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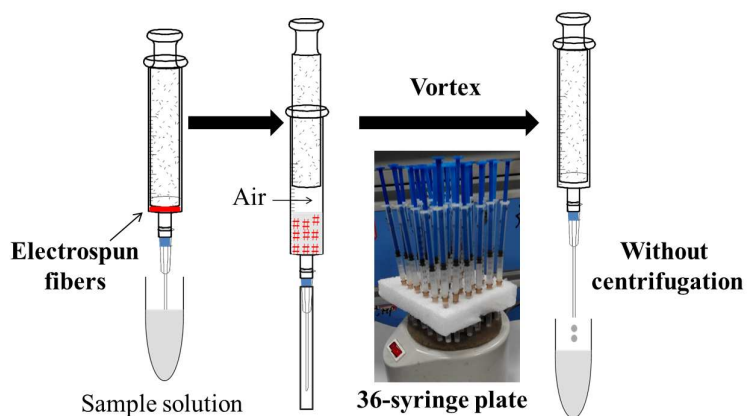
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The Table of Contents:

Vortex

Air

Electrospun fibers

Sample solution

36-syringe plate

Without centrifugation

A rapid and high-throughput in-syringe dispersive solid phase extraction (dSPE) system using electrospun fibers as adsorbent is presented.

Journal Name

ARTICLE

In-syringe dispersive solid phase extraction: a novel format for electrospun fiber based microextraction†

Gang-Tian Zhu, Xiao-Mei He, Bao-Dong Cai, Han Wang, Jun Ding, Bi-Feng Yuan, and Yu-Qi Feng*

A novel in-syringe dispersive solid phase extraction (dSPE) system using electrospun silica fibers as adsorbent was developed in the current work. A few milligrams of electrospun silica fibers were incubated in sample solution in the barrel of a syringe for microextraction assisted by vortex. Due to the benefit of dispersion and the high mass transfer rate of the sub-microscale electrospun silica fibers, the extraction equilibrium was achieved in a very short time (less than 1 min). Moreover, thanks to the long fibrous property of electrospun fibers, the separation of adsorbent from sample solution was easily achieved by pushing out the sample solution which therefore simplifies the sample pretreatment procedure. Besides, the analytical throughput was largely increased by using a multi-syringe plate to perform the extraction experiment. The performance of the in-syringe dSPE device was evaluated by extraction of endogenous cytokinins from plant tissue samples based on hydrophilic interaction. Six endogenous cytokinins in 20 mg of *Oryza sativa* L. (*O. sativa*) leaves were successfully determined under optimized conditions using in-syringe dSPE combined with liquid chromatography-mass spectrometry analysis. The results demonstrated that the in-syringe dSPE method was a rapid and high-throughput strategy for the extraction of target compounds, which has great potential in microscale sample pretreatment using electrospun silica fibers.

Introduction

Solid phase extraction (SPE) is one of the most important sample preparation methods with the advantages of rapid extraction, low consumption of organic solvents, and high recovery and enrichment capability.[1-4] Over the years, significant progress has been made in the development of SPE methods, including simplification, automation and miniaturization.[5-7] The adsorbent and operation format are two interactive topics in development of SPE methods.[8-11]

Novel adsorbents could benefit the establishment of new SPE formats. Electrospinning is a simple and versatile technique that can easily produce one-dimensional continuous nanofibers on an industrial scale and with controllable diameters, compositions and morphologies.[12-14] Up to now, electrospun fibers have been widely used in tissue engineering[15], drug delivery[16], energy technology[17] and catalysis[18]. However, the application of electrospun fibers in sample preparation field was less explored. In fact, as a candidate for SPE adsorbent, the electrospun fibers with nanoscale structure possess large specific surface area, allowing the use of small amount adsorbent in miniaturized SPE.[19, 20] In addition, different from nanoparticles, the continuous and integral structure offers the electrospun fibers two merits. Firstly, the fibers can serve as

