Microcapsule identification methods

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Review

Controlled release and protection of the active agent from premature release into the environment are the main roles of the microcapsules. In order to determine the amount of release of the active substance in any situation, e.g. during care, drying, carrying, closet storage, etc., appropriate microcapsule analysis is required. In order to achieve this, it is important to know the composition and constitution of the active agent and membrane designed to protect active substances from various impacts. This paper presents selected qualitative and quantitative methods of identifying microcapsules themselves and microcapsules applied on textile.

Key words*: textiles, microcapsules, release, identification methods*

1. Introduction

For the past 25 years intensive research has been implemented on the use of microcapsules in agriculture, food, cosmetic and textiles industry [1]. The size of microcapsules ranges between 1 to 1000 μm, and they consist of an isolated liquid, solid or gasous core and a membrane (wall, shell) protecting the core from external conditions. Microcapsules and encapsulation have been known since the 30's of the last century.

Encapsulation is an interesting method of *storing* active agents since it enables controlled release of the core into the environment due to: friction, pressure, temperature change, diffusion through the polymer membrane, dissolution of the polymer membrane and biological decomposition. Depending on the demands and use in textile industry, it is possible to encapsulate a broad spectrum of active agents, *i.e.* cosmetic preparations, fragrances, antimicrobial agents, softeners, vitamins, repellents, pigments, dyes, monomers, catalysts, flame retardants and other [2, 3].

A great number of encapsulation methods have been developed with the goal of inserting different active agents inside the membrane made of various materials. Sinthesized microcapsules vary according to size, thickness and permeability of the membrane [4]. The size of a microcapsule, consisting of a core with an active agent and a membrane, plays an important part in diffusion, permeability and/or controlled release [1]. The rate of releasing the active agents, *i.e.* oil, is influenced by the membrane's thickness, polymer concentration in the membrane and oil type, where the oil's hydrophilicity is an important property [4].

The most common are the morphology analysis, chemical and physicochemical analysis of the microcapsules. Since the microcapsules' morphology (microsphere, multi-layered microcapsule, multi-shelled and multi-core micro-spheric) varies, it is difficult to determine the size of the core, therefore, the size of the microcapsules is the subject of most measurements.

Researchers and textile producers are becoming more interested in textiles containing cosmetic preparations in direct contact with the skin – cosmetotextiles. Gradual release of small quantities of a cosmetic preparation is more effective than a single application of cosmetic preparation in greater quantity. The demands imposed on cosmetic preparations on

textiles are multiple, and they manifest through their contribution to comfort, transfer onto the skin when wearing a textile, minimal damage during treatment process, durability during laundering and stability during storage. The greatest technical challenge is resistance to laundering and the property of controlled release [5]. It is necessary to characterise the microcapsules before and after applying them onto the textile with the goal of determining their effectiveness.

This paper describes the most common microcapsule analysis: morphology (size and shape) and determination of microcapsules chemical structures and physico-chemical characterisation.

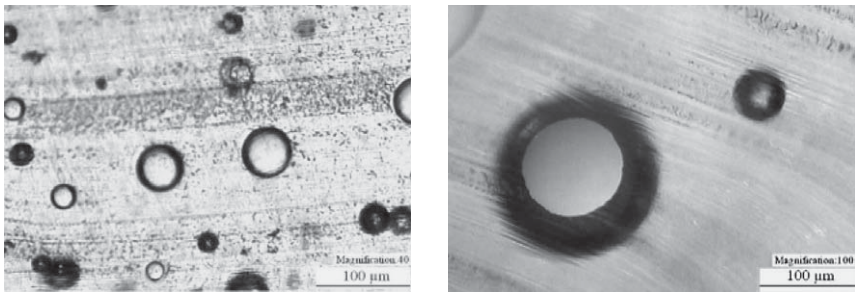
2. Methods of morphological analysis

One of the simplest methods of morphological analysis of sinthetized microcapsules is performed using an optical microscope which gives insight into the number, size and shape of the microcapsules. Analysis by laser dispersion (DLS) is used quite often for its high precision. Analysis by scanning electronic microscope (SEM) is the most common method used for providing a detailed analysis of the microcapsules' surface since it has a magnification up to 1.000.000x. Surface, roughness, holes, cracks and pores on the microcapsules can be additionally analysed using a cryo-SEM microscope.

Using confocal microscopy, it is possible to analyse microcapsules containing fluorescent compounds purposefully added into the core-membrane system to facilitate the analysis.

2.1. Optical microscope analysis

When observing microcapsules with an optical microscope it is possible to determine the microcapsules' shape *(i.e.* spherical) so as the size. One example of using the optical microscope to analyse the microcapsules in a solution is a microscope with suitable software where the samples are analysed by the transmission of light [6].



a) b)

Fig.1 Microcapsules recorded with optical microscope: a) 40x magnification; b) 100x magnification [7]

The thickness of the microcapsule's membrane affects the microcapsule's mechanical properties and the model of releasing the active substance inside the core, and it mainly depends on the membrane-core ratio of the material and the speed of mixing during the synthesis of the microcapsules. The sample is prepared by putting the microcapsules in epoxy resin at room temperature. After it becomes hard, a thin layer is cut off and analysed with a microscope. Fig.1 shows poly (urea-formaldehyde) microcapsules filled with epoxy resin and recorded using an optical microscope. The dark circles present membrane material and in this case the thickness of the microcapsules' membrane ranges between 5 and 82 µm [7].

2.2. Dynamic light scattering (DLS) analysis

Size distribution of the particles of the synthetised fragrant microcapsules can be analysed by laser distribution where it is possible to determine the quantity of the particles' total volume and the number of the particles on the surface [6].

