SEMARAK ILMU PUBLISHING

Journal of Advanced Research in Applied Mechanics

Journal homepage:
https://semarakilmu.com.my/journals/index.php/appl_mech/index
ISSN: 2289-7895

Morphological Assessment of Thymol Essential Oil using Different Solvents and Stirring Time

Nur Ain Arina Johan[1], Siti Hana Nasir[1,*], 'Aisyah Mohamed Rehan[1], Nik Normunira Mat Hassan[1], Abdul Waqar Rajput[2]

[1] Faculty of Engineering Technology, Universiti Tun Hussein Onn Malaysia, Pagoh Campus, Edu Hub Pagoh, KM1, Jalan Panchor, 84600 Pagoh, Johor, Malaysia
[2] University College of Textile Engineering, Bahauddin Zakariya University, Multan, Pakistan

ARTICLE INFO

Article history:
Received 22 June 2024
Received in revised form 2 August 2024
Accepted 10 August 2024
Available online 30 August 2024

Keywords:
Microcapsule; Thymol essential oil; Stirring time; Ethanol; Methanol; Morphology; SEM

ABSTRACT

Essential oils (EOs) have been used in numerous industries for ages including pharmaceutical, textile and food. Microencapsulation formulation and techniques are important to enhance the EOs characteristics and stability which could directly influence the yield and quality of EOs. This research paper evaluated the effect of using different solvents and stirring time on thymol EO microcapsules. Ethanol and methanol were used as solvents to encapsulate the core and wall material using a simple coacervation method. The microcapsules were collected at various time intervals which were 15, 30, 45, 60, 90, 120, 150, and 180 minutes. It has been noted that the stirring time influenced the size of microcapsules. Using Thymol oil, ethyl cellulose, methanol and polyvinyl alcohol (TM) produces the most microcapsules at 120 minutes of stirring time. The biggest microcapsule diameter was recorded at 90 min stirring time using formulation of thymol oil, ethyl cellulose, ethanol, and polyvinyl alcohol (TE). The encapsulation efficiency (EE) of both samples are 64.55% and 72.16%, respectively.

1. Introduction

Essential oils (EOs) are aromatic volatile compounds that are extracted from plants. Due to their diverse biological functions, EOs have played critical roles in a variety of sectors including pharmaceuticals (functional properties), textiles (aromatic, antibacterial, insect repellent), food applications (natural flavour, preservatives, edible packages) and home (as fragrance and cleaning products) [1]. The use of EOs has gained interest and is recognized as a promising alternative for synthetic chemicals, providing valuable health benefits to global consumers as well as minimizing environmental impacts [2–5]. EOs can be extracted or sourced from petals and flowers, roots, stems or barks, leaves, and fruits or seeds of plants [6].

* *Corresponding author.*
E-mail address: sitihana@uthm.edu.my

https://doi.org/10.37934/aram.124.1.6170

Thymol is derived from thyme (Thymus vulgaris L.) plants, and is a type of EO that has been widely used in traditional medicine for its diverse therapeutic properties [7]. In addition, Thymol is also utilized in cosmetics, foods, and perfumes. Few studies highlighted its effectiveness as an expectorant, anti-inflammatory, antiviral, antibacterial, and antiseptic agent for upper respiratory ailments [8,9]. One of the drawbacks of EO is their hydrophobicity, instability and high volatility, thus the encapsulation method and techniques are important to enhance their stability, shelf-life and properties [4].

Numerous processes and techniques have been developed and modified to produce microcapsules with specific materials and desired properties, yet not all methodologies are suitable for textile applications [10]. For textile applications, it is critical to encapsulate thymol EOs in suitable shell materials before their application, allowing them to regulate release under ideal conditions. Researchers have used several methods such as spray drying, solvent evaporation, simple and complex coacervation and polymerization to stabilize and regulate the volatility of EO active ingredients. Microencapsulation involves the production of tiny particles, in spherical or irregular shapes. It contains active ingredients within a polymeric shell, facilitating precise control over the containment and release timing of oil substances. Microcapsules offers various benefits across agriculture, cosmetics, health, medicine, and textile sectors [11–13]. Simple coacervation method is when a single polymer is used and is precipitated out by the influence of electrolytes (such as sodium sulphate) or is desolated by the introduction of a water-miscible nonsolvent (like ethanol) or through temperature changes [14]. It is one of the most widely used method for producing microcapsules. In this study, simple coacervation method was employed to encapsulate the Thymol EO. The wall constituents are dissolved within a solvent, while the core materials are emulsified. Subsequently, the active ingredient is encapsulated within the shell material as the solvent evaporates.