adsorbents in packed SPE (pSPE) with low back-pressure.[21] Several formats of electrospun fibers based pSPE have been developed, including disk format[22, 23], microcolumn configuration[24-27] and in-syringe SPE[28, 29]. Secondly, the fibers are easy to separate from sample solution in dispersive SPE (dSPE), which has been proved in the previous work.[30] Each of the SPE formats using electrospun fibers has its own merits and drawbacks. The pSPE assays are easy-operation and rapid. However, in pSPE, the potential extraction area is not fully employed because of the aggregate of adsorbents, and the equilibration time is too long that often cause the extraction without reaching equilibrium. These phenomena become critical when the amount of adsorbent is reduced to the microscale, which may restrict the application of miniaturized pSPE based on electrospun fibers.[5] In dSPE, vortex and shaking normally are used to assist the extraction to increase the contact area and the mass transfer rate between the sample and the extractant phase. But high-speed centrifugation is usually inevitable in dSPE, which is time-consuming and may lead to coprecipitation of unwanted interferents and loss of target analytes.[31]

In this work, using electrospun silica fibers as adsorbent, a novel in-syringe dSPE method combining the advantages of pSPE and dSPE was developed for the first time. Furthermore,

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to examine the feasibility of the proposed in-syringe dSPE method, a home-made 36-syringe dSPE array was employed for the extraction of endogenous cytokinins with mesoporous silica fibers. Six endogenous cytokinins in 20 mg of *O. sativa* leaves were successfully determined using the in-syringe dSPE combined with liquid chromatography-mass spectrometry. Our results showed that the proposed in-syringe dSPE method possesses the following advantages: (i) feasibility for vortex, which ensured the sufficient contact area and fast mass transfer rate between two phases; (ii) easy operation without any centrifugation step; (iii) ability to scale-up; (iv) miniaturization.

Experimental

Chemicals and Materials
Polyvinyl alcohol (PVA, average MW 110,000), cetyltrimethylammonium bromide (CTAB), ethanol, orthophosphoric acid (H_3PO_4, 85 wt. % in H_2O), sodium hydroxide (NaOH), tetraethyl orthosilicate (TEOS) and hydrochloric acid (HCl, 37 wt. % in H_2O) were supplied by Shanghai General Chemical Reagent Factory (Shanghai, China). HPLC grade acetonitrile (ACN) was obtained from Fisher Scientific (Pittsburgh, Pennsylvania, USA). Cotton wool was supplied by Xuzhou Hygiene of Material Factory (Xuzhou, China). Cytokinins standards: N^6-isopentenyladenine (iP), isopentenyladenineriboside (iPR), N^6-isopentenyladenine 9-glucoside (iP9G), trans-zeatin (tZ), trans-zeatin-riboside (tZR), trans-zeatin 9-glucoside(tZ9G), dihydrozeatin (DHZ), dihydrozeatin riboside (DHZR), and stable isotope-labeled standards: $[^2H_6]$iP, $[^2H_6]$iPR, $[^2H_6]$iP9G, $[^2H_5]$tZ, $[^2H_5]$tZR, $[^2H_5]$tZ9G, $[^2H_3]$DHZ, $[^2H_3]$DHZR were purchased from Olchemim (Olomouc, Czech Republic). Purified water was obtained with a Milli-Q apparatus (Millipore, Bedford, MA, USA).

Preparation of silica fibers by electrospinning
The mesoporous silica fiber (MSF) was prepared using electrospinning combined with pseudomorphic synthesis according to our previous work.[28] The amorphous silica fiber (ASF) was produced by electrospinning. The precursor sols for electrospinning were prepared by the following procedure. First, 16 g of TEOS was added to the mixture of 5 mL of ethanol and 15 mL of water. Then 0.2 mL of H_3PO_4 was added dropwise to the solution. After stirring for 5 h, 30 g of 8 wt. % PVA solution was added into the silica sol to make the viscosity suitable for electrospinning. The reactant mixture was further stirred for 8 h to get a spinnable sol. In electrospinning process, the sol was driven by a four channels syringe pump with a flow rate of 0.4 mL/h. A grounded aluminum sheet covered with tinfoil served as collector and counter electrode and the distance of tip-to-collector was 15 cm. A high voltage of 15 kV was applied to the needle and a jet was moved and covered on the tinfoil. The fibers were collected and dried at 60°C for 12 h. The PVA were removed after calcination at 550°C for 8 h. ASF and MSF were treated with 6 M HCl and then were washed with water to neutral pH before use.