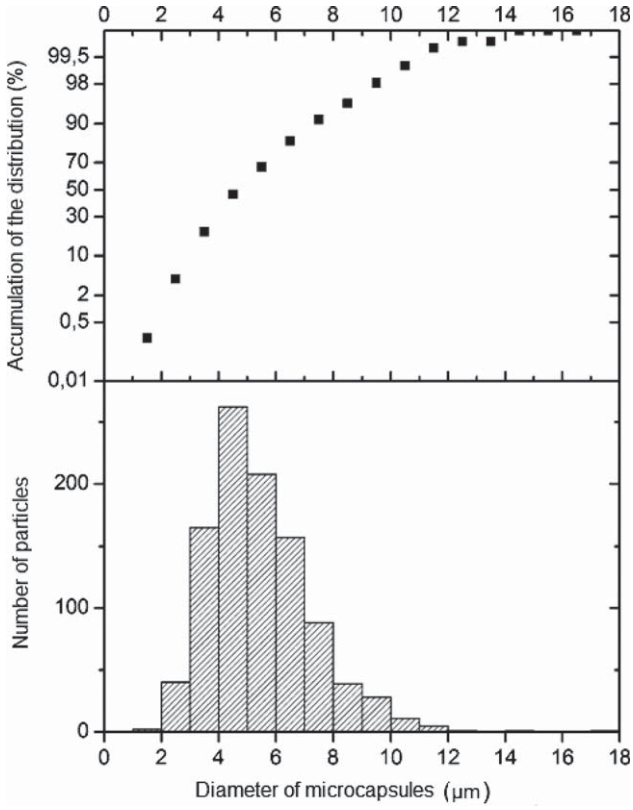


Fig.2 Distribution histogram of size and accumulation distribution of microcapsules with citronella essential oil [8]

Fig.2 presents a histogram and the corresponding accumulation distribution of the microcapsules containing citronella essential oil. The results show that microcapsules have limited distribution with a diameter between 1 and 18 μm with the biggest share of particles (approximately 90%) in the interval with the diameter from 3.5 to 7.5 μm. Homogenous distribution was confirmed by cumulative distribution of the particles' size which shows that only small fractions of the microcapsules have either small or big diameter. Average diameter of the microcapsules totals 5.435 μm [8].

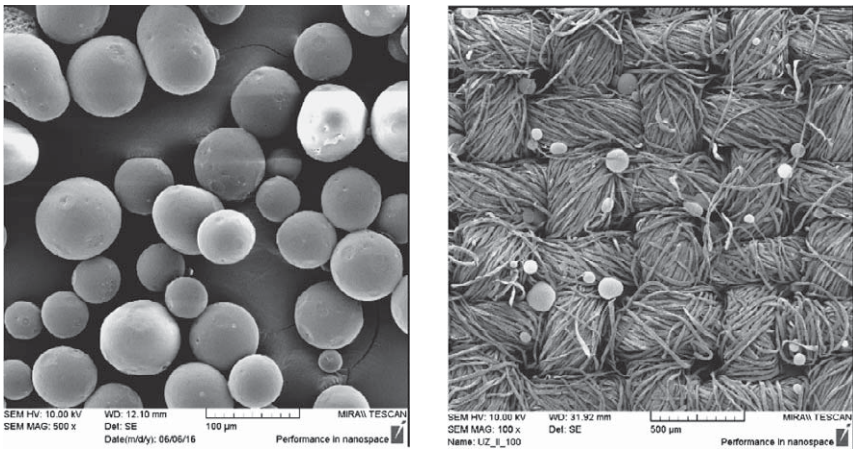
2.3. Scanning electron microscope (SEM) analysis

Scanning electron microscope (SEM) allows detailed characterisation of materials as well as analyses of the resulting changes on the textiles and other substrates. The used microscope can magnify up to 1.000.000x, and standard detectors, SEs (Secondary Electrons) and BSEs (Back-scattered Electrons) enable sample observation at very short working distance with high resolution [9]. It is necessary to prepare the samples appropriately to obtain a high-resolution image. Since electro-conductivity of the observed sample is a precondition for electronic microscopy, a sputter coater is used which coats the non-conductive samples with electro-conductive particles of gold, palladium or carbon [6, 10, 11].

SEM image of the synthetized ethyl cellulose microcapsules (Fig.3a) and the microcapsules after impregnation on the cotton fabric (Fig.3b) [6, 10].

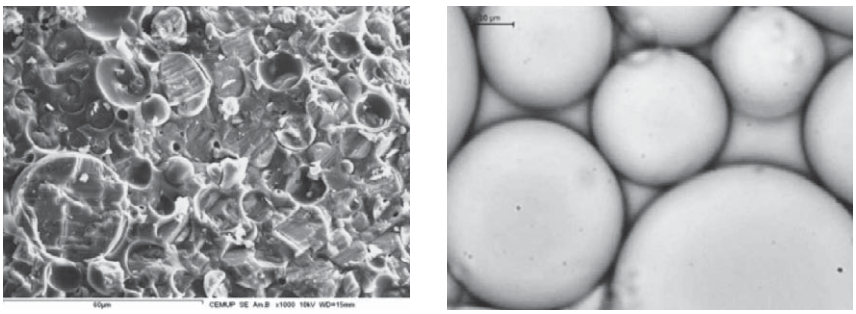
Using SEM analysis, it is possible to determine the quality of the synthetized microcapsules, their spheric shape, microcapsules' diameter at interval from 1 to 1000 μm and the microcapsules' adhesion to the textile material [6, 10, 12-15].

