New Fiber Coating with Silica-Coated γ-Fe$_2$O$_3$ Nanoparticles for Extraction and Determination of Volatile Compounds of *Ziziphora tenuior L.*

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ARTICLE INFO

ABSTRACT

The present study synthesized the silica-coated γ-Fe$_2$O$_3$ nanoparticles (NPs) with a large surface area and employed it as a novel fiber coating for extracting and determining volatile compounds from *Ziziphora tenuior* in a minimum period of time without using solvents with head solid-phase microextraction. The simplex approach was applied to optimize all parameters concurrently. Major optimized parameters include temperature, extraction time, and sample weight. In optimal circumstances, the repeatability for one fiber (n=5) was at the range of 2.8 – 9.2 percent for the sample compounds, which is given as relative standard division (R.S.D. %). The approach suggested in this work provides advantages such as simple usage, short analysis time, cheaper equipment, high relative recovery, and fiber thermal stability compared to common analysis approaches. Our findings indicated that γ-Fe$_2$O$_3$@SiO$_2$-PW fibers are appropriate for the HS-SPME analysis of essential oils.

INTRODUCTION

The genius *Ziziphora* L. is a member of the Lamiaceae Mint family, composed of 4 species: *Z. clinopodioides* Lam., *Z. tenuior* L., *Z. persica Bunge, and Z. capitata* L. Bunge in Iran. In Persian, *Ziziphora* plants are called "Kakouti," and they are considered as aromatic and medicinal species which have long been utilized for treating high blood pressure, edema, fever, neurasthenia, heart disease, insomnia, lung abscess, hemorrhoids, and tracheitis. In addition, they are used as stomachic, carminative, wound healing, and aperitive materials in Turkish and Iranian folk medicines. Iranian and Turkish people use the plants' dried aerial parts as flavouring materials, particularly in cheese and yogurt. Phytochemical studies concerning the *Ziziphora* genus chiefly focus on essential oil composition of this plant. Further, the *Ziziphora* species are a rich source of flavonoids, essential oils, caffeoyl derivatives, sterols, and fatty acids. Based on the previous studies, the *Ziziphora* oil is also a rich source of pulegone [1, 2].

It has been known that green materials serve as a set of principles eliminating or reducing the use or development of dangerous materials during the lifetime of chemicals. Hetero poly acids (HPAs) are considered as green materials which are made of hydrogen and oxygen with particular metals and non-metals. Two well-known classes of HPAs are on the basis of Dawson (H$_n$X$_2$M$_{18}$O$_{62}$) and Keggin

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($H_nXM_{12}O_{40}$) structures (where M=heteroatom and X= addenda atom). The common usage of HPAs is as heterogeneous and homogeneous catalysts, especially HPAs which are on the basis of the Keggin structure since they have attributes such as low toxicity, high oxidizing capacity, high thermal stability, high acidity, and high stability toward humidity. A polyoxometalate is the conjugate anion of an HPA. The early transition metals, including Ta, Nb, V, W, Mo, in the highest oxidation state, form oxygen-metal cluster anions and the link between them is the shared oxygen atoms for forming a 3D structure. Furthermore, their basic features such as solubility, elemental composition, size, reduction potential, shape, and charge density can be changed considerably [3-13]. Abolghasemi et al [3] synthesized Silica-Coated γ-Fe₂O₃ Nanoparticles and used it to extract polyphenols. The present study used this synthesized substance to extract volatile compounds from Ziziphora species.

EXPERIMENTAL
Chemical and Reagents
The analytical grade reagents were $FeCl_2.4H_2O$ (99%), $H_3PW_{12}O_{40}$ (>99%), $FeCl_3.6H_2O$ (99%), tetraethyl orthosilicate (TEOS, 98%), concentrated ammonium hydroxide (25%), as well as other solvents and reagents, prepared by Aldrich, Merck, or Fluka, without further purification.

Plant materials
When the plants flowered, the aerial parts of *Ziziphora tenuior* were gathered in summer 2019. The plants were gathered from the western region of Iran. They were shadow-dried at room temperature. Using the code 1812 ZT, a voucher sample was deposited at lab's chemistry herbarium.

Essential oils isolation
100 g of dried aerial parts (leaves and stems) of *Ziziphora tenuior* were grounded to obtain a fine powder. Then, the powder was placed into a 1000 ml distillation flask, and 500 ml of distilled water was added. In addition, hydrodistillation was applied for two hours using a Clevenger-type device as instructed by British Pharmacopeia. The oil in the condenser was gathered, dried over sodium sulfate anhydrous, and the resulting yield was approximately 0.30% based on the sample's dry weight. The resulting essential oil was kept at 4 ˚C to be analyzed later by GC–MS analysis.

