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Development of a Nanoparticle-Assisted Fabric Phase Sorptive Extraction Technique Coupled with High-Performance Liquid Chromatography for Sensitive Determination of Aflatoxins in Food Samples

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Fabric phase sorptive extraction (FPSE) is a new extraction method that has the advantages of permeable sol-gel derived hybrid organic-inorganic sorbents with flexible and permeable fabric substrates, which leads to high efficiency and high sensitivity of this method. This research aims to improve the FPSE method and design a modified technique called nanoparticle-assisted fabric phase sorptive extraction (NFPSE)using carbon dot nanoparticles to improve the sorbent efficiency. In this study, we focused on the determination of aflatoxins as one of the carcinogens that are abundant in nature and severely contaminate the food sources of humans and animals. The study focused on 4 types of aflatoxin B2, B1, G2, and G1. Various parameters that were effective in NFPSE were optimized. Under the selected conditions, extraction yields ranging from 80% to 95% for Aflatoxins were obtained with acceptable repeatability on the food samples. The calibration curves of the analytes were calculated by good correlation coefficient values ($R^2 > 0.990$). Limits of detection (LOD) were calculated in the range of 0.12-0.51 ng ml^{-1} and limits of quantification (LOQ) were considered in the range of 0.37-1.56 ng ml^{-1}. Intraday precisions were found in the range between 3.9% and 5.3% (RSD, n = 3). The within-laboratory reproducibility was investigated in one month and the results for all 4 types of aflatoxins were from 6.9-15%. The developed method was successfully used on food samples.

Keywords: Nanoparticle-assisted fabric phase sorptive extraction, HPLC, Carbon dot, Aflatoxins, Food sample

INTRODUCTION

Aflatoxins are secondary fungal metabolites produced by several species of Aspergillus, A. flavus and Aspergillus parasitics are the two major species that produce aflatoxins. These toxins poison a wide range of grains such as peanuts, cotton, and corn. In addition to having a very negative impact on grain production, they produce harmful products for humans and even livestock with very little acceptable range [1]. Due to the high stability of aflatoxins in various conditions, their complete elimination from the human diet and animal feed and poultry feed seems impossible, so aflatoxins cause many problems such as liver cancer. There are about 13 different types of aflatoxins, the most well-known of which are aflatoxins B1, B2, G1, and G2, of which B1 is the most toxic [2]. The degree of intoxication of the studied mycotoxins is as follows: AFB1 > AFB2 >AFG1 > AFG2 [3]. The International Agency for Research on Cancer (IARC) states that there is sufficient evidence for the carcinogenicity of G1, B1, and M1 aflatoxins in humans and laboratory animals. Therefore, there is a great demand for research and study on aflatoxins to create appropriate methods for measuring, identifying, and accurately detecting aflatoxins in food to ensure the health of consumers. Several analytical methods are mainly used to measure aflatoxins in foodstuff and animal feed, each of which differs in sensitivity, cost, and ease of use, and each of these methods

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has its advantages and disadvantages.

One of the common analytical techniques for aflatoxins was thin-layer chromatography (TLC), which is less commonly used these days. This technique has advantages such as its reliability if paired with densitometry and having an official reference for aflatoxins, and its disadvantages include the use of outdated equipment and destructive sample preparation, and generally today High-performance liquid chromatography(HPLC) has replaced it [4]. Another widely used chromatographic method in the analysis of aflatoxins is gas chromatography. Flame ionization detector (FID), electron capture detector (ECD), and mass spectrometry (MS) detector can be used for gas chromatography (GC). Gas chromatography also requires an initial stage of pre-analysis cleaning, which is why it is limited to analyzing several types of mycotoxins, Even in these analyses, disadvantages such as the nonlinearity of the calibration curve, the effectiveness of previous samples, and the lack of repeatability and reproducibility are included [5].

