Preparation and application of poly 3, 4-ethylenedioxythiophene (PEDOT) nanofibers in the pretreatment of samples before the determination of elements in children fingernails

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Abstract. Solid phase extraction (SPE) has been used widely for sample preparation in the analytical process. Many efforts have focused on developing novel adsorbents to enrich and purify the analytes effectively. In this study, poly-3, 4-ethylenedioxythiophene (PEDOT) nanofiber was prepared and used as the SPE adsorbent. The fiber performed good in extraction of metal ions, Cd, Sn, Hg, Pb, Al, and As, with the extraction recoveries ranged from 53.9% to 99.6% in the wet digested samples of fingernails. A PEDOT nanofibers SPE column coupled with ICP-MS was established for assay of elements in fingernails. The levels of elements (Cd, Sn, Hg, Pb, Al, and As) in the fingernails of 77 healthy Chinese children (6-7 and 10-11 years) were determined. Independent t test shows that no significance has been found between boys and girls. On the contrary, there was obvious difference on the levels of most elements between the two grade groups.

1. Introduction
Biological information from the subject can be obtained from fingernails. Children fingernails have been recognized as biochemical indicators, which have prominent advantages for monitoring children physical health. Compared to blood and urine, fingernails grow at a rate of approximately 1-1.4 mm per week, thus, the analysis and detection of fingernails has certain advantages, including easier sampling, transfer, and storage [1].

Elements in fingernails indicate exposure to hazardous material during the previous 2 to 18 months. Fingernails analysis is useful for detecting the levels of elements in the body, which keep information on the nutrition, habitat, and other environmental conditions.

Before determination, fingernails must be wet digested. However, direct determination of metal ions at trace levels in digested samples may be limited due to their low concentrations and matrix
interferences. Pre-concentration and separation methods have been routinely used to eliminate matrix effects and cope with low metal levels. Solid-phase extraction (SPE) is a cost-effective technique which is used not only to extract traces of targets from samples, but also to remove the interfering components of the complex matrices. In recent years, conductive polymers have attracted great interest for the extraction of target compounds due to a variety of advantages. The main advantage of using conducting polymers in SPE is that the charge of the sorbent can readily be controlled by oxidation and reduction of the polymers [2].

In this paper, poly 3, 4-ethylenedioxythiophene (PEDOT) nanofibers were prepared and used as the SPE absorbents. The fibers were prepared by electrostatic spinning of polystyrene as a template and in situ polymerization. Owing to their large surface area to volume ratio, electrospun nanofibers facilitate the miniaturization of SPE when they are packed into a mini column as the sorbent beds. Packed-fiber solid-phase extraction (PFSPE) has been successfully applied in various sample matrices. However, most of the published work has concentrated on the extraction of organic compounds [3]. Its application for the extraction of metal ions is still limited. In this work, coupled with this PFSPE column, the levels of elements in the fingernails of 77 healthy Chinese school children (6-7 and 10-11 years) were determined by Inductively Coupled Plasma Mass Spectrometer. The data is analyzed to find out whether sex and grade (age) are the factors influencing the content of elements in children’s bodies.

2. Materials and methods

2.1. Sample collection
77 healthy Chinese school children (6-7 and 10-11 years) were recruited from pupils living in Nanchang city, Jiangxi province. Fingernail samples were collected using stainless steel nail clippers and sealed in labeled polyethylene zip lock bags. Informed consent was obtained from each surveyed participant following guidelines approved by the school and parents. The age range spanned 6-11 years.

2.2. Equipment and reagents
Inductively Coupled Plasma Mass Spectrometer (ICP-MS), model agilent 7700, was used for analytical determinations. Double distilled water and guarantee reagents, HNO₃ and H₂O₂, were used to prepare the solutions. Methanol (chromatographic purity) was from Chengdu Xindu area of the industrial development zone (Chengdu, China). Dimethylformamide (DMF), Ferric chloride, Ethanol and Tetrahydrofuran (THF) were from Nanjing Chemical Reagent Company (Jiangsu Province, China). Buffer solution (Borax/boric acid, pH 7.5-8.0) were prepared using water as the solvent. Polystyrene (PS, Mw=185,000) was from Shanghai Institute of Chemical Reagent (Shanghai, China). 3, 4 – ethylene dioxythiophene monomers was from Suzhou Yake Reagent Company (Jiangsu Province, China).