This analysis shows both damaged and empty microcapsules, which occurs often after the application (impregnation) on the textiles. This is useful data for the microcapsule's utilisation degree [6].



a) b)

Fig.3 SEM images: a) synthetized ethyl cellulose microcapsules magnified 500x; b) ethyl cellulose microcapsules on the textile magnified 100 x [10]



a) b)

Fig.4 Images of poly(lactic acid) (PLA) microcapsule: a) using crio-SEM microscope, 1000x magnification; b) using conventional SEM; 1000x magnification [4]

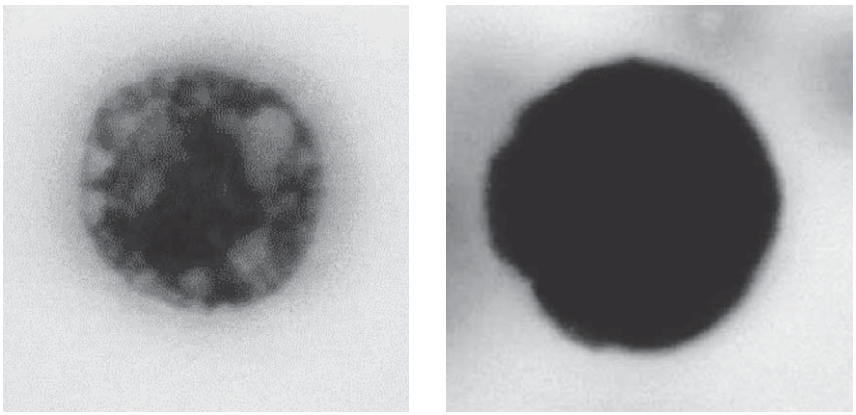
SEM can also measure thickness of the microcapsule's membrane [16, 17]. This is important because if damages occur during the synthesis, it can be concluded that the membrane is too thin. In case of any undesired damages, the active agent can be released from the membrane in an unwanted moment. On the other hand, an overly thick membrane can completely block the release of the active agent [16].

2.4. Crio-SEM microscope analysi

Crio-SEM microscope is a transmission electron microscope (TEM) where the sample is analysed at low temperatures (temperature of liquid nitrogen). This microscope allows better visibility of the surface compared with the one obtained using SEM. Fig.4a) shows rough surface of the microcapsules with expressed holes, cracks and pores. The conventional SEM image of the same microcapsules shows the microcapsules' spherical shape and it can be determined that the microcapsules did not agglomerate (Fig.4b) [4].

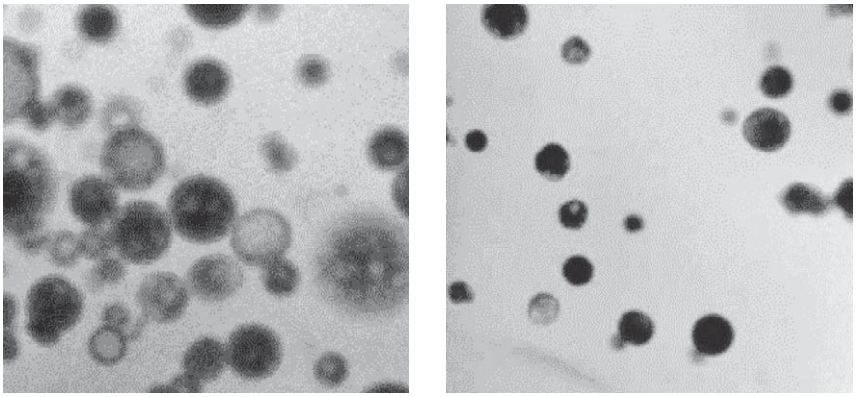
2.5. Confocal microscope analysis

Microcapsules for textile can be analysed with a confocal laser search microscope (CLSM) [14]. This is a light microscope used for observing and recording microscopic samples using fluorescency and in reflected light. Using this technique, the sample is slowly illuminated, and the emitted or reflected light is detected, creating an image in the memory of a digital computer. The main advantage of confocal microscopy, compared to



a) b)

Fig.5 Microcapsules recorded using confocal microscope with added fluorescein: a) containing essential oil; b) empty microcapsule [14]



a) b)

Fig.6 Microcapsules applied onto the fabric with added fluorescein, recorded with confocal microscope: a) containing essential oil; b) empty microcapsule [14]

classic-type light microscopy, is the option of optical dissection, *e.g.* obtaining a sharp image of a thin layer when recording a thicker sample [18]. The images of the microcapsules containing rosemary oil and the empty ones were recorded using the confocal microscope (Fig. 5a and 5b). When microcapsules contain hydrophobic rosemary essential oil, hydrophilic fluorescent dye cannot penetrate the capsule [14]. Fig.6 presents microcapsules containing rosemary oil applied onto the textile (Fig.6a) and empty microcapsules (Fig.6b).

The subject of the investigation was the application of confocal laser search microscopy during the analysis of polylactic (PLA) microcapsules which contain thyme oil for the purpose of identifying PLA membrane. Coumarin 6 dye was additionally added to the PLA solution, and the recordings showed partially dyed microcapsules where PLA polymer was green and thyme oil was colourless (Fig.7) [4].

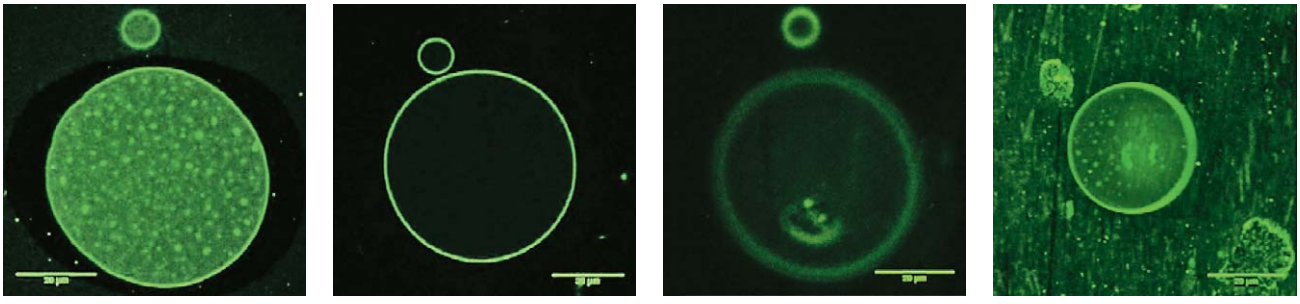
3. Physico-chemical methods

Physico-chemical methods which are used most frequently for microcapsules characterisation are the following: liquid chromatography (HPLC), gas chromatography (GC) and Fourier transformation infrared spectrophotometry (FTIR) with pre-preparation of the sample. Detailed description of the methods and the examples are provided below.