The reference methods for the detection of aflatoxins are based on chromatography, more precisely HPLC is used. HPLC-Fluorescent detection (FLD) and HPLC-MS/MS systems can be used in most cases [6]. In general, high-performance chromatography has good sensitivity and selectivity, as well as good repeatability for aflatoxins. In recent years, there have also been many attempts to create fast and reliable immunochemical techniques, such as the enzyme-linked immunosorbent assay **(**ELISA), which is more suitable for aflatoxins analysis. But it has matrix interference problems and generally requires further analysis [7]. To properly take advantage of these analytical techniques, especially under-diagnosed aflatoxin species such as M1, it is essential to thoroughly purify and prepare the sample to minimize the effects of the sample matrix [8].

Perhaps the most important point in developing an analytical method for aflatoxins analysis is the issue of sample preparation and purification. Solid Phase Extraction (SPE) is one of the oldest and most common methods of sample preparation, it has advantages such as low cost, ease of operation, and good stability, but the problem is that the adsorption between aflatoxins and sorbents in SPE is not usually satisfactory, so they are generally combined with new techniques such as molecularly imprinted solid-phase extraction (MISPE) [9]. Another common method is liquid-liquid extraction (LLE), which is fast, robust, and high throughput, which has been used, for example, to measure aflatoxin M1 in milk [10]. Today, the most common method of clean-up and preparing samples in complex aflatoxin matrices is the immunoaffinity method, which is used before injection into HPLC. Although this method has very high accuracy, it also has disadvantages, for example, the immunoaffinity process is a time-consuming method with high solvent consumption, requires a high-quality antibody and it also uses expensive disposable cartridges [11].

Considering the disadvantages and advantages of all the preparation techniques, in this study, we used a new and simple method of fabric phase sorptive extraction developed by Kabir and Forton [12]. Since the introduction of FPSE in 2014 as a sample preparation technique, it has attracted the attention of many scientists active in the field of separation [13].

A major part of the increasing popularity of the FPSE extraction technique goes to sol-gel coating technology. Along with this, the high loading capacity, the possibility of using any organic or aqueous-organic solvent mixture, and the capability to extract materials with different polarities and different acidic and basic properties, are other advantages of this method [13,14]. Figure 1 presents a graphical schematic of a typical FPSE workflow [15].

However, in this study, we did not suffice with the same method of sample preparation and tried to improve this method by using nanoparticles to improve the texture of the fabric to better absorb the analytes during the extraction stage. In this study, we used carbon dots as a coating on the fabric texture during the sol-gel process and named the

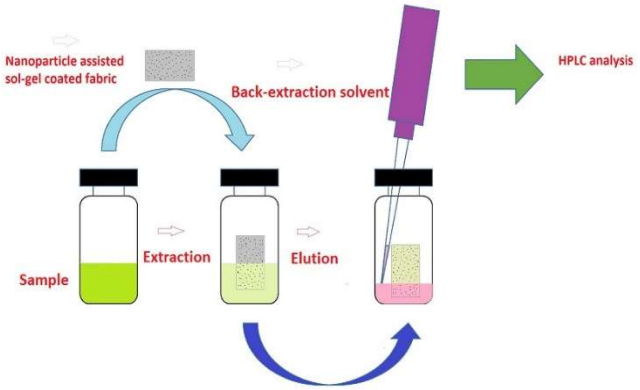

Fig. 1. Typical FPSE workflow [15].

method nanoparticle-assisted fabric phase extraction (NPAFPSE).