Characterization of the prepared materials
Scanning electron microscopy images were taken using Quanta200 scanning electron microscopy (SEM) (FEI, Holland). Nitrogen sorption measurement was performed at 77 K using a JW-BK surface area and pore size analyzer (JWGB Sci. & Tech., Beijing, China). The composites were activated by evacuating in vacuum and heating to 423 K for 6 h to remove any physically adsorbed substances before analysis. The specific surface area value was calculated according to the BET (Brunauer–Emmett–Teller) equation at P/P_0 between 0.05 and 0.3. The pore parameters (pore volume and pore diameter) were evaluated from the desorption branch of isotherm based on BJH (Barrett–Joyner–Halenda) model.

Preparation of in-syringe dSPE device
As shown in Fig. 1, a small piece of cotton wool (approximate 1 mg) was packed in the hub between the barrel and the needle of a 1 ml syringe. Then 15 mg of silica fibers were added into the barrel. The sorbent bed was compacted at the end of the barrel by the plunger.

Sample pretreatment
O. sativa was grown in green house at 33°C under 16 h light/8 h dark photoperiods. Seven-day-old *O. sativa* were harvested and immediately frozen in liquid nitrogen, and stored at -80°C until used. *O. sativa* leaves (20 mg of fresh weight) were frozen with liquid nitrogen, ground into powder and then transferred into a 1.5 mL centrifuge tube. The stable isotopes labeled cytokinins (0.04 ng) were added to the samples to serve as internal standards (IS) for the quantification. Then 0.2 mL of ACN was added into the mixture. After maceration at -20°C overnight, the supernatant was collected upon centrifugation under 0°C for 20 min. The supernatant was then drawn into the in-syringe dSPE device without any pre-condition step. A home-made 36-syringe plate was used for the extraction experiment. After vortex for 1 min to allow analyte adsorption, the sorbent bed was washed twice with 0.2 mL of ACN. Subsequently, 0.2 mL of ACN/H_2O (60/40, v/v) was used to elute out the cytokinins and the eluate was evaporated to dryness under a mild nitrogen stream at 35°C. The residues were redissolved in 50 μL of water and 10 μL was subjected for the analysis of cytokinins by UPLC-MS/MS.

UPLC-ESI (+)-MS/MS System
Analysis of cytokinins was performed on a UPLC-ESI (+)-MS/MS system consisting of a Shimadzu MS-8040 mass spectrometer (Tokyo, Japan) with an electrospray ionization source (Turbo Ionspray), a Shimadzu LC-30AD HPLC system (Tokyo, Japan) with two 30AD pumps, a SIL-30AC autosampler, a CTO-30A thermostat column compartment, and a DGU-20A$_{5R}$ degasser. Data acquisition and processing were performed using LabSolutions 5.53 SP2 software (Tokyo, Japan).

The HPLC separation was performed on a Shim-pack XR-ODS Ⅲ (50 mm × 2.0 mm i.d., 1.6 μm) purchased from

Shimadzu (Tokyo, Japan) at 40°C. A 22-min gradient of water (A) and ACN (B) was employed for the separation at a flow rate of 0.4 mL/min. A gradient of 2 min 5% B, 4 min 5%–7% B, 2 min 7%–20% B, 4 min 20%–30% B, 1 min 30%–80% B, 4 min 80% B, 1 min 80%-5% B and 4 min 5% B was used.

Multiple reaction monitoring (MRM) of $[M+H]^+$ and the appropriate product ions were chosen to quantify cytokinins. The optimized conditions for selective MRM experiments were the same as our previous study.[32]

Results and discussion

Synthesis and characterization of silica fibers
Four spinnerets were used for electrospinning simultaneously, which allowed the rapid and large-scale preparation of MSF and ASF. The SEM images showed that both ASF (Fig. 2a) and MSF (Fig. 2b) possessed continuous one-dimensional morphology. The size of ASF was similar to that of MSF with a diameter ranging from 300 to 500 nm. The Brumauer–Emmett–Teller (BET) surface area and total pore volume of ASF were 30 m^2/g and 0.03 cm^3/g, respectively, and the pore structure was amorphous (Fig. S1b2, ESI†). The surface area and total pore volume of MSF were 903 m^2/g and 0.81 cm^3/g, respectively. N_2 sorption–desorption isotherms revealed type-IV curve for MSF (Fig. S1a1, ESI†), which indicated the presence of mesostructure. The narrow and sharp pore size distribution curve of MSF (Fig. S1b1, ESI†), with the center at the mean value of 2.5 nm, suggested that the mesopores had very uniform size.

