A solid-phase microextraction platinized stainless steel fiber coated with a multiwalled carbon nanotube-polyaniline nanocomposite film for the extraction of thymol and carvacrol in medicinal plants and honey

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A B S T R A C T
A mechanically hard and cohesive porous fiber, with large surface area, for more strong attachment of the coating was provided by platinizing a stainless steel wire. Then, the platinized stainless steel fiber was coated with a multiwalled carbon nanotube/polyaniline (MWCNT/PANI) nanocomposite using electrophoretic deposition (EPD) method and applied for the extraction of thymol and carvacrol with direct-immersion solid-phase microextraction (DI-SPME) method followed by high-performance liquid chromatography-ultraviolet detection (HPLC-UV) quantification. To provide a larger coarse surface for the tightened attachment of coating on the fiber, a stainless steel wire was platinized using a suitable optimized EPD method. Different experimental parameters were studied and the optimal conditions were obtained as: pH of the sample solution: 2; extraction time: 60 min; salt content in the sample solution: 1% w/v NaNO₃; desorption time: 60 min; type and volume of the desorption solvent: acetonitrile, 100 μL. Under the optimized conditions, limits of detection (LODs) were 0.6 and 0.8 μg mL⁻¹ for thymol and carvacrol, respectively. Linear dynamic range (LDR) for the calibration curves of both analytes were 1–80 μg mL⁻¹. Relative standard deviation (RSD%, n = 6) was 6.8 for thymol and 12.7 for carvacrol. The proposed fiber was successfully applied for the recovery and determination of thymol and carvacrol in thyme, savory, and honey samples.

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1. Introduction
Antioxidants are compounds that counteract chemically active products of metabolism. Due to the presence of antioxidants (e.g., phenolic compounds), especially thymol and carvacrol, some plants play an important role in the avoidance of various diseases [1]. Therefore, interest in extracting thymol and carvacrol from the plants has increased, and their essential oils and extracts are widely used in many fields [2]. Thyme (Thymus vulgaris L.) and summer savory (Satureja hortensis L.) are well-known plants that contain thymol and carvacrol along with their principal components [3]. Therefore, the development of simple efficient methods for the extraction and quantization of their thymol and carvacrol has attracted the attention of scientists.

Solid-phase microextraction (SPME) was introduced by Pawliszyn in 1990 [4] as a simple, fast, effective, easily automated, and relatively inexpensive solvent-free sample preparation method. Due to its solvent-free nature, it has been widely applied for pharmaceutical, biological, food, and environmental analyses [5]. In this technique, volatile and semi-volatile analytes can be analyzed by using headspace (HS) strategy and non-volatiles are the subject of direct-immersion (DI) or membrane-protected (MP) modes of SPME [6,7]. SPME can be easily coupled to gas chromatography (GC), high-performance liquid chromatography (HPLC), and supercritical fluid chromatography (SFC) techniques [8–10].

Many researchers have utilized polyethylene glycol [11], hydroxyl fullerene [12], crown ether [13], poly-3-methyl thiophene [14], polypyrrole [15], polyaniline [16], and nanomaterials such as single-walled carbon nanotubes (SWCNTs) [17], graphene [18], and multiwalled carbon nanotube/polyaniline (MWCNT/PANI) for preparation of hand-made SPME fibers. Compared to commercial

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fibers, nanomaterial coatings can absorb/adsorb analytes more efficiently, due to their high area-to-volume ratio and other unique physiochemical characteristics. Among nanosorbents, the composite of MWCNT and PANI has attracted high interest due to their unique features [19]. MWCNT/PANI has been coated on different conductive rods such as stainless steel [16] and platinum [20–22]. On the other hand, there are different methods for the preparation of hand-made SPME fibers coated with nanomaterials such as sol–gel [23] and electrophoretic deposition (EPD) [20]. EPD has several additional advantages over other coating processes. It is scalable, as demonstrated by applications in ceramics and coating industries, where films are deposited onto substrates as large as automotive bodies and as small as nano-scale electrodes.