3.1. Sample preparation - extraction

To determine the exact quantity of the cosmetic preparation applied on the textile, it is necessary to isolate the active agent, and one of the most common of preparation is the extraction of that active agent from the cosmetotextile.

The extraction method should be suggested by the producer of the cosmetic substances or the supplier of microcapsules. The extraction of the cosmetic preparation can be implemented using a validation method, i.e. accelerated solution extraction, extraction in Soxhlet, extraction using an electric shaker or ultrasound. The selection of the solution used for the extraction depends on the preparation subject to the analysis [19].



a) b) c) d)

Fig.7 PLA images of microcapsules with thyme oil recorded with fluorescent confocal microscopy: a) upper plane as the level of initialisation; b) medium plane; c) lower plane; d) 3D visualisation [4]

In addition to the extraction procedure, it is recommended that producer analyses the cosmetic preparation (i.e. with GC or HPLC). The quantity of the cosmetic preparation can be determined by the combination of extraction and HPLC on the cosmetotextile, before and after a certain number of laundering cycles [20].
If a cosmetic preparation is a compound of several ingredients, the selected leading molecules in the compound are analysed and used as surrogate markers [20]. It is not always necessary to analyse each molecule since the analysis of the surrogate markers provides data for other molecules as well.

3.1.1. Accelerated solution extraction

During the extraction of a textile treated with microcapsules containing fragrance *(i.e.* linalyl acetate), the microcapsule should burst and the capsulated linalyl acetate should be completely extracted. Therefore, it is recommended to extract the fragrance using accelerated solution extraction (ASE). It has been verified that acetone is the best solution for extracting linalyl acetate. In ASE extraction, high pressure and high temperature is used which allow the polymer membrane of the microcapsule to crack and linalyl acetate to extract completely, which is then quantified using GC [20].

3.1.2. Soxhlet extraction

For textiles treated directly with the cosmetic preparation (without capsule and binder), it is possible to apply the Soxhlet extraction using a solution *(i.e.* ether, alcohol) which dissolves the cosmetic preparation which is analysed using gas or liquid chromatography.
One example of good practice is the extraction of textiles treated with a cosmetic preparation in combination with cyclodextrin methanol for 90 minutes (ISO/TR 5090:1977) [21]. The obtained extracts are analysed on HPLC.

3.1.3. Extraction using electric shaker

The extraction was performed in methanol using an electric shaker. For the purpose of monitoring the speed at which the active substances are released, the aliquots were taken at the beginning and after 15, 30, 60, 120 and 180 minutes of mixing, and they were analysed with HPLC [21].

3.1.4. Extraction with ultrasound

The extractions of the capsulated active agent, ethyl-hexyl-methoxycinnamate (EHMC) (EHMC) in the microcapsule or liposome – suntanning filter were performed in different solutions and systems, with isopropanol in ultrasonic bath, with isopropanol/water mixture in the Soxhlet extractor.
After the extraction, the extracts were analysed on HPLC and on the UV-ViS spectrophotometer. The quantity of the product (liposome or microcapsules with EHMC) extracted using the two mentioned methods was similar [22].
However, it can be said that the Soxhlet extraction with the isopropanol/water mixture at high temperatures is more appropriate on cotton and polyamide, and the extraction in the ultrasonic bath with isopropanol is better for more hydrophobic fibres, *i.e.* polyacrylic and polyester. Full (100%) extraction was achieved on cotton and polyester, and it was slightly below 100% in the case of polyamide and acrylic fibres [22].
Rosemary oil, as active agent in microcapsules, can be determined by extraction in cyclohexane using an ultrasound and confocal microscope [14].

3.2. High-performance liquid chromatography characterisation

High-performance liquid chromatography (HPLC) is used to separate the components in the compound based on chemical interactions between the analysed substances and the stationary phase in the column [11].
Depending on the analyte an appropriately equipped device should be used (corresponding column and detector) as well as suitable mobile phases in order to perform the analysis. There are many other conditions depending on the analysed analyte, so a special method is required for each sample type. The results are processed using a computer programme and the obtained data are used for calculating the concentration using standards of high purity which show a certain linearity.
HPLC analysis is quite common method for analysing tocols – primary antioxidants. Tocols are stable under HPLC conditions and it is easy to dissolve them in suitable solutions. Fluorescent detector (FLD) and ultraviolet detector (UV) are often used for tocol analysis [23-25].
Examples of HPLC α-tocopherol analysis:

1. Cosmetotextile containing α-tocopherol as an active agent was subject to quantitative analysis using HPLC analyser as well as suitable computer programme and UV detector. The used column is 4 μm EC-C18 (4.6 x 250 mm), and the suitable mobile phase is a mixture of 97:3 v/v methanol/water. The tocopherols are registered at the wavelength of 292 nm [21, 26].
2. The analysis of the suntanning filter can be performed with the HPLC equipped with RP-18 (5 μm) column and UV-ViS detector or UV-Vis spectrophotometer [22].

3.3. Gas chromatography characterisation

Gas chromatography (GC) is an analysis used for measuring the composition of different components in the sample. The principle of gas chromatography is that the sample is injected inside the instrument, and then enters the gas flow which transfers it to the separation pipe – column. Carrier gases are helium and nitrogen. Different components are separated inside the column. The detector measures the quantity of the components

exiting the column. To measure a sample of unknown concentration, a standard sample of a known concentration is inserted in the instrument. Standard retention time of the sample excess and the surface are compared with the test sample, and then its concentration is calculated [27].

