Results of Parallel streak method on untreated cotton and cosmetotextiles are presented at Figure 57.

Three types of bacteria were used for this analyse: *Klebsiella pneumoniae* (KP), *Acinetobacter baumannii* (AB) and *Staphylococcus aureus* (SA). After conducted analyses, result was that analysed samples did not show antibacterial or bactericidal properties on listed bacteria. All tested samples did not prevent the growth of microorganisms, as can be seen from the lines touching the textile samples. According to the literature, treated fabrics showed antimicrobial activity against both SA and Escherichia coli at a 10 % concentration of the microcapsules [140].

Cosmetic products for personal hygiene (including the genital area) claim the soothing and antimicrobial properties of the essential oil of *Helichrysum italicum* contained in their formulas, so testing cosmetotextiles in this study was natural. In addition, *Helichrysum italicum* had been described as an antimicrobial agent in *in vitro* studies [76, 120]. The nature and concentration of the herbal constituents and consequently their antimicrobial

activity depend strongly on the way the essential oil is prepared. One of the major limitations of the available scientific data on *Helichrysum italicum* is that it is often not stated which subspecies were used in each study, making comparison between them difficult [76].

a.

b.

c.

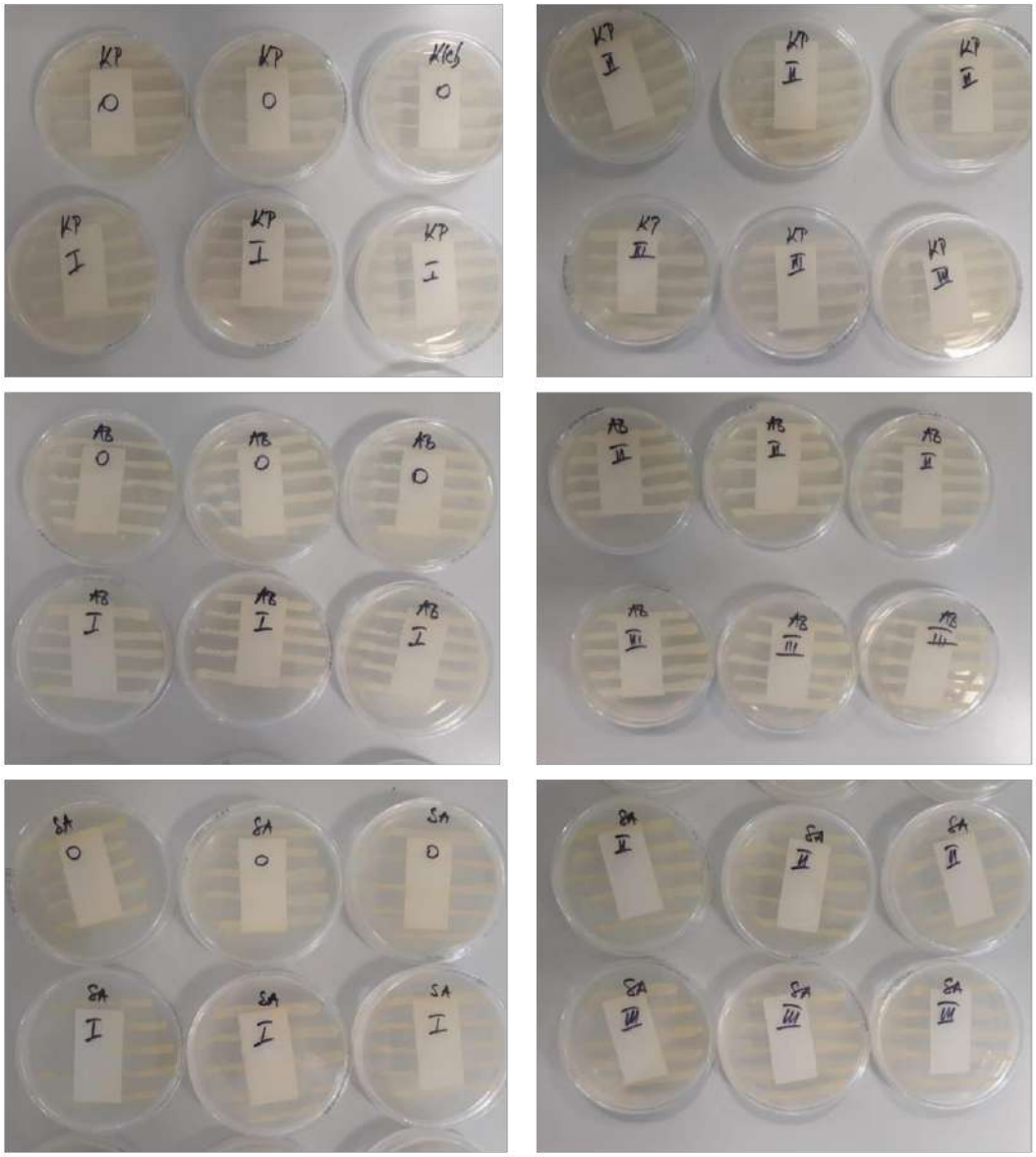


Figure 57 Parallel streak method of analysed non-terated cotton fabric (O) and cosmetotextiles with three different concentration of EC-EOI microcapsules (I, II and III) using bacteria: a. *Klebsiella pneumoniae* (KP); b. *Acinetobacter baumannii* (AB) and c. *Staphylococcus aureus* (SA)

4.15 Results of dermatology test - Patch test

Non-dyed cosmetotextile with EC-EOI microcapsules and dyed (with natural dye Cochineal) cosmetotextile with EC-EOI microcapsules, applied by exhaustion, were analysed by Patch test.

All tested patients (Table 19) signed consent to be tested for dermatology test and all results of Patch test were negative. Also, all tested patients have sensitized skin so it can be concluded that tested cosmetotextiles were hypoallergenic. This meant that the analysed samples reduced or minimised the possibility of an allergic reaction by containing relatively few or no potentially irritating substances.