In microcapsules, the selection of solvents are crucial as it can impact encapsulation efficiency (EE), yield, and quality of the microcapsules generated [15]. While this parameter's effect is intricate, further study into it could improve the conditions to generate microencapsulation methods economically feasible. Extensive research has been conducted in the field of microencapsulation studies to optimize various process variables and achieve the most efficient encapsulation of oils. Among the focal points of prior research efforts is enhancing the effectiveness of encapsulating EOs [16]. However, it is important to recognize that EE is influenced by a multitude of process parameters, including equipment settings and characteristics of the wall matrix [17].

To our knowledge, no prior investigation has explored the pivotal role of stirring time in the application of thymol EO-based microcapsules, especially in the context of textile applications. The primary objective of this research was to develop microencapsulated thymol EO with different solvents using a simple coacervation method. This study aims to illuminate the previously unexplored influence of stirring time by using different solvents on the characteristics of the resulting microcapsules, providing novel insights for potential advancements in the smart textile industry.

2. Methodology

2.1 Material

Thymol EO, which was 100 % pure and certified organic was purchased from US Organic, (New Jersey, USA) used as core material. Ethyl cellulose (EC) (Aldrich Chem.) was used to form the shell and encapsulate the core material. Ethanol (CH_3CH_2OH) or methanol (CH_3OH) were used as solvents according to their sample codes. Poly (vinyl alcohol) (PVA, Polysciences Inc., Mw = 78,000 Da, degree of hydrolysis of 88 mol%) was used as the wall-forming polymer. All chemicals in this study were analytical reagent grade and used as received.

2.2 Preparations of Microcapsules

A simple coacervation method as in Julaeha *et al.,* [13] research was applied in this study. The process was performed using the phase separation technique and separated into two stages. In the first stage (the oil phase), 5 g of EC powder was gradually dissolved in 100 ml of solvent using a magnetic stirrer for 10 minutes. Two types of solvents, which were Ethanol and Methanol were used in this study to measure the effect of using different solvents on microcapsule quantity and diameter. Next, 0.5 g of Thymol EO was gradually added to the solution for 30 minutes. Throughout the process, the system was kept at room temperature with continuous stirring at 700 rpm.

In the second stage (the water phase), the oil solution produced above was poured into 100 ml of 1%wt polyvinyl alcohol (PVA) solution. Two droplets of solution containing microcapsules were collected while being constantly stirred using a dropper at different time intervals of 15, 30, 45, 60, 90, 120, 150 and 180 minutes. The droplets were used for microcapsule characterization on an optical Microscope and Scanning Electron Microscopy (SEM). The samples were left to dry for characterization to determine the effect of the time taken for the EO to be encapsulated. The high-resolution imaging capabilities of scanning electron microscopy (SEM) allow it to provide detailed information about the shape, structure, and elemental makeup of surfaces [18]. To simplify the sample types, sample codes will be used hereinafter, as shown in Table 1.

Table 1
Sample code for each type of sample

Sample code	Sample type
TE	Thymol oil + EC + Ethanol + PVA
TM	Thymol oil + EC + Methanol + PVA

2.3 Microcapsules Characterization

The morphology of the microcapsule solution was observed using MOTIC Model BA210LED optical microscope with 10x magnification at different time intervals (15, 30, 45, 60, 90, 120, 150 and 180 minutes). The surface of the microcapsule solution was covered with glass fibre so that the solution did not encounter the microscope lens. For observation of the microcapsule's morphology, the droplet was dropped onto cotton fabric, and placed on the SEM stubs using two-sided adhesive tape and then sputter coated with gold. The diameter size of microcapsule solution droplets was determined by image analysis using the ImageJ software. The measurements were carried out in triplicate with three repetitions, and the mean diameter was measured. The EE was calculated according to Eq. (1).

$$EE\ (\%) = \frac{mass\ (total) - mass\ (non - encapulated)}{mass\ (total)} x\ 100 \quad (1)$$