The structure and properties of the cast film can be tuned to targeted values by manipulating the process’ variables such as applied AC/DC voltages, frequency, and nanocrystal surface chemistry, thereby confirming the process flexibility. Finally, by preparing suitable templates as electrodes, patterned films can be cast. These combined characteristics make EPD an ideal deposition scheme to produce robust nanoparticle thin films [24]. Due to smooth surfaces of fiber and lack of proper adhesion between fiber base and coating film, the hand-made coatings prepared for SPME fibers are often unstable and breakable. Many of these coatings are easily removed during the handling process as well as their leaving and entering into the needle of SPME holder. Therefore, we decide to make a mechanically hard and cohesive porous fiber base, with large surface area, to improve the adhesion of coating on the fiber bed. According to this strategy, for the first time, the surface of stainless steel wire was platinized using a suitable optimized EPD method. Therefore, the fiber bed became very porous, with large surface area, and remarkably resistant to corrosion. After preparation of the platinized stainless steel wire, it was coated with an MWCNT/PANI film using a suitable EPD technique.

The proposed SPME fiber was prepared by coating the MWCNT/PANI nanocomposite film on the platinized stainless steel rod and was used to extract PAHs from polluted soil samples followed by determination using gas chromatography-flame ionization detection (GC-FID) [25]. It was successfully applied for more than one hundred analyses without any damage or change in its extraction efficiency. The proposed fiber’s coating had significant stability and durability. Thus, after a year of preparation, it never lost its effectiveness. In this research, for further study, we attempt to test the stability and durability of the proposed SPME fiber in the solution. The proposed SPME fiber prepared by coating the MWCNT/PANI nanocomposite film on platinized stainless steel wire was applied for the extraction of thymol and carvacrol in aqueous solutions using DI-SPME strategy followed by high-performance liquid chromatography-ultraviolet detection (HPLC-UV) quantification.

2. Experimental

2.1. Chemicals and materials

Thymol (≥98%) and carvacrol (≥99%) standard materials were purchased from ROTH (Karlsruhe, Germany). Stock solutions of thymol and carvacrol (100 μg mL⁻¹) were prepared by dissolving 10 mg of each compound in 100 mL ethanol and stored at 4 °C in refrigerator. The standard working solutions were prepared daily from these stock solutions using double distilled water. Analytical grade potassium hexachloroplatinate (IV) (K₂PtCl₆), acids, bases, and organic solvents such as acetoneitrile (analytical grade), aniline (≥99.5%), hydrochloric acid (HCl, 37%), sulfuric acid (H₂SO₄, 98%), and nitric acid (HNO₃, 70%) were purchased from Merck (Darmstadt, Germany). MWCNT with purity higher than 95% (10–20 nm O.D., 5–10 nm I.D., 10–30 μm length) was purchased from Plasma Chem (Berlin, Germany).

2.2. Instrumentation

High-performance liquid chromatography (HPLC) was carried out using a Shimadzu system composed of two returning pumps, an SPD-10A UV-detector, a high pressure manual injection value (with 20 μL injection loop), a DGU-14A in line degasser, and a CT10-10AC column oven. All injections were made by overflowing the fixed volume loop. The separation was performed on an RP-C18 analytical column (Shim-Pack CLCC18, with 15 cm length and 3.9 mm I.D.) packed with 5 μm particles. The mobile phase consisted of 70% acetonitrile and 30% (v/v) water, delivered from separate pumps. The flow rate was kept at 1 mL min⁻¹. Data collection and processing was performed with Class-vp v.R-6.1 software. The UV detector wavelength was fixed at 277 nm, and the column temperature was set at 40 °C. Fourier transform infrared spectra were recorded by an FT-IR 4400 spectrometer (Shimadzu, Japan) in the transmittance mode, and employed to characterize the oxygen-contained functional groups. A VEGA\ U TESCAN scanning electron microscope (SEM CM120, Czech) was used to investigate the morphology of the MWCNT/PANI fiber coating surface. A hand-made electrode rotator was fabricated and used for spinning the electrode during EPD process to ensure uniform deposition of nanocomposite film on the surface of fiber.