Mobile phase in gas chromatography is an inert gas which eluates the components of the compound in the column filled with a stationary phase. As oppose to liquid chromatography, in gas chromatography the analyte does not react with the mobile phase so its speed of moving through the column does not depend on the chemical structure of the mobile phase. Stationary phase can be of the following types:

1. *for separating components of small molecule mass;* in that case the stationary phase is a solid of large specific surface onto which the analysed components are adsorbed;
2. *for separating components of large molecule mass;* the stationary phase is a liquid phase applied onto the surface of a strong carrier using adsorption or chemical bonding [28].

Gas chromatography enables the separation of active agents which allows their qualitative and quantitative analysis. The methods are widely used for analysing tocols, perfumes and essential oils [5, 6, 23, 24, 29].

We selected an example of a microcapsule containing fragrance as an active agent; qualitative and quantitative analysis was performed on the GC instrument using a suitable computer programme which controls the FID detector. Carrier gas was N60 helium at constant flow speed of 1 ml/min. The temperature of the furnace was programmed as follows: isotherm (50 °C) for 5 minutes, then increase from 50 to 200°C with gradient of 2 °C/min and maintains isotherm (200 °C) for 25 minutes. The injector was set at 240 °C with split ratio of 1/50. The FID detector is maintained at 250°C. Non-permeable nozzle (SGE, 1 ml) is used for analysing free space using the gas phase method. The sample's volume is injected into the 0.5 ml of free space. The sample is injected manually at the rate of 0.1 ml/s [6]. Figure 8 shows a vial with the 2 present phases.

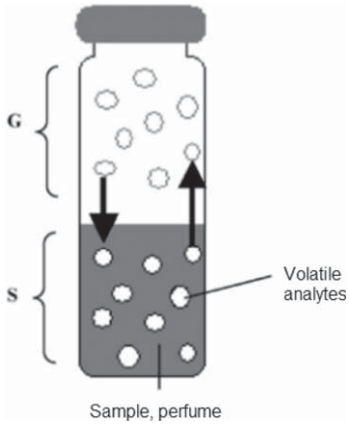


Fig.8 Vial for steam phase analysis;
G = gas phase (free space);
S = sample phase [6]

Gas chromatography is used for analysing tocols, but in that case it is necessary to derivate the analyte since the analyte could decompose due to excessive temperature [23-25].

When analysing microcapsules, gas chromatography is often used to perform quantitative analysis of the active agent, i.e. microcapsules resistance analysis to laundering [5, 29].

The microcapsules containing α-tocopherol as well coconut oil in the core and chitosan as membrane and microcapsules containing neroli fragrance were analysed using GC. The peak of the neroli appears at 25 minutes and it is still present on the textile substrate after 20 laundering cycles [29].

3.4. Fourier transform infrared spectrometer (FTIR) characterisation

FTIR is an instrument used for identifying unknown materials when determining certain components in a sample and its quality as well as consistency [11].

Two techniques are available for performing FTIR spectroscopy: ATR and KBr, where ATR can be used for performing non-destructive sample analyses, *i.e.* in forensics and restorations; and with KBr technique the sample must be shaped into a plate for perform testing [30].

FTIR-KBr technique is used for analysing the chemical structure of microcapsules consisting of a core – neroli fragrance and bio-polyurethane membrane [29].

FTIR-ATR technique is used for the qualitative analysis of microcapsules, *e.g.* agents used for synthesis of microcapsules and finally synthetised microcapsules. It is possible to compare the spectres of the chemicals used for the synthesis of the microcapsules and the microcapsules alone. This method helped to determine and see a characteristic spectre of the active agent (inside the micro-

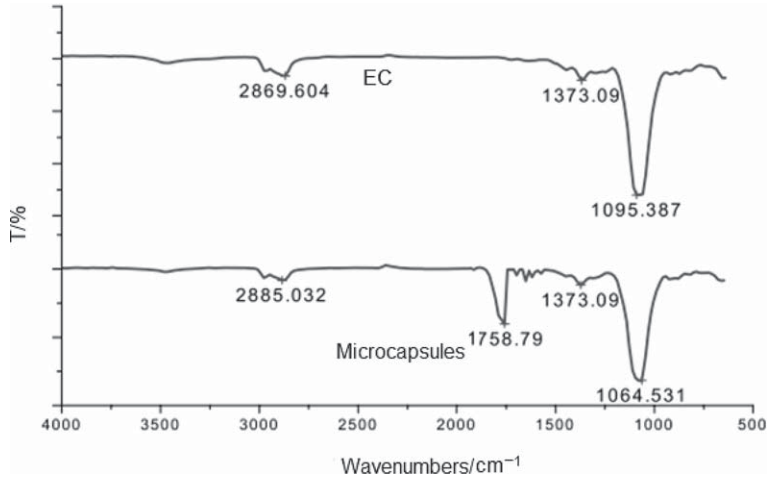


Fig.9 FTIR spectre of the fragrant microcapsule and ethyl cellulose (EC) [31]

capsule) since the ATR measures the surface of the tested sample and the obtained result is expected – overlapping of the majority of the components' peaks used for the membrane of the microcapsule and the microcapsule itself. Using another method (drop test) and the same mentioned case, the presence of the active agent (α-tocopherol) was determined, proving that the microcapsules contain α-tocopherol even though the FTIR did not show the characteristic peaks [10].