2.3. Preparation of solid-phase extraction column packed with PEDOT nanofibers
The polystyrene (PS) was added into a mixed solvent composed of dimethylformamide and tetrahydrofuran (4: 6, v/v) and then stirred slowly at room temperature for 10 h. Therefore, a 10% (w/v) solution of polystyrene was prepared. After dissolving totally, as can be seen in figure1 the solution was removed quickly and put in the syringe with the stainless steel needle connected with the positive pole of 15 KV high voltage power. The collection screen which collected to the negative power was placed at a distance of about 20 cm from the stainless steel needle. Until now, a fall mesh was shown in the collection screen.

After PS nanofibers were gained, it would be firstly cleaned with ethanol and water. 3, 4 - ethylene dioxythiophene monomer (EDOT) was dissolved with a small amount of ethanol and then added into 50 ml distilled water. The PS nanofibers were put into the solution mentioned above and mixed for 15 minutes. 50 ml of 0.25 g/ml ferric chloride solution was added into the solution mentioned above and then placed in 60°C water bath for 24 hours [4]. Lastly, the PEDOT nanofibers should be dried after
being washed with ethanol and distilled water. The solid phase extraction column used in this study was prepared by filling 5 mg PEDOT nanofibers in the thin bottom of the column.

![Schematic view for electrostatic spinning apparatus](image1.png)  
**Figure 1.** The schematic view for electrostatic spinning apparatus (a) and SEM graph of polystyrene nanofibers (b).

![Graph of PEDOT nanofibers and solid phase extraction column](image2.png)  
**Figure 2.** The graph of PEDOT nanofibers (a) and the solid phase extraction column (b).

### 2.4. Sample pre-treatment and analysis

The fingernail samples were immersed in the mixture of cleanser and deionised water and then placed in an ultrasonic bath for 10 min. After sonication, the solution was discarded and the nails were rinsed thoroughly with deionised water (18 MΩ), then dried at 60°C for 2 h. Following cleaning, 10mg nail sample was accurately weighed into Teflon beaker, 5 ml of 65% HNO₃ and 2 ml H₂O₂ were added into each beaker, the beakers were capped and put on the far infrared electric heating plate. After heating at 100°C for 0.5 h, open the lips and continue heating at 150°C until the nitric acid is steamed off completely [5].

The columns in figure 2 and the liquid storage cartridge were washed with 0.1 mol/L nitric acid and deionized water for cleaning up the interfering ions. 2 mol/L sodium hydroxide aqueous solution and deionized water were used sequentially to activate the nanofibers. Then, the remaining digested liquid for each fingernail sample along with the water for washing the beaker was transferred into the column, the pH value of which was adjusted to 7.5-8.0 by the buffer solution. After loading and elution, the column was desorbed with 0.1 mol/L nitric acid solution in a small volume (1 ml).

The concentrations of elements were assayed in triplicates by use of ICP-MS with a flow injection system. A series of standards were prepared for instrumental calibration by diluting in commercial standards containing 1000 µg/L of the metals. Standard and blank samples were determined in the same manner as the samples.