Although the FPSE method has important advantages, we achieved much better results by combining it with nanoparticles. Following the previous works, we have considered the base of the FPSE method and tried to develop it in order to innovate a more efficient method with lower detection limits. It must be noted that; in our work incorporation of carbon dots as a relatively emerging member of the carbon material family which was first reported in 2004 [16,17], into the extractor phase significantly increases the contact surface and its homogeneity. Chemical bonds between analyte and extractor are stronger and thereupon, the enrichment factors are higher than what was previously reported for Feathering, carbon dots/SiO_2 nanocomposites were synthesized by surface coating of SiO_2 with carbon dots through a modified sol-gel approach using tetraethyl orthosilicate (TEOS), as the raw material for covering the fabric surface with them for the first time. Furthermore, the porous aperture structure of carbon dots with a large surface area contributes to the adsorption of the analyte's molecules. The adsorption activity of SiO_2 was largely improved through the addition of carbon dots. The reason can be concluded as follows: firstly, carbon dots can adsorb aflatoxins on the outside of its surface due to the large surface area; secondly, carbon dots are eminent electronic conductors that can orderly export electrons from the surface of SiO_2 and quickly reduce electronic accumulation on SiO_2.

EXPERIMENTAL

Instrumentation

The analysis was performed on an HPLC (Waters 1525 Binary HPLC pump) equipped with an Ultimate 3000 Fluorescence detector. AnInert Sustain Swift C18 (GL Sciences INC) column (4.6 × 150 mm, 5 µm) was used for the separation and a thermostat was used to control the column temperature at 30 °C. The mobile phase was composed of acetonitrile, methanol, and water at a volume ratio of60/10/30, and the composition of the mobile phase is kept constant throughout the elution process (Isocratic mode). The flow rate of the mobile phase was 1.0 ml min^{-1}. The fluorescence detector was set at wavelengths of373 and 450 nm for excitation and emission, respectively.

A GT Sonic VGT-1620T professional Ultrasonic cleaner is used to disperse the solutions and make no bubble-free sol solution. Centrifugation of different solutions was carried out in the Orto arista digicam 21 model. AD8000 a microprocessor-based pH meter was used to adjust the pH of the solutions. A universal oven UF55 from Memmert Company was used for the experiments.

Materials and Reagents

Ingredients for sol-gel preparation included tetraethyl orthosilicate (TEOS), ethanol, de-ionized water, and hydrochloric acid, all of which were purchased from Merck (Darmstadt, Germany). In the preparation of nanoparticles process, sulfuric acid, phosphoric acid, and sucrose were used and in the process of FPSE and fabric activation, sodium hydroxide, methanol, acetonitrile, and sodium chloride were used, all of them were supplied by Merck (Darmstadt, Germany) too. The fabrics used as media in the fabric phase sorptive extraction were made from the local markets (Mashhad, Iran). Aflatoxin standards were received as a gift from the Research Institute of Food Science and Technology, Mashhad, Iran. The standard aflatoxins solution was prepared as a mixture of aflatoxins G1, G2, B1, and B2 1000 mg l^{-1} and stored at -4 °C, away from light.

Standard Solution

The stock solution of mixed aflatoxins (5 µg l^{-1}) was prepared by dissolving the standard solution in deionized water .The stock solution was stored in the refrigerator at -4 °C and away from sunlight. The standard solution was used for daily analytes solutions with different concentrations.

Preparation of Carbon Dot

CDs can be synthesized mainly via two routes: (i) top-down approach and (ii) bottom-up approach. The top-down approach refers to breaking down larger carbon structures via chemical oxidation, discharge, electrochemical oxidation, and ultrasonic methods. The bottom-up approach refers to the conversion of smaller carbon structures into CDs of the desired size. The bottom-up approach is consisting of hydrothermal treatment, ultrasonic treatment, thermal

decomposition, pyrolysis, carbonization, microwave synthesis, and solvothermal method to synthesize CDs [18].

In this work a single-step process bottom-up method was employed using strong acids ($H_2SO_4$and H_3PO_4), to carbonize carbon-rich starting precursor (sucrose, $C_{12}H_{22}O_{11}$) into C-dots. Synthesis of CDs in this method involves a series of processes that includes hydrolysis, polymerization, particle growth, and oxidation. Thus, H_2SO_4 and H_3PO_4mixturemay lead to more effective carbonization.