Development of the in-syringe dSPE method
The electrospun fibers were further used as an adsorbent for in-syringe dSPE. As mentioned in the previous works, electrospun fibers, which possessed the sub-microscale structure as well as the long fibrous property, could be easily used for pSPE with low back-pressure.[21, 28] As shown in Fig. 1, the electrospun silica fibers were added in the barrel of a syringe, and then the sample solution and excess air was sucked into the barrel in sequence. For instance, when loading 0.2 mL of sample solution, the plunger was drawn to the graduation marked with 0.4 mL. The excess air could provide the space for vortex. After vortex for a certain time, the solution was pushed out of the barrel. Benefiting from the special morphology of electrospun fibers, the aspiration and drawing out of solutions were fast and smooth with the in-syringe dSPE system. In order to increase the throughput of the dSPE, a home-made 36-syringe plate was used in the vortex step (Fig. 1).

Extraction of cytokinins using the in-syringe dSPE
Cytokinins are a vital family of phytohormones that involved in a wide variety of physiological and development processes.[33, 34] Due to the low-abundance of cytokinins and complex matrix in plant samples, sample preparation prior to instrumental analysis is indispensable.[32, 35, 36] Herein, the developed in-syringe dSPE method was applied to the extraction of cytokinins with silica fibers as adsorbent based on hydrophilic interaction.

The extraction efficiencies of ASF and MSF were compared. Equal amounts (20 mg) of ASF and MSF were used to extraction of cytokinins in ACN (0.2 mL). The results showed that the extraction efficiencies with MSF were 1.6-6.7 times higher than that of ASF (Fig. 3). The difference of the extraction efficiencies may be ascribed to the difference of surface areas of ASF (30 m^2/g) and MSF (903 m^2/g). Therefore, MSF was chosen as adsorbent for the extraction of cytokinins.

A series of parameters, including adsorbent amount, extraction time, desorption solvent and desorption time, were investigated to achieve the best extraction efficiencies of cytokinins.

Adsorbent amount. Different amounts of MSF (0 to 20 mg) were used to evaluate the extraction efficiencies of cytokinins (Fig. 4a). When no MSF was used for the extraction, cytokinins could also be detected, which may be due to the hydrophilic interaction between the analytes and cotton in the hub, but the recovery was poor. The extraction recovery reached a plateau with 15 mg of MSF. Thus, the adsorbent amount of 15 mg was chosen for the following experiments.

Extraction time. The effect of the extraction time on the extraction efficiencies of cytokinins was investigated from 0.5 to 20 min (Fig. 4b). It can be seen that the extraction equilibration of all cytokinins investigated could be reached within 1 min. The fast equilibrium benefits from the sub-microscale structure of the electrospun fiber and the vortex step that can increase the contact area and the mass transfer rate between two phases. Therefore, extraction time of 1 min was selected for further experiments.

Desorption solvent. Cytokinins were extracted with MSF by hydrophilic interaction; therefore the content of water in the solution was a key factor to the retention of cytokinins on the adsorbent. In this respect, different ACN contents ranging from 0%-100% (v/v) in desorption solvent were tested (Fig. 4c). The results showed that the desorption efficiencies of cytokinins decreased with the ACN content increased from 60% to 100% (v/v), and the recoveries of cytokinins kept almost constant at the ACN content from 10% to 60% (v/v), indicating that the hydrophilic interaction was dominant factor in the capture of cytokinins on the adsorbent. On the other hand, because of the weak hydrophobic characteristic of MSF at high water content, when the desorption solvent was pure water, the adsorbed cytokinins could not be eluted completely. Considering the extraction efficiency and time for evaporation to dryness, ACN/H_2O (60/40, v/v) was used to further experiments.

Desorption time. Desorption time was investigated ranging from 0.5 to 20 min using ACN/H_2O (60/40, v/v) as the desorption solution. As shown in Fig. 4d, high desorption efficiencies of the target analytes could be achieved within 1 min. Thus, desorption time of 1 min was chosen for further experiments.