3. Results and Discussion

Figures 1 shows the optical microphotographs of TE microcapsules at different time intervals. It was observed that all microcapsules were round-to-spherical in shape, which indicated that the microcapsules had formed [19,20]. No free oil material is seen in the optical micrographs, indicating that the oil is well contained inside the shell and that no air bubbles are present inside the microcapsule. As shown in Figure 1, the size of the microcapsules of sample TE increases from

minutes 0 to 90 minutes. The diameter ranges from 2.506 to 35.001 µm. However, after 90 minutes, the diameter started to decrease, and the diameter ranges from 2.750 to 22.689 µm. The biggest microcapsule of TE was noted at 90 minutes, and the smallest microcapsule was at 15 minutes. Although the diameter of the microcapsules at 90 minutes was the biggest, the shell was noted to be quite thin. It was also observed that the shell of the microcapsule was thickest at 180 minutes. This shows that stirring time did influence the size of microcapsules [21].

TM microcapsules at different time intervals (0-180 minutes) was depicted in Figure 2. The size of microcapsules starts to increase at 15 minutes and decreases after 120 minutes. The diameter ranges from 3.752 to 24.187 µm from 0 to 120 minutes and decreases to 3.606 to 16.00 µm after 120 minutes. The biggest diameter for sample TM was recorded at 120 minutes, with a mean size of 13.76 µm. The outer shell of microcapsules of TM at 90 minutes (Figure 2(e)) and 120 minutes (Figure 2(f)) were thicker than other microcapsules. It was also observed that the microcapsules of TM in Figure 2 have many thick shells compared to the TE samples in Figure 1. This is similar to the findings reported in previous research by Martins *et al.,* [22], where the use of different solvents and surfactants influences the thickness and size of the microcapsules produced. When comparing Figures 1 and 2, it was found that the best solvent to encapsulate thymol oil was using TM at 120 minutes.