In addition to tested fabrics, in accordance with standard protocol, all patients were tested for a basic series of allergens containing: bichromate, cobalt chloride, nickel sulfate, odor mixture, epoxy resins, paraphenylenediamine, Peruvian balm, rubber antioxidant, mercapto compounds, thiuram compounds, carbamates, paraben mixture, charcoal tar, neomycin sulphate, benzocaine, rosin, formaldehyde, thimerosal, phenyl mercury acetate, sesquiterpene lactone mixture, clioquinol, quaternum 15, primine, budesonide, thixocortol-21-pivalate, methylisothiazolinone + methylchloroisyl, lanolin, linalool hydroperoxide, limonene hydroperoxide, 2-mercaptobenzothiazole, textile dye mixture, hydroxyisohexyl 3-cyclohexene carboxydehyde.

5 CONCLUSIONS

Cosmetic sector is continuously developing by using new raw materials, natural preparations, as well as by designing and formulating new active and auxiliary substances. The application of cosmetic preparations onto textiles has opened a new area of research, production and application, so that cosmetotextiles present an innovation in technical and bio-technical areas and in medicine. The interest of scientists is aimed at investigating the possibilities of incorporating sustainable cosmetic preparations into textile products of high added value and longer life cycle. Processing techniques have been developed accordingly, mechanisms of release controlled, and methods tested for objective and subjective evaluation of cosmetotextiles efficiency. Obviously, the development, improvements and commercialisation of cosmetotextiles ask for a continuous cooperation of researchers, manufacturers and end-users.

Several types of active substances (α-toc, EOI and combination of α-toc and EOI) were used in this research and for final prototype EOI was chosen as the best one.

Optimal results in synthesis were obtained under the following conditions:

- solvent evaporation method according to patent No.: US 6932984 B1 for synthesis of microcapsules [50, 51],
- oil:EC rate 1:3,
- stirring speed in synthesis 400 rpm.