Under the optimized conditions, the whole extraction process could be accomplished within 4 min. The preparation reproducibility of the MSF was evaluated by calculating the relative standard deviations (RSDs) of the extracted amounts while performing the extraction of cytokinins from standard

solutions. The results showed that the batch-to-batch RSDs were 3%-13% (n=5), indicating the good preparation reproducibility of the MSF. The recoveries of cytokinins were found to be 55%–89% in standard solution and 12%–58% in O. sativa leaves extract spiked with cytokinins standards (Table S1, ESI†).

Validation of the in-syringe dSPE method

The calibration curves were constructed by plotting the peak area ratio (analytes/IS) against the cytokinins concentrations ranging from 0.001 to 20 ng mL^{-1} with triplicate measurements. As listed in Table S2 (ESI†), good linear correlations were obtained with correlation coefficients (R) greater than 0.9954. The limits of detections (LODs) and quantifications (LOQs) were calculated as the signal to noise ratios of 3:1 and 10:1, respectively. The results showed that LODs and LOQs for the eight cytokinins ranged from 0.3 to 9.0 pg mL^{-1} and 0.8 to 29.9 pg mL^{-1}, respectively, which can meet the requirement for the analysis of endogenous cytokinins in plant samples.[32]

The accuracy of the method was tested and expressed as recoveries. The precision of the method was evaluated by determining intra- and inter-day relative standard deviations (RSDs). Both recoveries and intra- and inter-day RSDs were calculated with cytokinins spiked in O. sativa leaves samples at three different concentrations (0.5, 5, 50 ng g^{-1}). It was found that the relative recoveries were between 77% and 107%, and the intra-day and inter-day precisions of the eight cytokinins were between 0.6% and 17.1% (Table 1), indicating that the reproducibility of the current method is satisfactory for the determination of cytokinins in plant samples.

The endogenous cytokinins in O. sativa leaves were determined by the developed in-syringe dSPE method. As listed in Table 2, six endogenous cytokinins in 20 mg of O. sativa leaves were detected, indicating the potential of the in-syringe dSPE system in analysis of endogenous cytokinins in plant samples. Compared to the method we previously developed for the detection of cytokinins in plant samples[34, 36], the in-syringe dSPE method showed better extraction performance and shorter time, lower cost and smaller amounts of adsorbent and solvent.

Conclusions

In summary, we presented a new in-syringe dSPE method for the extraction of endogenous cytokinins with mesoporous silica fibers prepared by electrospinning. Benefiting from the vortex-assisted dispersion and the sub-microscale structure of the electrospun fibers, the extraction equilibrium can be accomplished within 1 min. The in-syringe dSPE was demonstrated to be rapid (within 4 min), high-throughput (36-syringe plate), easy-operation (without centrifugation), cost-effective and solvent-saving. The excellent performance in microextraction of endogenous cytokinins suggested that the miniaturized SPE format was sensitive and reproducible for dealing with small amount of sample. Furthermore, the in-syringe dSPE approach may provide a general and promising method in the application of other electrospun fibers in microscale sample preparation field.

Notes and references

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† Electronic Supplementary Information (ESI) available. See DOI: 10.1039/b000000x/

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Table 2. Contents of measured endogenous cytokinins in *O. sativa* leaves.

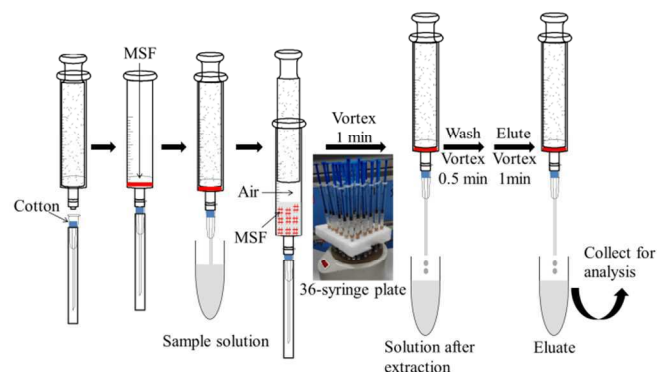| Analyte	tZ9G	tZ	tZR	DHZ	DHZR	iP9G	iP	iPR
Contents (ng/g)	0.062±0.005	0.081±0.003	n.d.	0.063±0.006	n.d.	0.418±0.039	0.128±0.010	0.173±0.011

n.d., not detected.