For the synthesis of the carbon dot, initially, a stock sucrose solution (1.0 M) was made by combining 3.42 g of sucrose with 10 ml of deionized water. Then 1.0 ml of H_2SO_4 (98%) and H_3PO_4 (85%) at a fixed concentration was added to 1.0 ml of the sucrose stock solution. The mixture was homogenized in a container and covered with aluminum foil. The container was placed in an oven set at 100 °C, for 30 min. Then the obtained brown solution was neutralized with NaOH, drop by drop, and slowly. The mixture was diluted to a final volume of 5.0 ml using deionized water and centrifuged to disperse the C-dots to give a colloidal solution. After that, the solution was centrifuged for 20 min at a speed of 4500 rpm. After the centrifugation process, the supernatant was poured into a clean vial [19].

Fabric Pre-treatment

First, the fabric was cut to a size of 100 cm^2 and placed in deionized water, and sonicated for 15 min. Then it was rinsed with a large amount of deionized water, and immersed in a 1 M NaOH solution for 1 h under constant sonication and again it was rinsed with large amounts of deionized water. Finally, it was immersed in a solution of 0.1 M HCL for 1 h under constant sonication and then rinsed with large amounts of deionized water and placed in an inert atmosphere to dry overnight, and the fabric was placed in a clean glass container to be ready for coating [13].

Preparation of the Sol Solution for the Coating Process

The sol solution designed for this experiment was an acid-catalyzed sol, which was briefly prepared as follows: first, 30 ml of TEOS and 30 ml of ethanol were combined and stirred by a magnetic stirrer. Ina container, 38 ml of distilled water was mixed with 3-4 drops of concentrated HCl. The acidic solution was then added to the precursor solution, which was constantly stirred. At first, the two solutions did not dissolve in each other, but after a few minutes, the final solution became completely homogeneous. The pre-prepared nanoparticles were then poured into the solution. After that, the fabric was placed in the solution. After 10 min, the fabric was removed and let it dried. After finishing the fabric coating process, we put it in an inert environment at a temperature of 50 degrees for 1 h. Then we divided the fabric into smaller parts of 2 × 2.5 cm and put it in a closed glass container in the desiccator for 1 day, then we kept it in a vacuum device so that it would not be contaminated.

Real Sample Preparation

To examine the real samples, two real samples were examined. A calf starter sample is available in the local market. 80% methanol and distilled water were used to wash the sample, respectively. Also, a high-consumption rice sample was used, which had already been proven to contain aflatoxin B1. Contamination of the samples had already been approved by the Iran Food and Drug Administration. The samples were prepared according to the procedure of the ASTM. Standard Guide [20]. After collecting and transferring rice samples to the laboratory, the samples have been washed with deionized water and dried in an oven for 48 h at 100 °C. The amounts of 2 g of rice milled samples have been selected. At first, 5 ml of methanol is poured on the sample and the obtained solution has been passed from Whatman 42 filter paper, fed into a 50 ml volumetric balloon, and diluted with an appropriate amount of deionized water. Then, the steps of extracting and spiking/extracting the analytes were performed by the NFPSE method. The results were mentioned in Table 5.

Analytical Procedure

The sample preparation and extraction process were performed as follows: 10 ml of an aqueous solution containing analyte or real sample extract was prepared and placed in a clean vial, then the pre-prepared and activated fabric was placed in the vial. The pH of the solution was then fixed with HCl and NaOH at 4 then the glass vial was stirred for 25 min At the end of the extraction step, the fabric was slowly and carefully removed from the solution and entered into another container containing 2 ml of optimized desorption solvent. The fabric and solution were then left for

30 min and then the back extraction solution containing the analyte was removed and injected into the HPLC. Aflatoxin was not exposed to direct light during all stages of sample preparation.

RESULTS AND DISCUSSION

Optimization of the Experimental Conditions

Univariate and multivariate analysis approaches were considered to optimize the FPSE process.

Acquisition and presentation of data. The experimental design was performed based on a standard orthogonal array and statistical treatment using Minitab V.17 (Minitab Inc., USA) software.