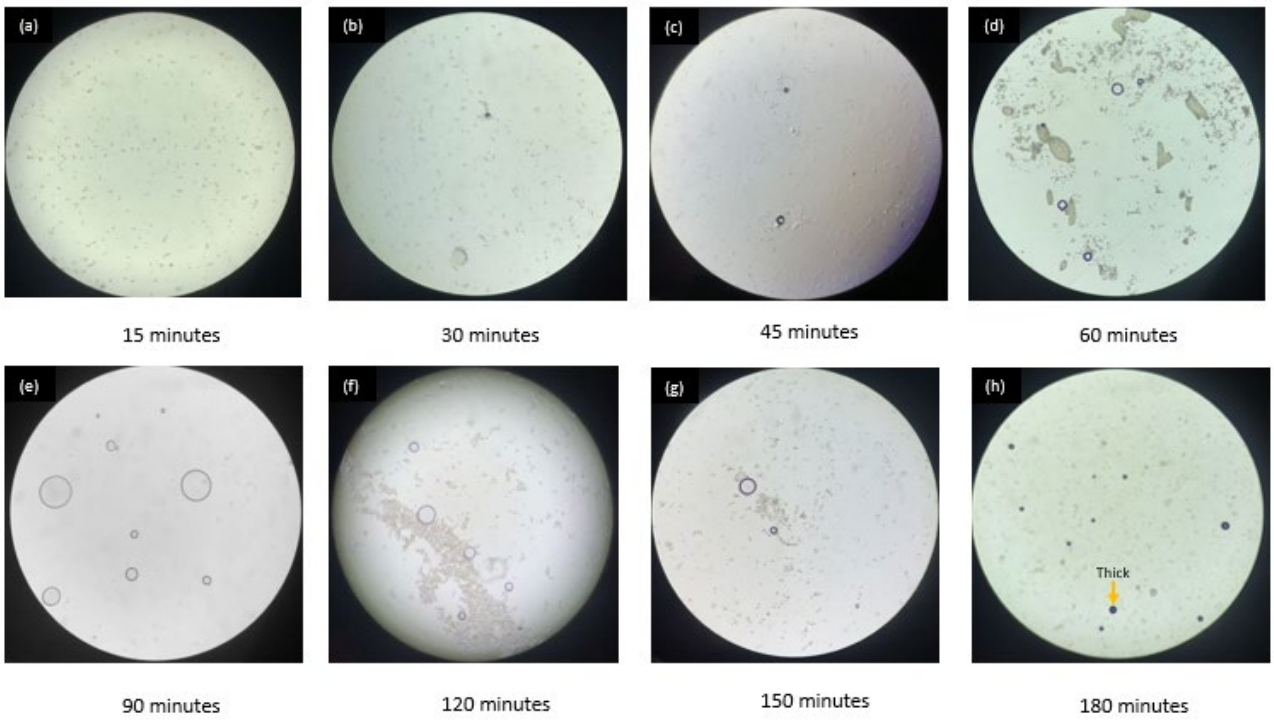

Fig. 1. Optical micrographs of TE at different time intervals; (a) 15, (b) 30, (c) 45, (d) 60, (e) 90, (f) 120, (g) 150 and (h) 180 minutes respectively (10x magnifications)

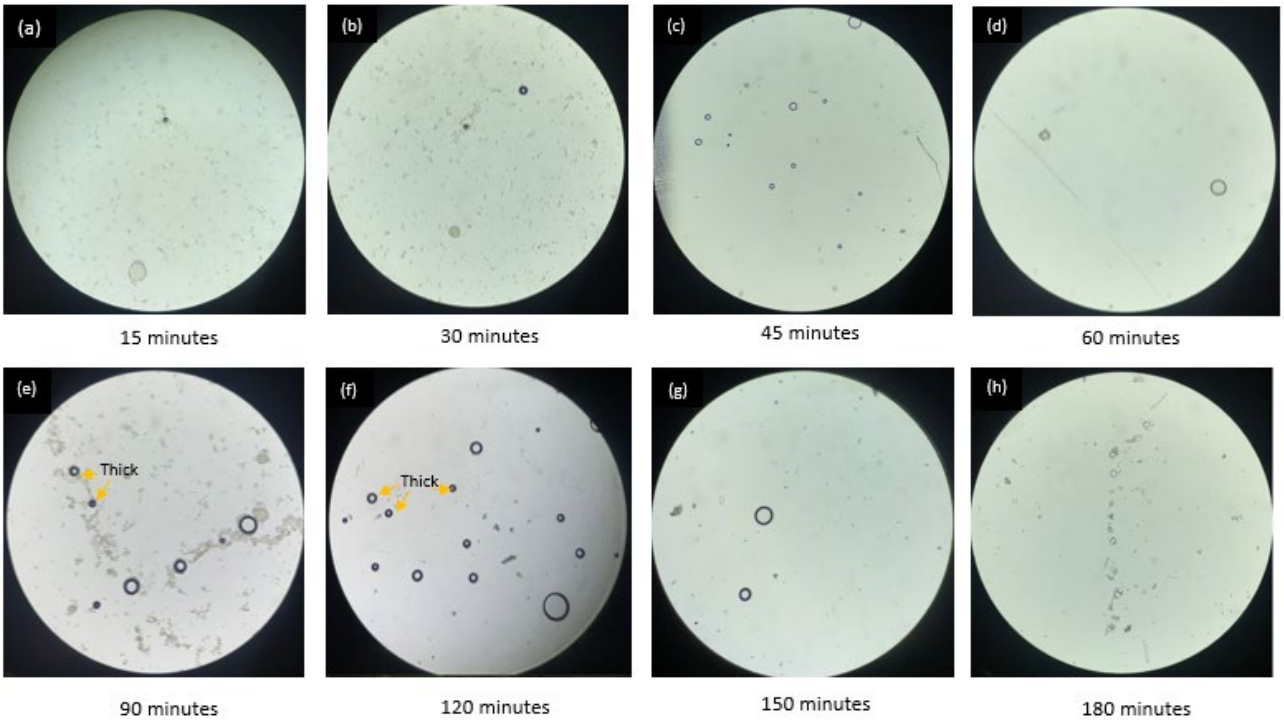

Fig. 2. Optical micrographs of TM at different time intervals; (a) 15, (b) 30, (c) 45, (d) 60, (e) 90, (f) 120, (g) 150 and (h) 180 minutes respectively (10x magnifications)

Figures 3 shows the SEM results of the microcapsule solution on cotton fabric of TE sample at different time intervals. The intricate interaction between the microcapsules within the fibre of the cotton fabric can be seen clearly in these figures. The distribution, shape, and adherence of the microcapsules on the fabric's surface are all clearly shown in these SEM images especially in Figure 3(a-f). The images from this study is similar to the findings in other studies [23–25]. It was observed that the shape of microcapsules was almost the same in all samples which is round to spherical, as also described in previous studies [26,27]. A spherical microcapsule can be clearly seen in Figure 3d. According to Hayden *et al.,* [28], a microcapsule that is spherical in shape is ideal for improving capsule storage and uniform distribution, increasing the interface between the core material and the matrix for coating applications.