Optimal results in application were obtained under the following conditions:

- exhaustion for application on textiles,
- binder Tubicoat WLI, in concentration 5.6 % on mass of material,
- concentration of microcapsules 8 % on mass of material.

Effects achieved were:

- hypoallergenic cosmetotextiles with EC microcapsules containing EOI in concentration of 8 % on mass of material,

- ten washing cycles durability proved with SEM and UV spectrophotometry analyses,
- optimal light fastness (55.74 % of active substance was present after the test) analysed by UV spectrophotometry,
- good rubbing fastness test showed that 73.77 % of active substance was released during the rubbing. Rubbing test was very important because if the active substance was not released during the use of the garment, it can't be called cosmetotextiles at all,
- no change in whiteness occurred after microcapsule application on cotton textiles,
- no morphology change occurred and microcapsules retained their spherical shape after the application on textiles which was confirmed by SEM.

According to the literature [120, 131] EOI possess good antibacterial activity but applied concentration of 8 % microcapsules on mass of material did not achieve antibacterial activity.

Future perspectives:

Additional improvements can be obtained with application of different binder which might improve bonding EC microcapsules on textile and prolong the life of microcapsules during washing cycles.

Furthermore, antioxidant analyses for microcapsules and cosmetotextiles could be increased. Recently, a new method for measuring antioxidant power (AP) has been applied in the Ruđer Bošković Institue. This method is based on the determination of antioxidant power by measuring antioxidant activity and antioxidant capacity of samples using ESR spectroscopy. Results of AP present absolute values expressed in AU units, where 1 AU corresponds to AP of the solution of vitamin C having a concentration of 1.00 ppm [137, 138]. It would be interesting to apply this method to the samples used in this work. Particularly, as research of special importance for practical application, it is planned to investigate AP of microcapsules embedded in textile fibers (cosmetotextiles).

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7 ANNEX

7.1 ANNEX I – List of figures and tables

List of figures

List of tables

7.2 ANNEX II – Abbreviations and symbols

α-toc	α-tocopherol
γ	mass concentration, expressed in μg/ml
ΔI	rest of the signal intensity after the reaction time t, expressed in %
A_{end}	absorption after 15/60 min
$A_{initial}$	initial absorption at 0 min
AB	bacteria *Acinetobacter baumannii* (AB)
Abs	absorbance
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
AP	antioxidant power
BST	black standard temperature
c	concentration, expressed in %
CHT	chamber temperature
ATR	Attenuated Total Reflectance
CLSM	Confocal laser scanning microscopy
DPPH	2,2-Diphenyl-1-picrylhydrazyl
E	irradiance
E-1 - E-6	synthesis of EC-toc microcapsules with increasing of α-toc and stirring speed
EA	ethyl acetate
EC	ethyl cellulose
EC-EOI MC	ethyl cellulose microcapsules containing EOI
EC-toc MC	ethyl cellulose microcapsules containing α-toc
ESR	electron spin resonance
EOI	essential oil immortelle
ex	exhaustion
FTIR	Fourier transform infrared spectrophotometer
HPLC	high pressure liquid chromatography
I, II, III	concentrations of microcapsules

I	signal intensity of DPPH in a sample solution measured at time *t*
I_0	signal intensity of DPPH at *t* = 0 min
IC	percentage of inhibition after 15 / 60 min / %,
KP	bacteria *Klebsiella pneumoniae* (KP)
M	modal fabric
m_0 - m_4	synthesis of EC-EOI microcapsules with different amounts of EOI
mAU*s	mili absorption unit per second
$m_{(MC)}$	mass of synthetized microcapsules / g
m_A	mass of filter paper/cosmetotextile after synthesis/application on textile / g
m_B	mass of filter paper/cosmetotextile before synthesis/application on textile / g
MC	microcapsules
MeOH	methanol
e	EC-toc microcapsules used in application on textile using exhaustion
EC-toc MC	ethyl cellulose microcapsules containing α-tocopherol
EC-EOI MC	ethyl cellulose microcapsules containing essential oil immortelle
EC-toc and EOI MC	ethyl cellulose microcapsules containing α-tocopherol and essential oil immortelle
oil-free EC MC	oil-free ethyl cellulose microcapsules
PEO	polyethylene oxide
PES	polyester
R	sample tested on rubbing fastness
RH	humidity in chamber
rpm	revolutions per minute
s0.5 - s4	symbols for EOI solutions with different concentrations of EOI
S	EC-EOI microcapsules used in application on textile using exhaustion