Fig. 1 Schematic diagram of preparation of the in-syringe dSPE device and the procedure for the extraction of endogenous cytokinins.
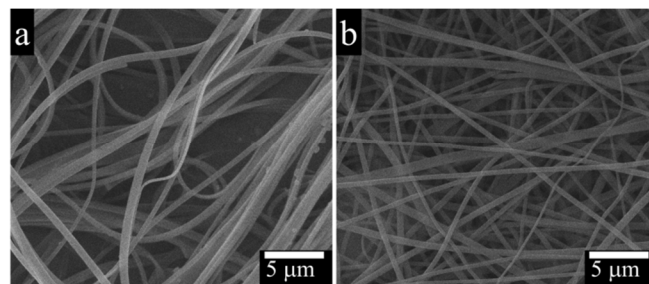

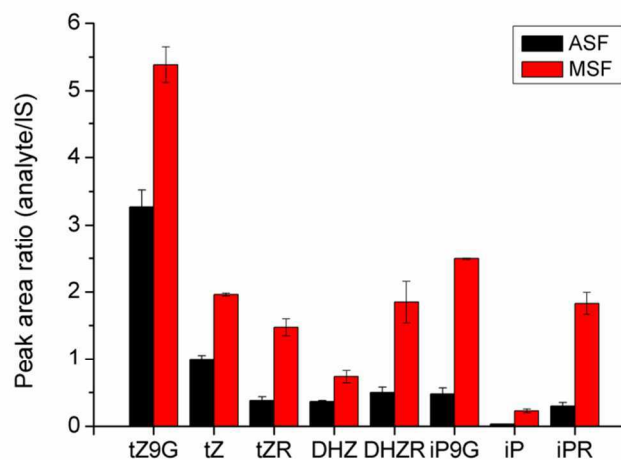**Fig. 2** SEM images of ASF (a) and MSF (b).

Table 1. Precisions and recoveries for the determination of cytokinins in *O. sativa* leaves.

Analytes	Intra-day precision (RSD, %; n=5)			Inter-day precision (RSD, %; n=3)			Recovery (%)		
	Low (0.5 ng g⁻¹)	Medium (5 ng g⁻¹)	High (50 ng g⁻¹)	Low (0.5 ng g⁻¹)	Medium (5 ng g⁻¹)	High (50 ng g⁻¹)	Low (0.5 ng g⁻¹)	Medium (5 ng g⁻¹)	High (50 ng g⁻¹)
tZ9G	10.0	5.3	2.3	6.8	1.7	5.2	90	80	87
tZ	10.1	5.7	8.4	6.7	4.4	5.2	99	89	90
tZR	12.4	14.7	13.0	13.2	4.3	4.8	96	107	100
DHZ	17.1	6.1	8.8	16.5	15.1	3.7	100	99	93
DHZR	14.7	7.5	17.1	3.1	13.4	12.8	88	95	99
iP9G	15.0	15.3	13.3	10.8	9.0	7.6	91	84	84
iP	5.2	12.9	13.2	4.8	8.3	8.0	84	78	77
iPR	6.6	5.1	9.6	3.1	4.3	0.6	10	85	83

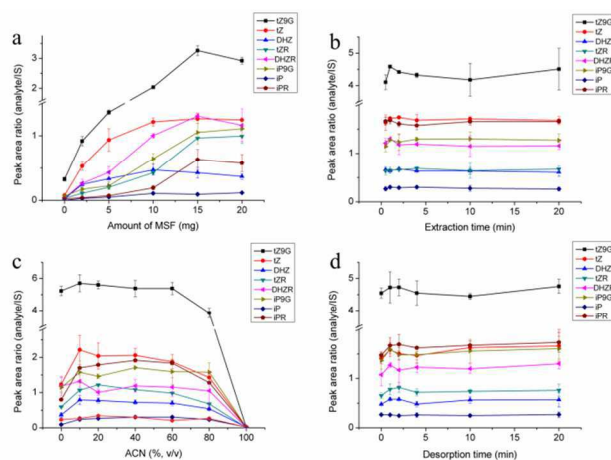**Fig. 3** Extraction efficiencies of cytokinins by ASF and MSF.