Apparatus

An Agilent 5975C mass-selective detector and a Hewlett-Packard Agilent 7890A series GC with a split/splitless injector were employed for the purpose of determination. The operating mode of MS was the EI mode (70 eV). He (99.999 %) served as a carrier gas with a flow rate of 1.1 mL min⁻¹. A 30 m×0.25 mm HP-5 MS column (film thickness: 0.25 μm) was used for separating phenolic compounds. The temperature of the column was 70 °C and it was raised to 110 °C at the rate of 25 °C min⁻¹. It was then increased to 140 and 180 °C at the rates of 3 and 20 °C min⁻¹, respectively, and the final temperature was maintained for one minute. The injector temperature was 275 °C, and the splitless mode was applied for all injections for two minutes. The temperatures of ion surface, GC–MS interface, and quadrupole were 230, 280, and 150 °C, respectively. The Wiley 7N Mass Spectral Library was implemented for identifying the compounds.

Preparation of silica-coated γ-Fe₂O₃ nanoparticles
$FeCl_3.6H_2O$ (5.4 g) and $FeCl_2.4H_2O$ (2.0 g) were separately mixed in water (20 mL). In the next phase, the resulting solutions were mixed by strong stirring (1200 rpm). Then, a concentrated solution of NH₄OH (25% w/w) was added to the mixture at ambient temperature so that pH =11-12 could be maintained. The obtained black dispersion was constantly stirred (1200 rpm, one hour) at ambient temperature. The mixture was warmed to reflux for one hour to obtain a dispersion in brown color. Then, the γ-Fe₂O₃ NPs were purified by using 4-time repeated centrifugation (3000–6000 rpm, 20 min), and re-dispersion cycle continued until obtaining a stable magnetic dispersion in brown. A dispersion of pure NPs (8.5% w/w, 20 mL) was mixed with ethanol (80 mL) for one hour at 40 °C in order to achieve a silica layer coating on the surface of γ-Fe₂O₃ NPs, and the resulting mixture was thenmixed with a concentrated solution of ammonia (15 mL), and stirred at 40 °C (800 rpm, 30 min). After that, TEOS (1.0 mL) was charged to the reaction mixture and stirring the solution continued for 24 hours at 40 °C (800 rpm). A permanent magnet was used for collecting γ-Fe₂O₃@SiO₂ nanocomposite. Afterwards, they were washed three times with diethyl ether, ethanol, and dried for 24 hours in a vacuum.

Keggin-type HPA Immobilization on surface of silica-coated γ-Fe₂O₃ NPs
γ-Fe₂O₃@SiO₂ (1.0 g) was impregnated with an

HPA solution (1.2 g in 50 mL of acetonitrile) and the mixture was stirred at about 60 °C for 24–72 hours for preparing supported HPA materials. Further, a rotary evaporator was used for drying the γ-Fe$_2$O$_3$@SiO$_2$-PW nanocomposite. Finally, the sorbent was further calcinated for two hours at 250 °C in air [3].

Preparing SPME fiber

A piece of stainless steel wire was cleaned (d: 200-μm) two times with methanol in an ultrasonic cleaner for 20 minutes and dried at 70 °C. Epoxy glue was used for liming 1 cm of the wire, and γ-Fe$_2$O$_3$@SiO$_2$-PW was immobilized onto wire. An oven was utilized for heating the coated wire for 48 hours up to 50 °C. It was then softly scrubbed so that non-bonded particles are removed and it is mounted to the SPME holder. Lastly, SPME fiber was placed into GC injection port for the purpose of conditioning and cleaning for one hour at 270 °C in a helium medium.

RESULTS AND DISCUSSION

Optimization

The γ-Fe$_2$O$_3$@SiO$_2$-PW fiber efficacy was examined for headspace SPME of phenolic compounds. Previous experimental studies have shown that the prepared fiber was successful in adsorption of all compounds under testing. We optimized desorption conditions of gathered analytes in GC–MS injection port and their appropriate separation over the column before optimizing the parameters of the extraction. To this end, various desorption times and injection temperatures were examined. The thermal stability of the fiber coating confines upper temperature used for desorption. After testing various desorption temperatures, we found that 262 °C is the most suitable temperature for efficiently desorbing the analytes from γ-Fe$_2$O$_3$@SiO$_2$-PW fiber without damaging the coating. We investigated desorption times at a range of 2-4 minutes at this temperature, and the results indicated that the time duration of 2.8 minutes offers complete desorption without any carry-over impact.