Design of experiments. The experiment was designed based on a standard orthogonal array to calculate the impact and evaluate various parameters in the testing process. The Taguchi method was used to calculate the signal-to-noise ratio and is commonly used to reduce errors and increase the efficiency of laboratory experiments [21]. This method uses a special design for orthogonal arrays to reduce the number of experiments, but full-factorial methods require more experiments, which greatly increases costs and time. There are three types of S/N ratios used in the Taguchi method and these are 'larger is better', 'nominal is best', and 'smaller is better' [22]. Since the present study requests maximum current, S/N ratio analysis (following equation) for the 'larger is better' theorem is applied.

$$\frac{S}{N} = -10 \log \left[\frac{1}{n}\sum_{k=1}^{n}\frac{1}{Y_{kj}^2}\right]$$

where y_{kj} is the j th experiment at the kth test, and n is the total number of the tests.

We first used Taguchi to screen the parameters at 2 levels to limit the final choice for the parameters and to identify the effective parameters. In the next step, we examined 3 of the most effective parameters that can be multi-level at different levels to obtain the most optimal conditions possible.

Optimization of FPSE Conditions

Initially, a few parameters were studied by the OVAT method (one variable at a time), these parameters including the type of fabric and the duration of contact of the fabric in the sol-gel. For the first parameter, there were 3 types of fabric, two of which were cotton with different textures and the other was polyester. Cotton and polyester were chosen as fabrics according to the observations of previous researchers [12,13,15]. It was found that fine-textured cotton fabric gives us a higher amount of adsorption than other fabrics, see Fig. S1.

NFPSE utilized a piece of fabric (cellulose, polyester) as the substrate with a sol-gel sorbent coating on its surface. The sorbent coatings should be chemically bonded to the substrate for high pH and sorbent stability. Thus, the fabric substrate plays a key role in the structure of the NFSPE. Unlike many extraction methods, the substrate in NFPSE is not just a host for the sorbent but also fabric actively contributes to the selectivity of the NFPSE *via* hydrophilic/hydrophobic interactions. On the other hand, the fabric should interact with modifier nanoparticles.

Since the sorbent loading on the NFPSE substrate depends on the concentration of sol-gel active functional groups, the amount of sorbent loading per unit area on cellulose fabric is substantially higher than on polyester fabric. It is important to note that the fabric networks chemically bind to the sol-gel active functional group, leaving the main part of the fabric substrate surface uncovered and available for interaction with target compounds.

Direct light exposure to solutions containing aflatoxins was avoided at all stages of the experiments even though the concentration of aflatoxins was low. There was a significant increase in the amount of adsorption in these conditions. The time of contact of the fabric with sol-gel was considered as 10, 15, 20, and 30 min, which showed in Fig. 2 that 15 min was the maximum adsorption time. Fortunately, in this method, due to the large contact surface area of fabric, it does not take much time for direct interaction with the analytes during the extraction process. The extraction kinetics is directly related to the primary contact surface area of the fabric with the analytes. Although, the extraction time is a very important parameter in the NFPSE procedure because it influences the partition of the target analytes between the sample solution and the fabric. The extraction efficiency of the NFPSE technique increased when the extraction time increased but, if the extraction time is too long, it leads to the return of the analyte to the solution. As well as, longer times

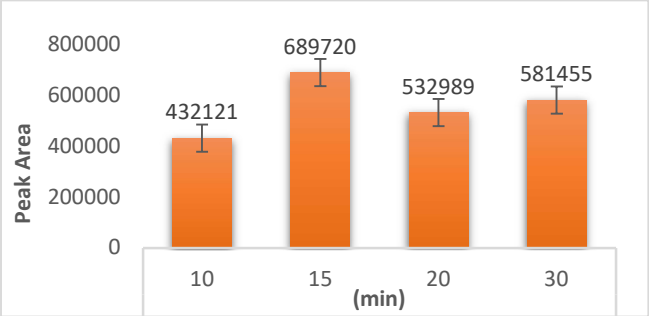

Fig. 2. Effect of fabric contact time with sol-gel, The amount of adsorption considered in this chart is the total adsorption of all 4 types of aflatoxins, including B1, B2, G1, and G2.