2.3. Preparation of SPME fiber coated with MWCNT/PANI by EPD

2.3.1. Modification of fiber base

For this purpose, a 0.01 mm O.D. and 4 cm-long stainless steel wire (Vita Needle Co., Needham, MA, USA) was washed with purified water and then dried at room temperature. Afterward, it was placed in a vacuum drying oven at 1100 °C for 35 min. It was then cooled to room temperature in a vacuum drying oven and stored in methanol, after removing from the oven. The platinizing suspension was prepared by dissolving 0.42 g of K₂PtCl₆ and 0.2 g of H₂BO₃ in 20 mL water. Then, the stainless steel wire was connected to the cathode of a DC power supply and immersed into the platinizing suspension. A normal stainless steel wire was used as anode and immersed into the suspension. The distance between the two wires was kept at 2.0 cm, and 1 mA DC electric current was applied to both electrodes for 5 s, while the cathode was spinning at 200 rpm. Finally, the platinized stainless steel wire was removed from the suspension, washed with water and dried.

2.3.2. Electrochemical deposition of MWCNT/PANI on platinized stainless steel wire

To synthesize MWCNT/PANI nanocomposite, 10 mg of MWCNT and 5 mL of aniline were refluxed in 2 mL mixture of concentrated nitric acid and concentrated sulfuric acid (1:3 v/v) for 3 h at 80–90 °C, which resulted in a dry solid voluminous precipitate. Then, 50 mg of MWCNT/PANI precipitate was ultrasonically dispersed in 5 mL of 1 M H₂SO₄ solution for 1 h. For coating the MWCNT/PANI film on the fiber bed, the platinized stainless steel wire was connected to the cathode and a normal stainless steel wire was connected to the anode of the DC power supply and both were immersed into the suspension, obtained from the last stage. The distance between the two stainless steel wires was kept at 2.0 cm and a DC voltage of 0.7 V was applied between them for 300 s, while the cathode was rotating with a speed of 200 rpm. To achieve a fiber’s coating with appropriate thickness, the EPD procedure was sequentially repeated three times.
2.4. SPME procedure

A 40 mL extraction vial (Supelco, Bellefonte, PA, USA) was used as the sample container. A 10 mL portion of the sample solution containing 10 µg mL\(^{-1}\) of thymol and carvacrol in double distilled water, with pre-established pH 2 and ionic strength (1% w/v NaNO\(_3\)), was placed into the extraction vial and stirred with a magnetic stirring bar. Then, the nanocomposite SPME fiber, handled with a suitable hand-made fiber holder, was immersed into the sample solution and the extraction was performed at room temperature with a stirring rate of 1000 rpm. After completion of the extraction (60 min), the fiber was placed inside a conical microvial containing 100 µL of acetonitrile for desorption of the analytes. After desorption of the analytes, 20 µL of the elution solvent was injected into the HPLC system and the peak area was used to evaluate the extraction efficiency of the DI-SPME procedure.

2.5. Preparing the essential oils of thyme and savory

Using a glass Clevenger-type apparatus (recommended by the European Pharmacopoeia), 100 g of each dried and ground plant material was subject to hydrodistillation for 4 h [26]. Then, the extracted essential oils were dried over anhydrous sodium sulfate followed by filtration. Finally, the essential oils were stored at 4 °C in the dark until tested.

3. Results and discussion

3.1. Characteristics of the prepared coating

To evaluate the oxygen-containing functional groups of MWCNT/PANI composite, the FT-IR spectra of MWCNT and MWCNT/PANI were recorded (Fig. 1). The spectra show a definite chemical interaction between MWCNTs and PANI in the constituted composite. Polyaniline structure can be clearly observed from the vibrational modes of ~1552 cm\(^{-1}\) (indicating the C=N bond) and ~2929 cm\(^{-1}\) (indicating the N–H bond).