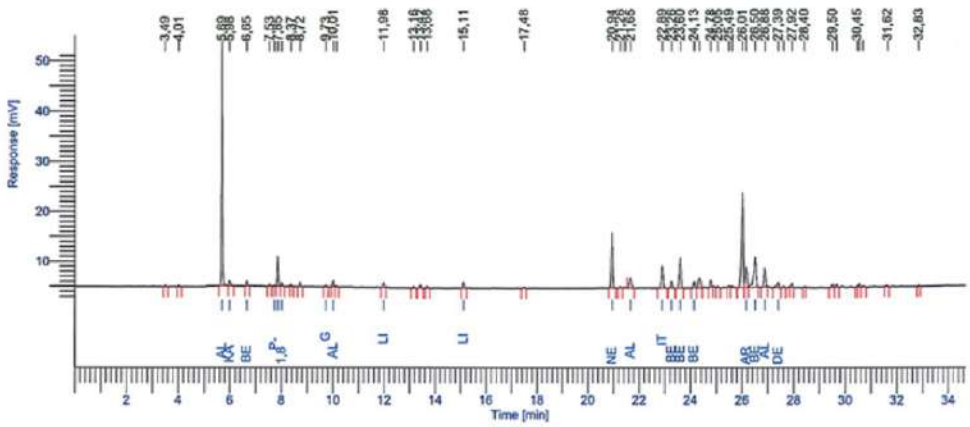
SA	bacteria *Staphylococcus aureus* (SA)
SDS	sodium dodecyl sulphate
SEM	Scanning Electron Microscope
TV	tint value
TD	tint deviation
toc	α-tocopherol
UPF	ultraviolet irradiation
UV/VIS	ultra violet/visible
w	mass fraction, expressed in %
W_{CIE}	whiteness degree
X	sample tested on light fastness
Y	basic whiteness

7.3 ANNEX III – Gas Chromatography of essential oil of immortelle (EOI)

Gas chromatography results of essential oil of immortelle analyse was supplied by Irex aroma d.o.o.

Software Version : 6.3.2.0646
Sample Name : ETU.ULJE SMILJE 2021
Instrument Name : AutosystemXL
Rack/Vial : 0/0
Sample Amount : 1,000000
Cycle : 1
Date : 23.07.2021 08:36:10
Data Acquisition Time : 23.07.2021 13:58:36
Channel : A
Operator : Sanja
Dilution Factor : 1,000000

Result File : d:\podaci\autosystemxl\smilje\uzorci\2021\20210723\20210723-2 001.rst
Sequence File : D:\sekvence\20210723-2.seq



REPORT

DEVELOPMENT: GAS CHROMATOGRAPHY LABORATORY Column: Elite-5

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]
3	ALFA-PINEN	5,693	123627,25	24,12
4	KAMFEN	5,985	3968,12	0,77
5	BETA-PINEN	6,647	2808,70	0,55
6		7,528	1204,71	0,24
8	LIMONEN	7,854	17382,61	3,39
9	1,8 CINEOL	8,005	2183,44	0,43
10		8,369	1186,20	0,23
11		8,715	2650,19	0,52
12	GAMA-TERPINEN	9,726	1448,30	0,28

23.07.2021 08:36:10 Result:
d:\podaci\autosystemxl\smilje\uzorci\2021\20210723\20210723-2_001.rst