Fig. 4 The effect of adsorbent amount (a), extraction time (b), ACN content in desorption solvent (c) and desorption time (d) on the extraction efficiencies of cytokinins.
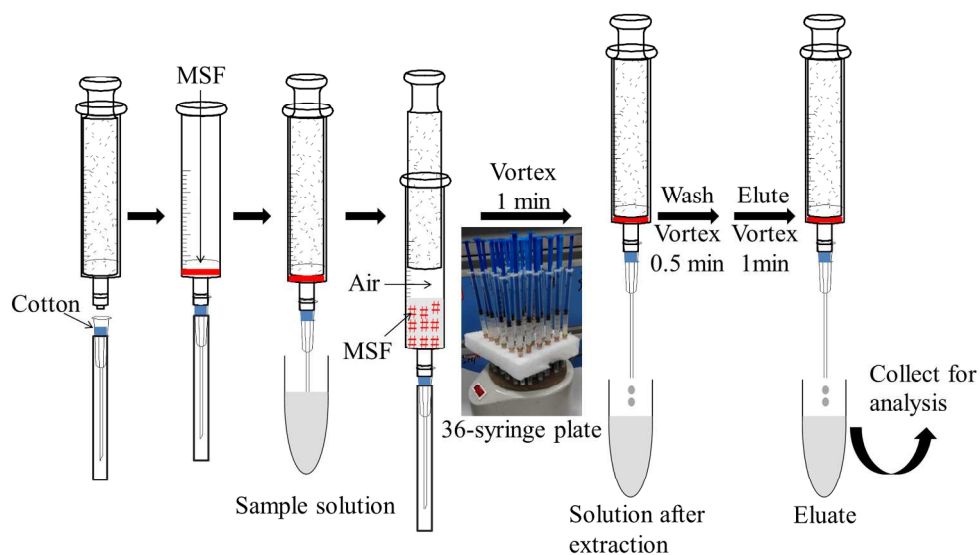
Fig. 1 Schematic diagram of preparation of the in-syringe dSPE device and the procedure for the extraction of endogenous cytokinins.
170x95mm (300 x 300 DPI)
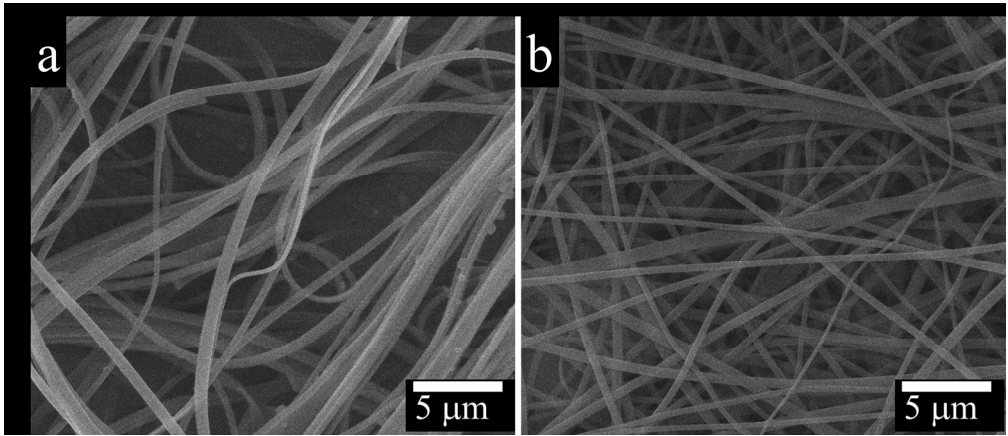
Fig. 2 SEM images of ASF (a) and MSF (b).
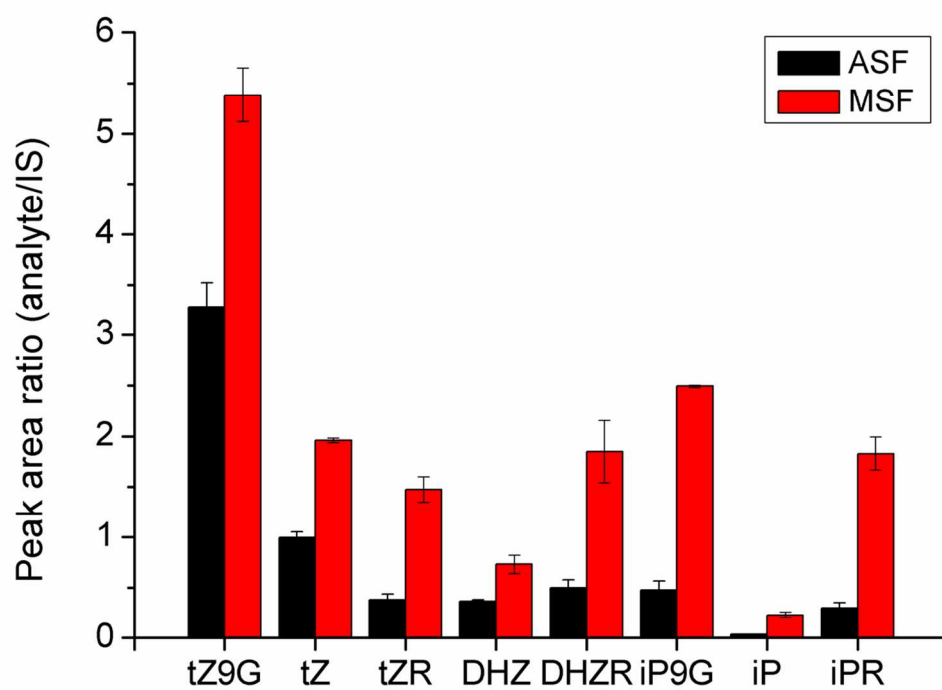219x94mm (300 x 300 DPI)