3.2. Characteristics of the prepared SPME fiber

The morphological surface structures of the platinized stainless steel wire and the MWCNT/PANI nanocomposite-coated fiber were evaluated using a scanning electron microscope (SEM). The right side of Fig. 2 shows the surface of the platinized stainless steel wire which has been completely porous and cohesive using the EPD platinization procedure. The middle and left parts of Fig. 2 are SEM images of the MWCNT/PANI composite, with two different magnifications, coated on the platinized stainless steel wire. It can be clearly observed that the surface of the nanocomposite coating is saliently porous. These images prove that EPD platinization has promoted the porosity and adherence of the stainless steel wire as well as its interaction with the nanocomposite coating. Other results of the proper cohesion of the stainless steel surface to the nanocomposite coating include higher mechanical strength, greater chemical resistance, more durability, and longer lifetime. On the other hand, severe porosity of the MWCNT/PANI nanocomposite fiber will result in larger surface area and, consequently, higher extraction efficiency.

3.3. Extraction of thymol and carvacrol using the MWCNT/PANI fiber

The proposed MWCNT/PANI fiber was applied to extract thymol and carvacrol from aqueous solutions. Thus, different experimental factors influencing the extraction efficiency for thymol and carvacrol such as pH of the sample solution, extraction time, ionic strength of the sample solution, type and volume of the desorption solvent, and the desorption time were evaluated and optimized.

3.4. Optimization of desorption conditions

Desorption of the extracted analytes was performed using a conical micro-vial in a stationary mode. First, using different times over
the range of 20–80 min, desorption time was optimized. The results demonstrated that 60 min was the optimum desorption time (Fig. 3a). Then, several solvents such as acetonitrile (ACN), methanol, ethanol, and water were evaluated as desorption solvents at a constant desorption time of 60 min. The highest extraction efficiency was achieved when ACN was used as the desorption solvent. Thus, it was selected as the optimal desorption solvent (Fig. 3b). Moreover, the volume of the desorption solvent was studied. For this purpose, different volumes of ACN including 2.0, 1.5, 1.0, 0.5, 0.2, 0.1, and 0.05 mL were evaluated over a constant desorption time of 60 min. The best response was obtained using 0.1 mL and it was selected as the optimum volume for the desorption solvent (Fig. 3c). The volume of the HPLC injection loop used in this study was 20 μL.

Accordingly, the volume of the desorption solvent could not be lower than 20 μL. To evaluate the memory effect on the desorption process; different consecutive elutions were carried out on an MWCNT/PANI fiber. The results indicated that more than 99% of the extracted analytes were desorbed from the fiber during the first desorption step.

3.5. Effect of the sample solution pH

Different sample solutions containing thymol and carvacrol with varying pH, over the range of 2.0–8.0, were evaluated using the MWCNT/PANI fiber in DI-SPME method. The results are summarized in Fig. 4. It can be seen that the responses of both thymol and carvacrol decrease with the increase of pH for all the target solutions. This fact is due to the presence of carboxyl groups on the MWCNT/PANI coating, which can be ionized at high pH. These polar acidic groups are in neutral form, at low pH, and suitable to adsorb polar analytes such as thymol and carvacrol. The highest extraction efficiency for the proposed nanocomposite fiber was achieved at pH 2. To avoid possible damage of the fiber coating, the pH values less than 2 were not examined. These results also demonstrated that the proposed MWCNT/PANI fiber is stable and applicable in a wide range of pH 2–8. A possible reason for this stability is the strong interaction between the MWCNT/PANI coating and the porous surface of the platinated stainless steel wire.