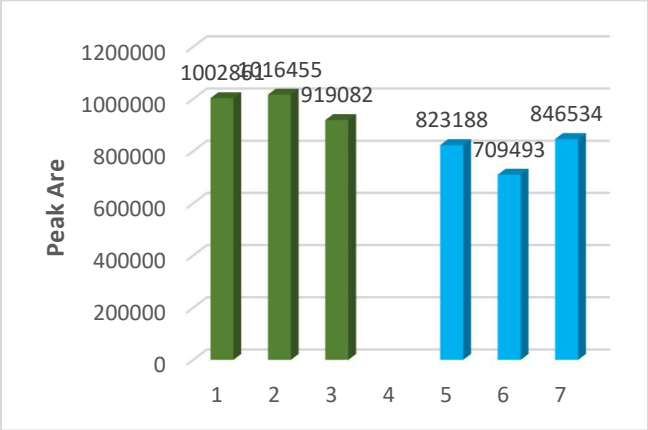

Fig. 3. Comparison of normal fabric and carbon dot reinforced fabric in the FPSE process: the first 3 graphs in green are related to carbon dot reinforced fabric and the next 3 graphs in blue are normal fabric.

have reduced the extraction efficiency due to the contact of the fabric with the walls of the container. Therefore, it should be optimized. Thus, the optimum extraction time was 25 min and 15.0 min was the optimal adsorption time.

Then to compare normal fabric and fabric reinforced with carbon dots, a comparison was made in an identical condition. 3 Measurements were performed for each fabric, and the analysis conditions were as follows: the volume of the solution containing all 4 types of aflatoxin is 10 ml, with a concentration of 5 ppb, with extraction and desorption times of 30 min, and the volume of the solvent is 5 ml, without adjusting the pH. As you can see in Fig. 3 the results for the carbon dot-reinforced fabric are better than the normal fabric under the same conditions.

Infrequently, high surface area carbonaceous nanoparticles such as carbon dots are also used in this research in combination with inorganic sol-gel to enhance the efficiency and selectivity of the extracting sorbents.

In this study, we considered 7 important factors including pH, solution volume, extraction time, desorption time, salt effect, type of desorption solvent, and volume of desorption solvent at two levels. Table S1. A 12-thigh Taguchi table was designed to examine all four types of aflatoxins present simultaneously in the analytes, and the uptake response of all thighs can be seen in Table 1. Signal-to-noise ratio values of all factors were examined and by calculating the delta (max-min (Δ)) meaning the difference between S/N values at the two levels of each parameter, the influence of factors on the adsorption response rate can be ranked (Table 2).

By examining the mean values of S/N, the levels of operational variables that show the highest response was considered as the optimal level. The highest value of delta represents the most effective parameter in the adsorption response rate, as seen in Table 2, the volume of desorbed solvent was identified as the most effective factor, and the factors pH, type of desorbed solvent, adsorption time, solution volume, salt effect and desorption time were ranked next. On the other hand, by looking at Fig. S2, we can determine the optimal values of each factor that when the signal-to-noise ratio is higher at a level, that level of the factor is considered as the optimal level. Thus, for the pH factor, a value of 2 was considered optimal and the absence of salt effect was considered one of the optimal conditions. Then the amount of desorption solvent was 2 ml and the volume of solution was 10 ml and the desorption solvent of type 2 (MeOH + ACN + H_20 with ratios of 10/60/30 in order) was used as the final solvent. The adsorption and desorption times were both 30 min. However, in this study, to increase the level of optimization, three effective factors that could be multi-level, including pH, solvent volume, and adsorption time, were finalized by designing a Taguchi table in 4 levels and 16 thighs. The adsorption responses obtained by HPLC are shown in Fig. S3. Based on the S/N values, the most optimal values were obtained for the better adsorption response, which was calculated as 2 ml for the desorption solvent volume and pH = 4 was the best pH for the study, and the optimal extraction time was 25 min. Table 3. Thus, the