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]
13	ALFA-TERPINOLEN	10,009	4575,37	0,89
15	LINALOOL	11,977	3494,05	0,68
16		13,161	1485,06	0,29
17		13,419	2813,59	0,55
18		13,678	1779,47	0,35
19	LINALIL ACETAT	15,113	4535,84	0,88
20		17,481	1220,06	0,24
21	NERIL ACETAT	20,935	44869,20	8,75
22		21,256	1445,91	0,28
23		21,441	1040,90	0,20
24	ALFA-KOPAEN	21,653	12634,88	2,46
25	ITALICEN	22,893	21410,50	4,18
26	BERGAMOTEN CIS-ALFA	23,261	5701,77	1,11
27	BETA-KARIOFILEN	23,598	27058,17	5,28
28	BERGAMOTEN TRANS-ALFA	24,130	5049,58	0,99
29		24,338	12241,29	2,39
30		24,779	5964,05	1,16
31		25,049	1243,63	0,24
32		25,486	1473,67	0,29
33		25,600	2150,09	0,42
34		26,005	93509,96	18,24
35	AR-KURKUMEN	26,146	20226,14	3,95
36	BETA-SELINEN	26,498	38911,12	7,59
37	ALFA-SELINEN	26,876	17724,70	3,46
38	DELTA-KADINEN	27,388	6734,84	1,31
39		27,601	1410,70	0,28
40		27,920	3069,13	0,60
42		29,495	3215,06	0,63
43		29,647	2817,11	0,55
44		30,448	1164,73	0,23
45		30,535	2271,87	0,44
46		30,667	1523,72	0,30
47		31,624	1373,82	0,27
			512599,47	100,00

ZAKLJUČAK:
Kromatografska analiza poslanog uzorka "[redacted] 2021" pokazuje da se radi o eteričnom ulju smilja (biljka: *Helichrysum italicum*). Identificirane sastavnice su tipične za eterično ulje smilja porijeklom iz Hrvatske (Dalmatinska zagora). Kromatografska analiza pokazuje da je eterično ulje 100 % čisto i prirodno, stabilno, ne oksidirano (bez raspada) bez primjesa stranih otapala i/ili ostalih neželjenih substanci, niti je miješano sa nekih drugim uljem. Postoci sastavnica prikazanih na kromatografskoj analizi ukazuju da kvaliteta ispitivanog eteričnog ulja smilja "[redacted] 2021" iznadprosječne kvalitete, i u skladu s zadanim intervalima.
Nadalje, napravljene su dodatne analitičke kontrole- relativna gustoća i indeks refrakcije, te organoleptička kontrola.
Dobiveni rezultati:
Relativna gustoća (referenti interval: 0,880-0,900 g/cm3)= 0,8901 odgovara
Indeks refrakcije (referentni interval: 1,470-1,490) = 1,4822 odgovara

Dokument je izrađen na računalu i stoga je pravovaljan bez žiga i potpisa.

7.4 ANNEX IV – Biography and list of papers

Biography

Iva Brlek was born in Zagreb, Croatia in 1988. After graduating from Grammar School, she enrolled Textile Technology and Engineering at the University of Zagreb, Faculty of Textile Technology. For her accomplishments during the undergraduate study she received the State scholarship (2008 - 2010) and for accomplishments during the graduate study, she received Scholarship of the city of Zaprešić (2010 – 2012). She graduated in February 2014, with the diploma thesis: FTIR atlas of textile fibres, and gained the professional title of graduated Master engineer of Textile Technology and Engineering. Since January 2015, she has been employed at the Faculty of Textile Technology, working on the project titled: Croatian Science Foundation 9967 "Advanced textile materials by targeted surface modification", coordinated by Prof. Sandra Bischof.