The SEM images of TM samples in Figure 4 revealed that the size and shape of the microcapsules greatly influence by encapsulation parameters. Microcapsules were present on the cotton fibres and in the interstices between the fibres. It also can be noted that the microcapsules formed on TM were smoother and more uniform compared to the microcapsules formed on TE. Figure 4(e-f) show good spherical microcapsules were formed between minutes 90 to 120. This result is consistent with the findings in the optical micrographs from Figure 2(e-f). The size of the microcapsules ranged from 3.333 to 24.187 µm. Nelson [29] also mentioned in the study that the typical size range of microcapsules is 1 to 20 µm. The smaller the capsule, the greater the product coating and the longer the fragrance will remain because it takes more force to physically shatter the capsules. Larger capsules, however, burst more quickly and release more aroma [29].

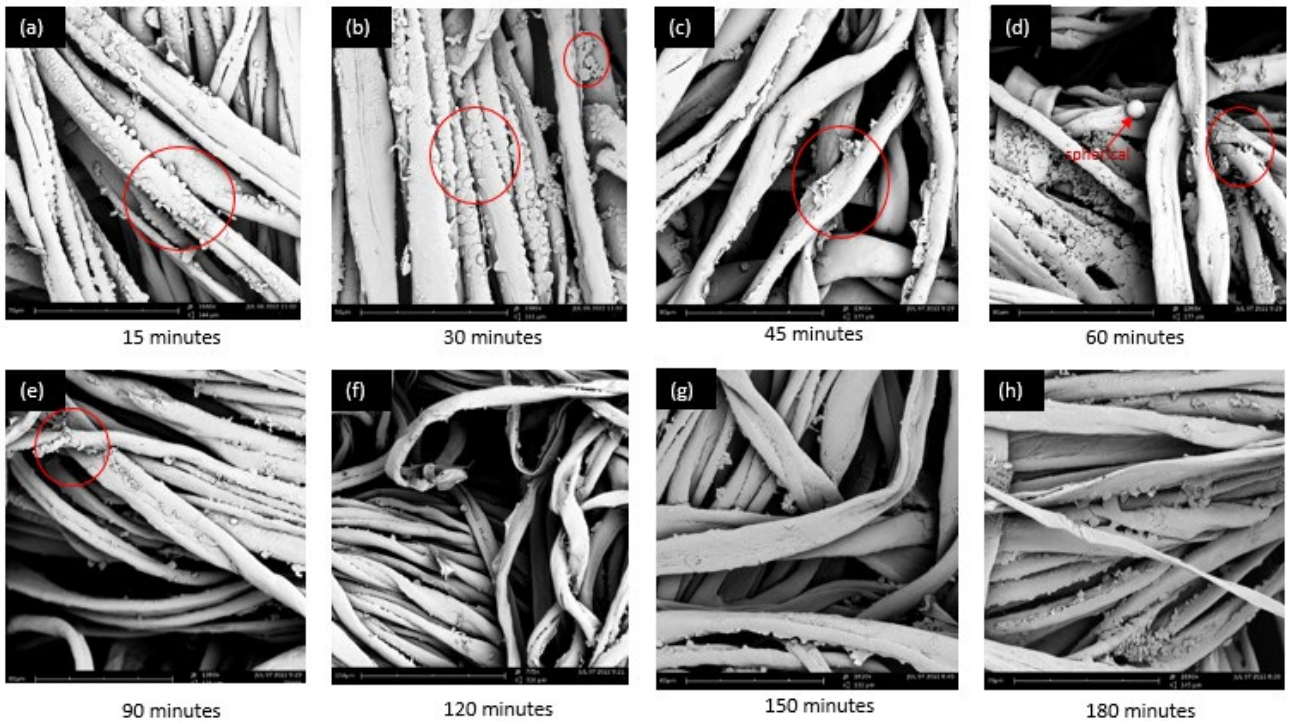

Fig. 3. SEM micrographs of TE at different time intervals; (a) 15, (b) 30, (c) 45, (d) 60, (e) 90, (f) 120, (g) 150 and (h) 180 minutes respectively. Microcapsules attached to the fibre in the fabric are depicted by the marked circles