Table 1. Factors and Optimized Values

Factors	Factors level		Optimized value
(A) pH	2	8	2
(B) Salt effect	0	120	0
(C) Solvent volume (ml)	2	5	2
(D) Adsorption time (min)	15	30	30
(E) Solution volume (ml)	5	10	10
(F) Solvent type	1	2	2
(G) Desorption time (min)	15	30	30

Note: solvent type 1 contains MeOH + ACN with ratios 80/20 and solvent type 2 contains MeOH + ACN + H_2O with ratios of 10/60/30 in order.

Table 2. Response Table for Signal-to-Noise Ratios (Larger is Better)

	Factors						
Level	A	B	C	D	E	F	G
1	104.65	103.06	105.41	102.04	102.05	101.85	102.40
2	99.75	102.16	99.81	103.19	103.18	103.37	102.83
Delta	4.90	0.90	5.60	1.15	1.13	1.52	0.43
Rank	2	6	1	4	5	3	7

Note: Refer to Table 1 to identify the symbol for each factor.

Table 3. Factors and Optimized Values of Second Multivariate Optimization

Factors	Factor levels				Optimized value
(A) pH	2	4	6	8	4
(C) Solvent volume (ml)	2	3	4	5	2
(D) Adsorption time (min)	15	20	25	30	25

final optimal conditions for the NFPSE method in the analysis of aflatoxins B1, B2, G1, and G2 are obtained.

As was mentioned, due to the chemical bond between the adsorbent coating and the analyte, the stability against pH changes is good in this type of adsorbent. As such, the strong covalent bonding between the hybrid coating and the fabric substrate, the coating demonstrates high chemical stability and can be exposed to highly acidic and basic environments. The pH solution changes the charge property of the surface of the coating, which is a primary factor that affects the adsorption efficiency of the aflatoxins. In the extraction of aflatoxins from food samples, attention should be paid to the physicochemical properties and lipophilicity (logP for B1 = 1.58, B2 = 1.57, G1 = 1.37, G2 = 1.36) [23] of the analytes. Aflatoxins are weak organic acids for the carboxyl group and phenolic hydroxyl group of the isocoumarin part of them. The extracted aflatoxins would be in their neutral form to be well extracted by the fabric. Therefore, for sufficient retention of them the fabricated fabric sorbent, the sample solution was acidified to optimal pH 4.0.

The preconcentration factor in extraction techniques is determined by the analyte recovery and by the volume of the

sample theoretically. As the volume of the sample increases, the preconcentration factor also increases. Decreasing the volume ratio of the desorption solvent and the sample phases can lead to an increase in extraction efficiency. Furthermore, a larger sample volume can even be disadvantageous due to poorer mass-transfer kinetics, resulting in worse extraction efficiency. Thus, the phase ratio of sample and desorption solution volumes should be optimized. The optimum desorption solvent was 2 ml and the volume of the solution was 10 ml.

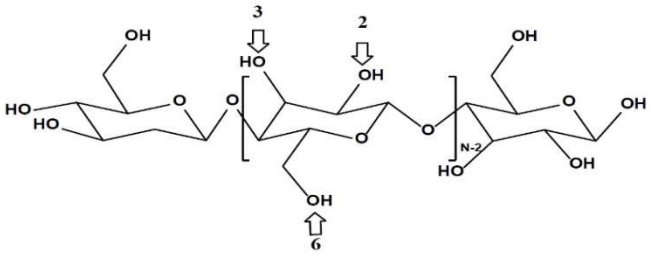

Fig. 4. Chemical structure of cellulose substrate illustrating available hydroxyl functional groups for harboring sol-gel inorganic-organic networks.