3.6. Effect of the sample solution ionic strength

The effect of ionic strength was evaluated by adding different values of NaNO₃ (over the range of 0–5% w/v) to standard sample solutions. The results showed that the extraction efficiency of the MWCNT/PANI fiber for thymol and carvacrol is affected by changing the NaNO₃ contents (Fig. 5). The extraction efficiency increases with adding NaNO₃ from 0 to 1%, and then decreases. Salt contents less than 1% cause an increase in the extraction efficiency, while higher values of NaNO₃ result in gradual decrease in the adsorption of thymol and carvacrol. A possible explanation for this fact is changing the physical properties of the Nernst diffusion layer [27]. Increase in the efficiency of SPME experiments for the majority of analytes with salt addition is undoubtedly a proved fact, due to the salting-out effect [4]. Increasing the ionic strength of an aqueous solution decreases the solubility of analytes by involving water molecules and releasing them to adsorb on the attracting extraction phase. However, this effect is variable for different analytes with different polarities. Otherwise, adding a high content of salt to the aqueous sample is often found to decrease the extraction
efficiency due to reducing the diffusion coefficients of analytes by mutating the physical characteristics of the Nernst diffusion layer. This effect is more serious for polar analytes due to compensation for the salting-out effect [28].

3.7. Effect of the extraction time

As a very significant parameter in SPME procedure, extraction time was evaluated by applying different times in the range of 20–80 min for the DI-SPME of thymol and carvacrol from aqueous solutions using the proposed MWCNT/PANI fiber (Fig. 6). The extraction efficiency increased by increasing the time up to 60 min and then decreased. These results demonstrated that 60 min is enough for the complete equilibration of the sample solution and the fiber coating. This optimum point can be presumed as a balance between the extraction amount and the extraction time. Thereafter, at the extraction times longer than 60 min, the extraction efficiency decreased probably due to the exchange of adsorbed analytes with other species present in the aqueous solution. However, 60 min was selected as the optimal extraction time for further studies. Finally, the optimal conditions for the DI-SPME of thymol and carvacrol in aqueous samples, by the prepared MWCNT/PANI nanocomposite fiber, was achieved as pH of sample solution: 2; extraction time: 60 min; salt content of the sample solution: 1% w/v NaNO₃; desorption time: 60 min; desorption solvent: acetonitrile; and volume of the desorption solvent: 100 μL.

3.8. Durability and extraction efficiency of the prepared fiber compared to commercial fibers

To assess the permanence of the prepared MWCNT/PANI fiber to extract thymol and carvacrol, different sample solutions spiked at a concentration range of 5–100 μg·L⁻¹ were examined under the optimized conditions. The results indicated that the extraction efficiency of the proposed fiber did not decline significantly even after being used 80 times. The proposed fiber was examined again after a year, and the results demonstrated that its extraction efficiency has not changed more than 3%. Therefore, it was proven that the prepared fiber has prefect persistence and an acceptable lifetime, compared to other hand-made or commercial fibers. However, these observations are consistent with our previous experiences about this fiber, which was used for the HS-SPME of PAHs from contaminated soil samples [25]. To ensure the increasing effect of EPD platination on the mechanical strength and durability of the fiber, an MWCNT/PANI fiber was made without platination and its extraction efficiency, mechanical strength, and durability were evaluated. Its mechanical strength was much lower than the platinated one. It was easily stripped by finger's rubbing and seen to be flaky after a few minutes of exposure to the aqueous solution. Finally, it was used to extract thymol and carvacrol in aqueous solutions under the optimal conditions, and the obtained results were compared with those of the proposed fiber (Fig. 7).

This substantial durability of the prepared MWCNT/PANI fiber is remarkable even compared with that of the commercial fibers. The experiments showed that a polydimethylsiloxane/divinylbenzene (PDMS/DVB) commercial fiber can only be used more than 40 times in similar conditions. Otherwise, in recent years, numerous fibers with different coating compositions have been introduced by researchers for the SPME of organic analytes. However, based on our knowledge, such good durability has never been reported.

Additionally, the extraction efficiency of the fiber was evaluated compared to different commercial types. Thus, four commercial fibers (i.e., PDMS, PDMS/DVB, CAR/PDMS, and DVB/CAR/PDMS) were applied to extract thymol and carvacrol in water samples under the optimum condition and the results were compared to those of the MWCNT/PANI fiber (Fig. 7). The results clarified that the proposed nanocomposite fiber is more efficient than the tested commercial fibers for the extraction of thymol. However,
for the extraction of carvacrol, this fiber is just better than PDMS, but not comparable to PDMS/DVB, CAR/PDMS, and DVB/CAR/PDMS.