In 2014, Iva has started the postgraduate course Textile science and technology at the University of Zagreb, Faculty of Textile Technology. During her work at the Faculty of Textile Technology, Iva Brlek has been involved in the following European and national projects: Advanced textile materials by targeted surface modifications, ADVANCETEX HRZZ - IP– 2014– 9967, Croatian Science Foundation, coordinated by Prof. Sandra Bischof (2014-2019); Modernisation of the textile science research centre infrastructure (MI-TSRC) KK.01.1.1.02.0024, coordinated by Prof. Sandra Bischof (2018-2020); Skrojene budućnosti UP.02.1.1.03.0043, coordinated by Tehnički muzej Nikola Tesla (2018-2019); „Techno-Past Techno-Future; European Researchers“ Night (TPTF_ERN), H2020-MSCA-NIGHT-2018/2019; ESF project: 'Internacionalizacija doktorskog studija Tekstilna znanost i tehnologija', coordinated by TTF (2019 - 2021).

During her PhD study, Iva attended CEEPUS mobility at University of Maribor, Faculty of Mechanical Engineering, Maribor, Slovenia in duration of one month.

Iva has been a member of the Organizing Committee of the International Textile, Clothing & Design Conference (2012) and has received Certificate of Excellence issued by DAAAM International, Vienna, Austria (2012) for the successful organization of that

conference. Additionally, Iva has been awarded with Award for the best e-course in ac. yr. 2017/2018 and Dean's Award in the category of assistants for publishing a scientific paper in the journal with the largest echo factor in ac. yr. 2020/2021. Also as an assistant, Iva Brlek has been involved in practical training at the graduate and undergraduate study for following courses: Textile Dyeing, Colour Metrics, Theory of Dyeing, Textile Dyeing D and Colour Metrics for Erasmus students. During her scientific research, Iva has published 8 reviewed journal papers, 9 scientific papers in conference proceedings, 3 published abstracts in proceedings and 2 other papers in conference proceedings.

List of published papers

Scientific papers:

- Čorak, I.; Brlek, I.; Sutlović, A.; Tarbuk, A.: Natural Dyeing of Modified Cotton Fabric with Cochineal Dye // Molecules, 27 (2022), 3; 1100, 13 doi:10.3390/molecules27031100 (international peer-review, article, scientific)
- Brlek, I.; Ludaš, A.; Sutlović, A.: Synthesis and Spectrophotometric Analysis of Microcapsules Containing Immortelle Essential Oil // Molecules, 26 (2021), 8; 1-9 doi:10.3390/molecules26082390 (international peer-review, article, scientific)
- Glogar, M. I.; Tančik, J.; Brlek, I.; Sutlović, A.; Tkalec, M.: Optimisation of process parameters of Alpaca wool printing with Juglans regia natural dye // Coloration technology, 136 (2020), 2; 188, 201 doi:10.1111/cote.12462 (international peer-review, article, scientific)
- Sutlović, A.; Brlek, I.; Ljubić, V.; Glogar, M. I.: Optimization of Dyeing Process of Cotton Fabric with Cochineal Dye // Fibers and polymers, 21 (2020), 3; 555-563 doi:10.1007/s12221-020-9153-z (international peer-review, article, scientific)
- Matijević, I.; Pušić, T.; Bischof, S.: Isolation of α-tocopherol from Cotton Cosmetotextiles // Fibres & textiles in Eastern Europe, 4 (2019) , 136; 38-42 (international peer-review, article, scientific)

- Brlek, I.; Pušić, T.; Bischof, S.: Metode identifikacije mikrokapsula (Microcapsule identification methods) // Tekstil 67 (2018) 3-4, 65-114, review paper
- Matijević, I.; Bischof, S.; Pušić, T.: Kozmetička sredstva na tekstilu: kozmeto-tekstilije (Cosmetic preparations on textiles: Cosmetotextiles) //Tekstil 65 (2016) 1-2, 1-24, review paper
- Pavunc, M.; Vujasinović, E.; Matijević, I.: Tekstil u kontekstu održivog razvoja // Tekstil 63 (2014) 5-6, 195-203

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