### 3. Results and discussion

The extraction recovery for the target objects is an important criteria for a material used as adsorbent. In this study, the extraction recoveries of metal ions by PEDOT nanofibers solid phase column were presented in table 1. As can be seen, most of the metal ions have high extraction recoveries.
Table 1. The extraction recoveries for the metal ions.

| Metals | Cd    | Sn    | Hg    | Pb    | Al    | As    |
|--------|-------|-------|-------|-------|-------|-------|
| Extraction recovery (%) | 93.8  | 85.3  | 88.7  | 99.6  | 53.9  | 65.3  |

Table 2 presents the results obtained from the monitoring of elements in the fingernails of boys and girls living in Nanchang city. Independent samples t test shows that no obvious effect has been found on the levels of elements except Cd. Also, boys spend more time playing outside than girls, so will be more exposed to pollutants. This may result in the high levels of Cd in the boy group. However, the mean levels of Sn, Al and Pb were lower in boy group. Girls like eating unhealthy snack food containing these metals may be one of the reasons for this phenomenon.

Table 2. Descriptive statistics of metal levels (μg/g) in fingernail samples of different sex pupil groups along with independent t-test (P = 0.05).

| Metal | Boys (n=35) | Girls (n=42) | t-value at P=0.05 | p-value |
|-------|-------------|--------------|-------------------|--------|
|       | Mean        | Range        | Mean              | Range  |
| Cd    | 0.16±0.05   | 0.0-1.4      | 0.04±0.01         | 0.0-0.2 | 0.01  | 0.01* |
| Sn    | 0.58±0.13   | 0.0-4.3      | 1.0±0.6           | 1.0±0.6 | 0.50  | N.S   |
| Hg    | 0.09±0.099  | 0.0-0.2      | 0.09±0.01         | 0.09±0.01 | 0.93  | N.S   |
| Pb    | 4.79±0.81   | 0.0-18.8     | 5.2±1.2           | 5.2±1.2 | 0.79  | N.S   |
| Al    | 48.2±9.0    | 0.0-239.6    | 52.2±12.0         | 0.0-483.7 | 0.79  | N.S   |
| As    | 0.29±0.05   | 0.0-0.1      | 0.28±0.05         | 0.0-1.4 | 0.89  | N.S   |

*aIndicate significant difference level at P = 0.05.

Table 3 compares element levels of fingernail samples (μg ± SE) in two grade (age) groups. It can be seen from P values, grade (age) has significant correlation with the levels of most elements. However, the trend for the levels of elements between the two groups is not the same because the factors affect the contents of elements are varied [6]. As can be seen, the levels of Hg and Cd were no obvious change. However, the differences for the levels of remain elements were remarkable. Currently, there is no hypothesis can explain these significant differences. The factors influenced the contents of elements are various and complex. It may be resulted from varied diet and living habits.

Table 3. Descriptive statistics of metal levels (μg/g) in fingernail samples of different age groups along with independent t-test (P = 0.05).

| Metal | Junior Group (n=39) | Senior Group (n=38) | t-value at P=0.05 | p-value |
|-------|---------------------|---------------------|-------------------|--------|
|       | Mean    | Range   | Mean    | Range   |        |        |
| Cd    | 0.1±0.04 | 0.0-1.4 | 0.1±0.02 | 0.0-0.8 | 0.02  | N.S    |
| Sn    | 1.2±0.6  | 0.0-23.6| 0.4±0.1  | 0.0-0.3 | 1.25  | 0.02*  |
| Hg    | 0.1±0.01 | 0.01-0.23| 0.08±0.01 | 0.0-0.2 | 1.61  | N.S    |
| Pb    | 3.5±0.7  | 0.0-24.1| 6.6±1.3  | 0.0-44.7| -2.15 | 0.04*  |
| Al    | 73.4±14.0| 0.0-483.7| 26.7±3.4 | 0.0-86.1| 3.21  | 0.002* |
| As    | 0.37±0.05| 0.0-1.4 | 0.2±0.04 | 0.0-1.0 | 2.38  | 0.02*  |

*aIndicate significant difference level at P = 0.05.

4. Conclusion

In summary, a convenient method was developed to prepare the PEDOT nanofibers, and it was successfully used as absorbents of SPE for the elements enrichment and purity. The levels of metals in fingernail samples between boys and girls were found to be no significant difference. Whereas, age has significantly influences on the levels of metals between two grade groups.
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