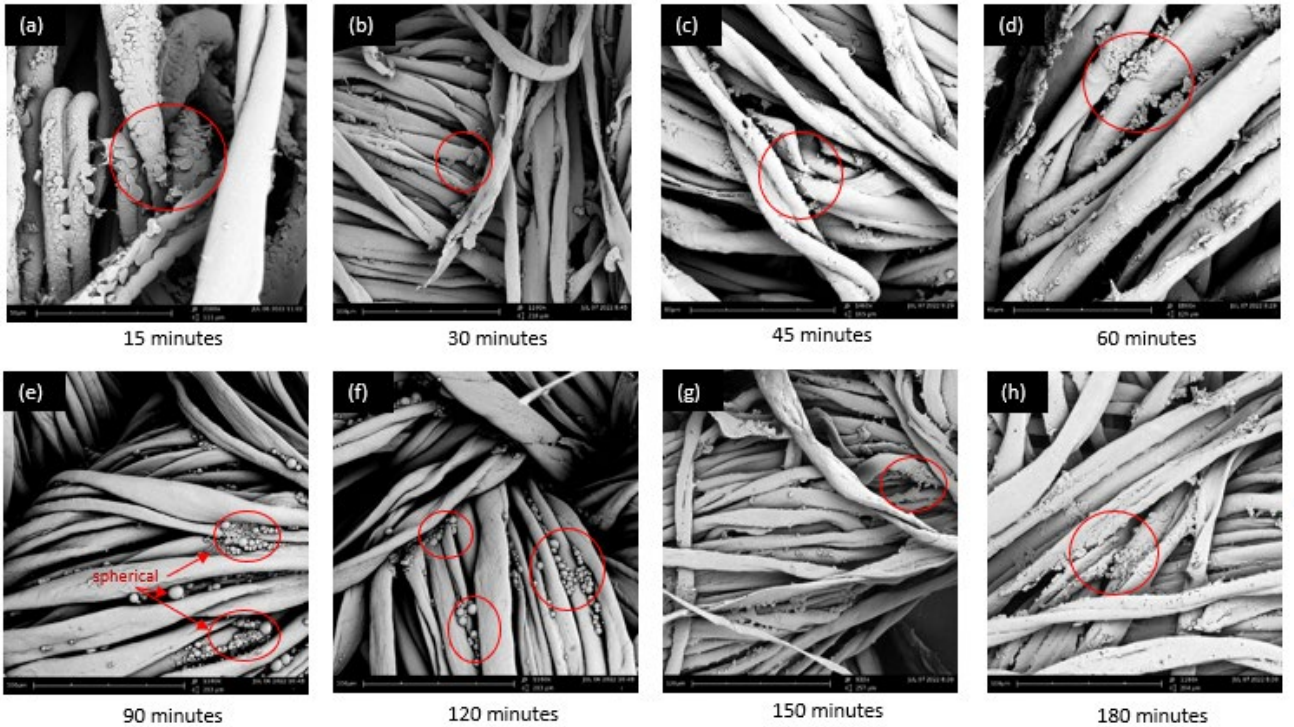

Fig. 4. SEM micrographs of TM at different time intervals; (a) 15, (b) 30, (c) 45, (d) 60, (e) 90, (f) 120, (g) 150 and (h) 180 minutes respectively. Microcapsules attached to the fibre in the fabric are depicted by the marked circles

Numerous factors influence the mean size of the microcapsules produced, and one of them is stirring time [26]. The effect of stirring time on microcapsule mean diameter can be seen in Figure 5.

It was discovered that the size of the microcapsules of TE increased at 0 to 90 minutes but started to decrease at 90 to 180 minutes. The mean diameter of TE was found to be the highest at minute 90, as can be also observed in Figure 1(e). While for TM sample, the size of the microcapsules increased from 0 to 120 minutes and start to decrease at 120 to 180 minutes as shown in Figure 5. The highest peak of mean diameter was found at 120 minutes, and it was noted that it rose significantly from minutes 60 to 120.