The selected analytes have relatively similar structures with benzene rings and hydroxyl groups, which may interact with CNT and functional groups on PANI through π–π and polar–polar interactions, respectively. However, under the experimental conditions of this study, carvacrol (with ortho-OH group) undergoes a keto–enol tautomeration more than thymol (with meta-OH group) (Fig. 8). The keto form has C–H, C–C, and C–O bonds whereas the enol has C=O, C–O, and O–H bonds. The keto form is the most stable for aldehydes and ketones in most situations. Thus, keto-form of carvacrol is less polar than its enol form, and its extraction is lower toward polar sorbents. This situation does not occur in thymol and can be extracted more than carvacrol using more polar extractants [29].

3.9. Analytical performance

Different replicated DI-SPME-HPLC-UV analyses were carried out using the MWCNT/PANI fiber in the optimized conditions. The results indicated that the recoveries of DI-SPME for thymol and carvacrol were within the range of 91–119%. Relative standard deviations (RSDs) for six replicate analyses of the sample solutions, containing 15 and 30 μg mL⁻¹ of analytes, were found to be 6.8% for thymol and 12.7% for carvacrol. The calibration graphs for both analytes were linear in the range of 1–80 μg mL⁻¹, with correlation coefficients greater than 0.99. Under the optimized conditions, the limits of detection (LODs) were found to be 0.6 and 0.8 μg mL⁻¹ for thymol and carvacrol, respectively.

3.10. Analysis of real samples

To demonstrate the reliability of the proposed MWCNT/PANI fiber, it was employed for the recovery of thymol and carvacrol in spiked honey, thyme, and savory sample solutions. Thus, different aqueous solutions of real samples were prepared and spiked with 5 μg mL⁻¹ of both thymol and carvacrol. Then, the spiked and non-spiked samples were examined using DI-SPME with the proposed fiber (Table 1). Additionally, thymol and carvacrol in both thyme and savory samples were analyzed through the hydrodistillation gas chromatography-mass spectrometry (HD-GC-MS) procedure as the standard method [26]. The results were compared with the proposed DI-SPME-HPLC-UV procedure (Table 2). The results showed that the MWCNT/PANI fiber can be successfully used to determine thymol and carvacrol in real samples by the proposed DI-SPME-HPLC-UV strategy.

### Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Thymol</th>
<th>Carvacrol</th>
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<tbody>
<tr>
<td></td>
<td>Added (μg mL⁻¹)</td>
<td>Found (μg mL⁻¹)</td>
</tr>
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<td>Honey</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>4.75</td>
</tr>
<tr>
<td>Savory</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>22.39</td>
</tr>
<tr>
<td>Thyme</td>
<td>–</td>
<td>8.02</td>
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<tr>
<td></td>
<td>5.0</td>
<td>13.97</td>
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### Table 2

<table>
<thead>
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<th>Sample</th>
<th>Thymol (w/w%)</th>
<th>Carvacrol (w/w%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DI-SPME-HPLC-UV</td>
<td>HD-GC-MS</td>
</tr>
<tr>
<td>Savory</td>
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<td>7.3</td>
</tr>
<tr>
<td>Thyme</td>
<td>58.7</td>
<td>62.1</td>
</tr>
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4. Conclusions

In this study, the surface of a stainless steel wire was made porous and cohesive through its platination with a proper EPD technique in a suitable fiber base for the firm attachment of coating. Then, a layer of MWCNT/PANI was coated on the stainless steel wire using a modified EPD method. The resulted SPME fiber showed substantial durability, long lifetime, and acceptable efficiency to extract thymol and carvacrol from aqueous solutions through a DI-SPME strategy. It was successfully utilized for the selective extraction and determination of thymol and carvacrol in real samples. The comparison of this novel fiber with some known commercial fibers has established its superiority to them.

### References