Fig. 3 Extraction efficiencies of cytokinins by ASF and MSF.
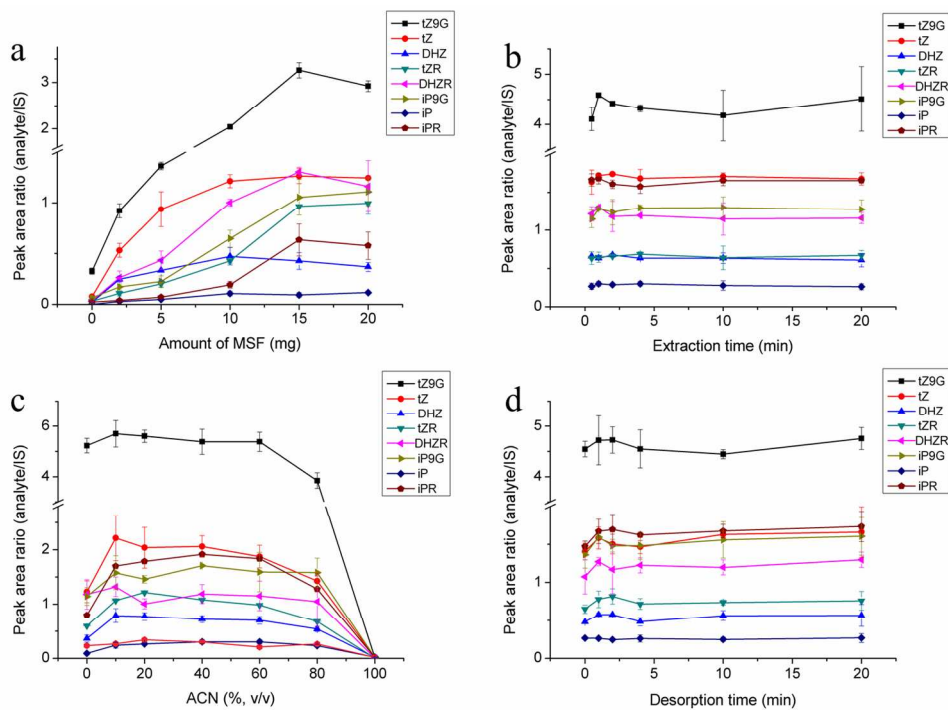113x82mm (300 x 300 DPI)

Fig. 4 The effect of adsorbent amount (a), extraction time (b), ACN content in desorption solvent (c) and desorption time (d) on the extraction efficiencies of cytokinins.
139x99mm (300 x 300 DPI)