It was difficult to identify the core of the microcapsule when it consisted of small molecules with volume share below 25% and an ethyl cellulose membrane since the most common adsorption peaks were covered with ethyl cellulose. If -COOH, -COOR, C=O etc. molecule groups are present in the active component in the polymer, the adsorption peak will appear at the wavelength of ~ 1700 cm^{-1} due to the vibrations produced by stretching of chemical bonds. The most important components of lavender essential oil are linalyl acetate, linalool, lavandulol, *etc*. which contain -COOR and C=O molecule groups. Their characteristic adsorption peaks can be identified using the IR spectre at the wavelength of 1740 – 1755 cm^{-1} due to strong vibration of stretching. The spectre of pure microcapsules shows a peak at 1758 cm^{-1} whereas the spectre of ethyl cellulosis does not show this peak (Figure 9). The variation in the shape of peak and the position of the peak suggest that ethyl cellulosis successfully treated the essential oil of lavender and that the scented microcapsules were generated [31].

There are several cases in which the presence of microcapsules on cotton was verified by FTIR:

The testing of the cotton fabric, treated with microcapsules, resulted in characteristic peaks of the cotton *(i.e.* stretching vibrations of hydroxyl groups (–OH–, stretching vibrations of methylene (–CH_2–, bending vibrations of methylene and stretching of etheric bonds (–C–O–C–) at 3340, 2917, 1429 i 1058 cm^{-1}) and tamoxifen (i.e. carbonyl group at 1640 cm^{-1} and aromatic rings of the tamoxifen at 1508, 827, 769 and 705 cm^{-1}). Tamoxifen is a synthetic drug used for treating breast cancer and infertility in women [32].

4. Risk assessment – textile analysis (cosmetotextile)

In some cases, textiles containing microcapsules should be additionally analysed. The best examples are microcapsules applied on textiles for cosmetic purposes (cosmetotextiles) and they must be subject to the analyses recommended by the Croatian Technical Report which prescribes safety assessments for protecting human health [20].

Primarily, quality control of textile used as a carrier of the active agent is recomended. This criteria for carriers and related standards for the analysis are described in Tab.1.

Cosmetic preparation (active agent) mainly consists of many various compounds. Therefore, it is required to make a toxicology profile for each preparation. Total toxicology analysis for certain cosmetic preparations is based on these profiles. In accordance with the Scientific Committee for Consumer Products (SCCP), risk assessment consists of the following steps:

a) hazard identification of all compounds,
b) assessment of reacting to dose,
c) exposure assessment,
d) risk characterisation [20].

Safety of bonds and microcapsules, membranes of the microcapsules as well as other supplementary substances used generally in the production of cosmetotextiles should also be subject of risk assessment. Textile used for cosmetotextiles must not contain substances above the allowed toxicological level [20].

Every statement on the properties and effects of the preparation should be credible and verified and should not be confusing to the consumer. General principle and procedure descriptions for elaborating the statements can be found in the European Cosmetics Directive 76/768/EEC and COLIPA guidelines (Colipa - Cosmetics Europe - personal care association) [20, 33]. The statements can be elaborated with different methodologies, i.e.:

a) experimental research on humans (list of typically used testing methods for clarifying cosmetic demands (Tab.2), *i.e.:*
   1) bioengineering methods and other objective technical methods;
   2) clinical testing with evaluators trained in risk assessment;
   3) sensory assessment with trained panel and users;
b) studies for testing users using subjective final points *(i.e.* preferences, opinions) of the user as a criterion;
c) *in vitro* testing or some other non-human or instrumental testing;
d) literature (scientific or academic nature);
e) generally acquired knowledge *(i.e.* shampoo cleans hair) [20].

It is possible to subjectively and objectively evaluate cosmetotextiles testing throught different cosmetic effects: chemical properties, toxicity,

Tab.1 Criteria for cosmetotextile quality control [20]

Criteria	**Standard**
Colour fastness to water	EN ISO 105-E01
Colour fastness to rubbing	EN ISO 105-X12
Colour fastness to perspiration	EN ISO 105-E04
Depending on care specifications:	
Colour fastness to domestic and commercial laundering	EN ISO 105-C06
Colour fastness to dry cleaning	EN ISO 105-D01

Tab.2 Examples of testing methods for verifying cosmetic statements [20]

Basic cosmetic functionality	**Classic experimental parameter**		
	method 1	**method 2**	**method 3**
Skin barrier function	Transepidermal water loss (TEWL)		
Moisturizing	Conductometry (measurement of skin water content trought its electrical conductivity, e.g. via corneometry)	Visual evaluation by a trained person	
Firming / improvement of skin elasticity	Cutometry	Ballistometry	Torquemetry
Skin surface pH	pH-meter		
Skin roughness / smoothness (skin topography; microrelief)	Visual assessment by a trained person	Imaging of the skin or skin prints (e.g. profilometry or fringe projection)	
Skin redness and pigmentation	Visual assessments by a trained person	Chromametry (e.g. Mexameter)	Evaluation via imaging
Assessment of the outer appearance of cellulite	Visual assessment of dimpling by a trained person	Evaluation of the improvement of the skin micro-relief (anti-dimpling effect) by profilometry or fringe projection of replicas or in vivo	Measurement of skin elasticity/ tone
Lightening/ whiteness	Colorimetric evaluation of skin + macrophotographs	Utilisation test – user self-evaluation + check by dermatologist in accordance with a pre-defined scale	
Anti-heavy / anti-tired legs	Measurement of the blood circulation in legs before / after treatment, e.g. via Laser- Doppler analyses		
Hair growth retarding	Measurement of hair growth on macrophotographs	Hair diameter measuring using microscope	
Deodorant / anti-perspirant	Anti-odour test: also called "sniff-test". Armpit odour is evaluated by a trained specialist.	Anti-perspirant: During this test, a cotton pad is put under the armpit. The volunteers rest for a certain time in a sauna. The sweat quantity is evaluated by gravimetry (weight of the cotton before/after the sauna period as well as left and right/ treated vs. untreated comparisons).	

vitamin E presence, efficiency, smell analysis, durability, marking [1].