We studied the main parameters that can influence extraction efficiency in headspace SPME. For selecting optimum extraction conditions, a simplex approach was employed. The Simplex approach should be used for achieving highest extraction efficiency and decreasing the number of experiments. During optimization, we monitored relative areas of major peaks (4 peaks) in the GC–MS chromatogram. In this approach, (n + 1) primary tests were designed, in which n denotes the number of parameters affecting extraction efficiency in HS-SPME, and the conditions corresponding to the worst response were specified. Furthermore, this process was iterated until the response no longer improved. When necessary, some reflections were adjusted.

The influence of temperature on the microextraction approaches is more significant as extracting analytes from the sample solution and sorption of analytes by SPME fiber are temperature dependent. The vapor pressure of the analytes increases at higher temperatures, resulting in increasing the concentration of analytes in headspace. Different temperatures at a range of 70-100 °C were used for samples to find the optimal extraction, and for this purpose, SPME fiber was exposed in the headspace at 87 °C.

HS-SPME is described as an equilibrium process, and mass transfer is a time-dependent process. During the extraction process, the sample compounds can transfer to headspace and HS-SPME device from aqueous phase to be collected. The duration of exposure of the fiber in headspace significantly influences achieving distribution equilibrium of analytes between the sample and fiber. Usually, the time given in headspace of samples is maintained as long as possible so that the equilibrium between the adsorbent and headspace is reached while the extraction efficiency is maximized.

Table 1 gives the conditions under which the primary tests and the designed ones for HS-SPME were conducted. A modified reflection approach was applied for obtaining the experimental conditions. In addition, the practical limitations of some factors were considered in modifications. Based on the results, the extraction efficiency of HS-SPME approaches is influenced by extraction time and temperature, desorption time, desorption temperature, and sample mass.

Fig. 1 indicates obtaining the maximum response for test No. 10. The experimental observations indicated that the optimal weight for the sample was 2.8 g. It is expected that in the HS-SPME, the extracted analyte amount increases with a rise in the extraction time of fiber in the sample headspace until 30 minutes. Following 30 minutes, the extraction rate remains constant. The most optimal responses for an extraction time of 30 minutes are

Table 1. Experimental conditions used and results obtained for the HS-SPME experiments performed in the simplex optimization procedure

Experiment number	Sample mass(g)	Extraction temperature(°C)	Extraction time(min)	Temperature desorption (°C)	Time desorption(min)
1	2	70	20	260	3
2	3	70	20	260	3
3	2	90	20	260	3
4	2	70	30	260	3
5	2	70	20	270	3
6	2	70	20	260	4
7(Reflection)	2.5	74	24	265	2
8(Reflection)	2.6	76	24	266	2.6
9(Reflection)	2.8	82	27	254	2.4
10(Reflection)	2.8	87	30	262	2.2
11(Reflection)	3	80	30	260	2
12(Reflection)	3	90	30	260	2
13(Reflection)	3	100	30	260	2
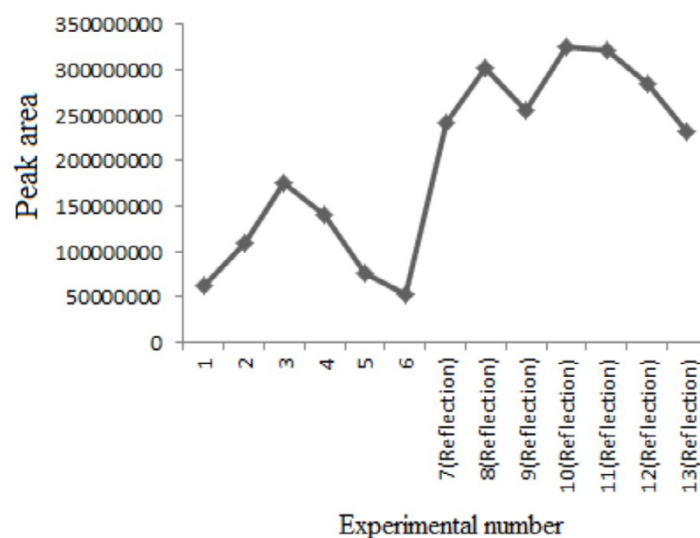
Fig. 1. The response (sum area of four main peaks of *Ziziphora tenuior* samples) for the designed experiments mentioned in Table 1.

given in Table 1. Moreover, the maximum relative peak area for volatile compounds was achieved at 87 °C. The heating system can evaporate the volatile oil compounds from the herbs at this temperature. Thus, approximately 2.8 g of the sample was needed for HS-SPME extraction, and the extraction lasted almost 30 minutes. Desorption time and desorption temperature were two minutes and 262°C, respectively, obtained by GC/MS.