According to previous studies, smaller microcapsules have a larger surface area-to-volume ratio. This can lead to quicker diffusion of the EO components through the capsule shell, resulting in a faster release [27,30]. Smaller capsules also tend to have a relatively thicker shell, which provides more protection to the EO components within, making them less prone to external factors that could trigger premature release. This can lead to a longer-lasting fragrance as the EO components are released more gradually. However, in the case of fragranced textiles, if the shell is too thick, the microcapsule will resist breakage under applied stresses. So the thickness of the shell has to be considered for the release of core material containing fragrance [31].

Encapsulation efficiency was also calculated based on the mass total of encapsulated microcapsules and the mass of non-encapsulated microcapsules. The results showed that the encapsulation efficiency of the TE and TM are 64.55% and 72.16%, respectively. Higher efficiencies were observed for TM compared to TE, attributed to its better solubility and evaporation properties. During the encapsulation process, methanol usually has a higher solubility in many organic compounds, which makes it easier for the active ingredients to dissolve and for a homogenous mixture to develop. Additionally, methanol has a lower boiling point compared to ethanol, which makes it simpler to eliminate during drying procedures leading to less residue and better encapsulation efficiency [32].

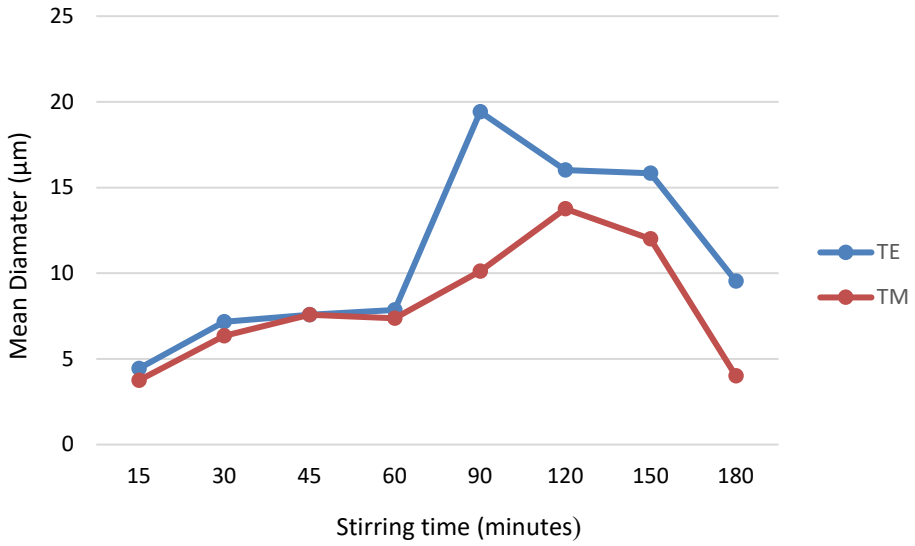

Fig. 5. The mean diameter of produced TE and TM microcapsules

4. Conclusions

In this study, a simple coacervation method was used to encapsulate microcapsules using thymol EO and two different types of solvents. Microcapsules' morphological structures was observed using optical microscope and SEM to evaluate the effect of stirring time and solvents. The microcapsules were successfully encapsulated and can be clearly seen on the cotton fibres and in the interstices

between the fibres. The study found that the size of thymol microcapsules diameter was influenced by the stirring time. The best time to encapsulate microcapsules was obtained between 90 to 120 minutes using TM sample. In addition, the best microcapsules characteristic was also found in TM sample. The diameter of synthesized capsules ranges from 2 to 35µm. The encapsulation efficiency of TE and TM are 64.55% and 72.16%, respectively. Higher efficiencies were observed for TM could be attributed to its better solubility and evaporation properties. Further studies need to be conducted to analyse the characterization of produced microcapsules in order to enhance the existing method and reinforce the capability of core-shell particle for further development in textile finishing.

Acknowledgement
This research was supported by Universiti Tun Hussein Onn Malaysia (UTHM) through GPPS (vot Q228) and Erasmus+ programme through Erasmus+ Smartex Project (610465-EPP-1-2019-1-EL-EPPKA2-CBHE-JP).

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