Certain objective assessment methods of cosmetotextiles used for testing the skin are shown in Tab.2. Corneometry (using Corneometer®), used for testing the skin's hydration effect; in vivo optical technique of the geometry of human skin (using Dermatop®, FOITS), used for testing the skin's roughness effect and determining the changes in transepidermal water loss (using Tewameter®), used for testing the function of the skin's barrier. Subjective methods, such as testing of end-users through a survey and/or talk are used for evaluating the effects like cooling and weight loss (anticellulite effect) [1, 20].

In 2005, the European Committee for Standardization (CEN) appointed a workgroup (WG) in charge of cosmetotextiles, CEN/TC 248/WG 25. The workgroup WG 25 was responsible for developing standards for cosmetic textiles. WG 25 identified some areas where standardisation is needed. In line with this, five subworkgroups were appointed for various areas of cosmetotextiles. The European Committee for Standardisation adopted a norm for cosmetic textiles CEN/ TR 15917:2009: Textiles – Cosmetotextiles (CEN/TR 15917:2010 [1, 34, 35].

The necessary normative references stated in the norms are:

- EN ISO 3175-1: Textiles -- Professional care, dry cleaning and wet cleaning of fabrics and garments -- Part 1: Assessment of performance after cleaning and finishing (ISO 3175-1; EN ISO 3175-1),
- EN ISO 3758: Textiles – Care Labelling Code Using Symbols (ISO 3758: 2005; EN ISO 3758:2005), EN ISO 6330:2003/A1en pr: Textiles – Domestic Washing and Drying Procedures for Textile Testing (ISO 6330:200/ Amd 1; EN ISO 6330:2000/A1),
- EN ISO 22716:2008: Cosmetics – Good Manufacturing Practices - Guidelines on Good Manufacturing Practices (ISO 22716; EN ISO 22716).

It is recommended to apply the stated normative references and harmonise them with the standards such as

Oeko-Tex® 100 and Oeko-Tex® 1000, which ensures high textile quality before the cosmetic preparation is even applied onto it and the cosmetotextile as a ready-made product.

Individual testing needs to be performed on cosmetic preparations in chemical industry, and when the ready-made product (cosmetotextile) is manufactured, the entire product requires testing with the help of general biological tests similar to antimicrobe tests.

The cosmetotextile should be tested in accordance with EN ISO 10993-10: Biological evaluation of medical devices - Part 10: Tests for irritation and skin sensitization (ISO 10993-10:10); and OE CD methods (OECD 405, 406, 407 & 471) [35, 36].

Five years ago, a team of scientists investigated the performance of 16 medicinal herbal extracts on seven selected diseases and applied on cotton garments. The testing was performed in accordance with the *American Association of Textile Chemists and Colorists* (AATCC) standards for antimicrobial activity. The following three antimicrobial testing methods were used: AATCC 100, Hohenstein modified requirement test; resistance to laundering tests AATCC 124. Agar diffusion methods were performed on parts of the fabrics and the percentage of the decrease in the number of bacteria was calculated and verified using the shaker test. The fabrics were subject to antimicrobial and antibacterial testing. Clinical testing confirmed the connection between the medicinal effect and the antibacterial effect. Antibacterial effect was verified by the qualitative method, SN 195920 Agar diffusion test. It was concluded that the use of herbal extracts on clothing as an alternative method of delivering drugs minimised the side-effects of oral use. In all cases, both clinical results of the testing and the doctors' evaluation, medicinal properties proved to be quite significant. In the case of testing antimicrobial efficiency on the *Staphylococcus aureus*, the bacteria on the medicinal materials decreased in a range between 82% and 98%; and for *Escherichia coli* 68-82% (both presented as gram of positive bacteria). It was verified that the effects on the clothing washed with standard detergent lasted 10-15 washing cycles. Cosmetotextiles can be used as supplementary treatment for certain illnesses [36].

5. Release of microcapsules

One special aspect of cosmetotextiles is the decrease intensity of their cosmetic effect during use and care. Literature suggests performing a simulation test to evaluate the resistance of the cosmetic preparation on the cosmetotextiles to care, *e.g.* whether enough quantity was applied at the beginning and whether the preparation is correctly bonded. This test is used for developing cosmetotextiles, for determining the quantity of the remaining preparation after care and for quality control [20].

Resistance to laundering is verified by determining the quantity of the remaining cosmetic preparation on the cosmetotextile and after a determined number of washing cycles. Care instructions are described in the standards applied to textile product care in accordance with EN ISO 6330 for laundering and HRN EN ISO 3175-1 for dry cleaning.

The suggested methodology is:

a) before the first washing cycle, the quantity of the cosmetic preparation on the cosmetotextile is determined by a validated method and if needed, another one suggested by the producer of the cosmetic preparation;
b) performance of a predetermined number of the washing cycles (in accordance with HRN ISO standards), and the number depends on the anticipated number of the washing cycles of the cosmetotextile;
c) after the performed washing cycles; the quantity of the remaining cosmetic preparation on the cosmetotextile to be verified using the same methods as before the washing.

Materials and reagents used in care procedures are described in EN ISO 6330 and prEN ISO 3175-1.

An example from literature relates to care procedure for cotton containing microcapsule where short programme was used on Linitest, 45 minutes at 30°C, in accordance with ISO 105-C01. After the care cycles, the samples were left to dry on a flat surface. All the samples were tested after five and ten washing cycles using SEM. After 10 washing cycles the microcapsules became visible on the cotton fabric, which means not only did they adsorb into the fabric surface, but they also formed covalent bonds with the cotton fabric in the presence of binder, and the bonds lasted for over 10 washing cycles. This result is very important for drug transmission system [32].

Depending on the microcapsules' composition, some can even last more than 25-30 washing cycles. However, conventional ironing and other thermal processes (machine drying) can drastically decrease their performance [36-38].