Effect of humidity

Because of the γ-Fe₂O₃@SiO₂-PW structure, water vapor (humidity) may influence the ability of

the fiber for the adsorption of *Ziziphora tenuir* volatile compounds. Thus, under optimized conditions, varying volumes of water were added to the samples for evaluating the impact of humidity. Fig. 2 indicates the results of the four main compounds. Based on the results, the individual and total peak areas are reduced by the presence of water vapor in the atmosphere of headspace, which is consistent with the previous findings [3]. That is, the water molecules have the ability of deactivating the fiber surface; therefore, they block the active zones. Thus, the synthesized fiber is an appropriate adsorptive fiber in order to sample from the dried materials.

Table 2. Constituents of the oil of *Ziziphora tenuior L.*

No	Compounds	RI[a]	(HD) Area%[b]	SPME Area%[c]	Repeatability R.S.D.%[d]	Reproducibility R.S.D.%
1	α-pinene	939	1.86	1.34	4.5	8.2
2	Camphene	952	2.70	2.51	6.1	9.6
3	Sabinene	978	2.11	1.89	7.4	5.8
4	β-pinene	980	0.62	0.18	2.8	8.5
5	Limonene	1032	8.42	7.62	7.5	4.9
6	1,8-cineole	1035	4.02	3.98	5.8	9.7
7	Terpinolene	1090	0.85	0.91	7.2	7.1
8	2-nonanol	1092	0.02	0.05	6.3	10.2
9	Cis-limonene oxide	1131	0.11	0.05	8.1	8.4
10	Linalool (dihydro)	1135	0.18	0.08	4.7	10.3
11	Trans-limonene oxide	1140	0.22	0.10	9.2	9.6
12	Cis beta terpineol	1143	0.09	0.03	7.3	10.4
13	Benzyl acetate	1161	1.43	1.21	3.4	7.5
14	Neo iso isopulegol	1166	0.06	0.04	5.5	4.3
15	α – terpineol	1188	2.03	1.73	4.2	8.7
16	n-dodecane	1198	0.05	0.04	6.7	9.2
17	Pulegone	1236	55.72	48.83	9.2	7.3
18	Thymol	1291	5.43	4.26	7.3	10.8
19	α – copaene	1375	0.08	0.03	5.2	9.7
20	β- bourbonene	1385	0.31	0.27	7.6	8.2
21	Ethyl decanoate	1396	0.12	0.06	8.5	6.1
22	Caryophyllene (Z)	1402	0.80	0.12	7.6	9.5
23	Dodecanal	1403	0.05	0.14	5.2	10.3
24	E – caryophyllene	1420	0.41	0.17	4.6	10.5
25	β- Humulene	1441	1.53	1.51	8.1	9.7
26	Caryophyllene alchol	1565	0.78	0.42	7.5	6.5
27	Caryophyllene oxide	1580	4.94	3.88	8.6	8.7
28	1-hexadecene	1591	0.73	0.09	7.4	10.2

a) Retention indices (relative retention times normalized to closely eluting *n*-alkanes)
b) Relative area (peak area relative to total peak area) for hydrodistillation method
c) Relative area (peak area relative to total peak area) for SPME method
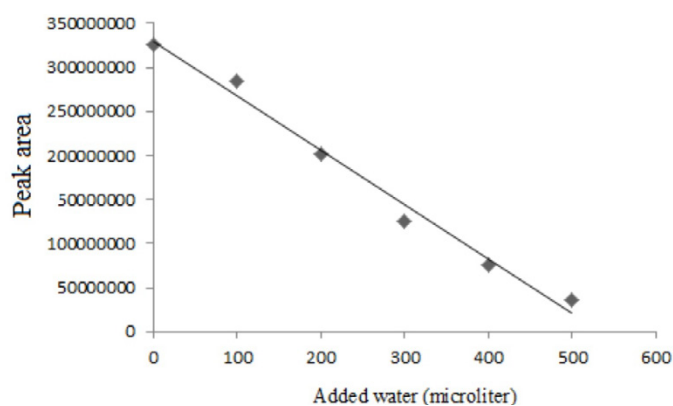

Fig. 2. Effect of water addition on the extraction efficiency of the solid-phase microextraction method.

CONCLUSION

In this study, we synthesized and tested the γ-Fe$_2$O$_3$@SiO$_2$-PW nanocomposite as a fiber for HS-SPME of volatile compounds in a medicinal herb. The developed approach is eco-friendly, as it does not use any toxic solvent. Based on the results of our experiments, synthesized fibers can be suitably applied for the HS-SPME analysis of volatile oils in medicinal herbs. Moreover, our findings showed that γ-Fe$_2$O$_3$@SiO$_2$-PW fibers

are appropriate for the HS-SPME analysis of essential oils.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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