The selection of the microcapsules' membrane significantly decreases volatility of the active substance and damages caused by light and oxygen. One of the examples is a carbohydrate membrane and an additional membrane based on lipids which guarantees a highly stable product with a prolonged life-span [37].

6. Specific analyses

It is sometimes possible to use an analysis method which is not typical for microcapsules or textiles treated with a cosmetic product. One such example is the analysis of cosmetotextile containing α-tocopherol (vitamin E) as an active agent, using the drop test. If the active agent is oil, the performance and the capacity of oil capsulation can be calculated.

Drop test is one of the preliminary tests for accelerated identification of α-tocopherol on a textile substrate. The test is based on the redox reac-

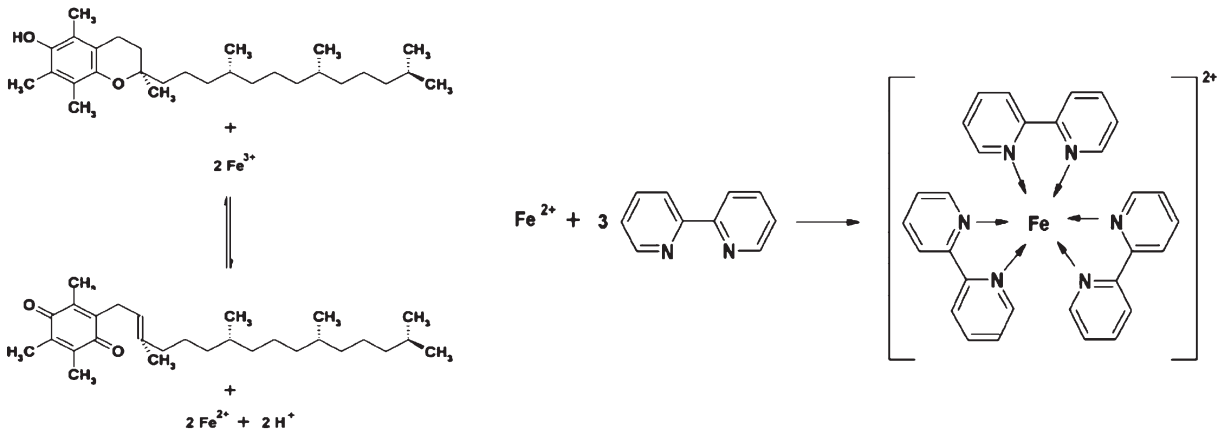
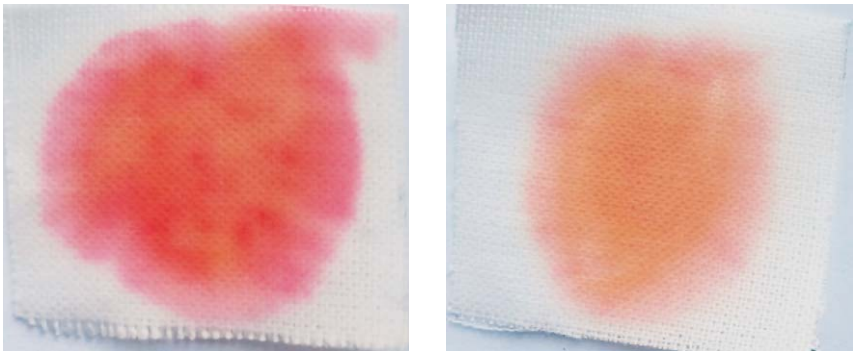


Fig. 10 Reaction mechanism: a) redox; b) complexation [41]



a) b)

Fig.11 Identification of α-tocopherol: a) cotton fabric treated with microcapsules containing α-tocopherol; b) non-treated cotton fabric [10]

tion between the α-tocopherol and iron(III) chloride where the iron is reduced ti iron(II) ion, and the α-tocopherol oxidises into Tokokinon. After addition of the dipyridyl solution, iron(II) ions form a red metal organic chelate complex with dypiridyl (Fig.10) [21, 39-43].

The presence of α-tocopherol was verified on the cotton fabric using drop test in the form of a red staining (Fig.11a). The microcapsules containing α-tocopherol active agent were applied onto this fabric. The fabric which was not treated with the active agent did not have a red staining, but a yellow one (Fig.11b).

7. Mathematical models for calculating the release of agents from the microcapsules

The mathematical release models are based on equations which describe real phenomena, such as mass transmission by diffusion, release of the active agent and *i.e.* transfer of polymer from glassy state to rubber state [4].

Some of the models used for calculating the release of certain active agents through the polymer membranes of the microcapsules are shown in Tab.3 [4].

One of the biggest challenges is a combination of mechanical theories which describe the release of the agents from the microcapsules and the mathematical models [44-45].

Tab.3 Release models of certain active agents through polymer membranes of microcapsules [4, 44]

Active agent	**Release model**
Fragrance	zero order model for film geometry
Drug	Diffusion according to the 2nd Fick's law – model for spherical geometry
Drug	one pellet model
Drug	multi-pellet model
	one pellet model
Dye (oil)	one membrane model
Propolis	2nd Fick's law – model for films geometry

8. Conclusion

Characterisation of microcapsules and the release method of the active agent from the inside of the microcapsule can be performed with different analysis methods, where each method has advantages and disadvantages. It is important to choose the method which can provide the answers to the following questions:

- what is the size of the microcapsules?
- what is the morphology of the microcapsule?
- how much active substance is contained in the microcapsule?
- how much of the active substances is released in to the environment / onto the skin during a certain period and under certain conditions?

The synthesis method and the components of the microcapsules direct-

ly affect the identification method and the above-mentioned parameters. Furthermore, additional analyses are performed, depending on the end-use of the textile, *i.e.* resistance to laundering, acceleration and the release method.

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