Chemistry of the NFPSE Substrates and the Sol-gel Coatings

Many synthetic and natural fabrics were considered in the market as fabric phase sorptive extraction options. Generally, all of these fabrics contain active sol-gel functional groups, but some of these fabrics need surface modification to be able to have active sol-gel groups. In general, cellulose and polyester fabrics have sol-gel functional groups, and both were the primary options. Cellulose fabrics are known as hydrophilic fabrics and polyester fabrics are known as hydrophobic types. The sol-gel substrate and coating play an important role in the polarity of the extraction medium and the final selection. Inorganic precursors such as TEOS also play a common role in sol-gel processes, as you can see in this study.

Surface chemistry of cellulose substrate. Cellulose is a hydrophilic linear polymer of β-D-glucopyranose, whose structure is shown in Fig. 4. Each dimer of cellulose contains three hydroxyl functional groups in positions 2, 3, and 6 that can participate in polycondensation during the sol-gel coating process at a varying degree of reactivity [23]. As such, cellulose represents to be an excellent candidate as a potential substrate for sol-gel sorbent coating.

The cellulose fabric used in this experiment was activated by treating it with 1 M NaOH solution for an hour under sonication. An important process that can improve chemical reactions is mercerization, which increases the availability of all hydro-cyclic groups in chemical reactions.

Fabric coating process. The creation of carbon dot-modified SiO_2 *via* sol-gel process, requires some reactions: (a) catalytic hydrolysis of the precursor, TEOS (b) polycondensation of hydrolyzed TEOS, leading to a growing silica network; (c) interpolation of C-dots nanoparticles randomly into the rapidly evolving sol-gel network; and (d) chemical bonding of the growing sol-gel network through condensation to the flexible cellulose fabric substrate.

Although the TEOS improved C-dot dispersibility in the organo-silica sol. The hybrid films for C-dots were prepared *via* the sol-gel reaction at a low C-dot concentration so that the as-prepared CDs could also be dispersed in the precursor sol.

Method Performance

The calibration curves using the standard aflatoxins B1, G1, B2, and G2 in the concentration range (1.00-15.00 ng ml^{-1}) were linear. Regression analysis of the result yielded linear calibration equation for B1, B2, G1, and G2 aflatoxins tested with r^2values > 0.99 and curve regression equation for AFG1, AFG2, AFB2 and AFB1 were Y = 59066X-91695, Y = 70247X-90232, Y = 95770X-154356, and Y = 123877X-175697, respectively. See Figs. S4-S7.

The LOD can be determined by a signal-to-noise ratio of 3:1. The lowest concentration level at which a measurement is quantitatively meaningful is called the limit of quantitation (LOQ). This is most often defined as 10 times the signal-to-noise ratio.

The method LOD values for aflatoxins G2, G1, B2 and B1 were 0.24 ng ml^{-1}, 0.12 ng ml^{-1}, 0.51 ng ml^{-1}, and 0.17 ng ml^{-1}, respectively. The LOQs were 0.74 ng ml^{-1}, 0.37 ng ml^{-1}, 1.56 ng ml^{-1}, and 0.52 ng ml^{-1} in the same order. The relative standard deviation calculated for data obtained within one day is often called intraday (within one day) precision, while that measured for days is termed interday (between days). Intraday precision was found to be in the

range between 3.9% and 5.3% (RSD, n = 3). Interday precision was investigated for one month and the results for all 4 types of aflatoxins were from 6.9 to 15%.

To confirm the NFPSE method in the extraction of aflatoxins, a comparison was made with several studies that showed the effectiveness of this method in the extraction of aflatoxins from foodstuff samples. See Table 4.

In comparison with the other conventional sample preparation methods, the developed method has the merits of considerable analysis speed, good separation efficiency, improved pre-concentration, notable precision, and high sensitivity. As compared to the other method low detection limits and acceptable recovery in the complex matrices are readily achieved in the present work.

Table 4. Analytical Performances of Method and Comparison with other Studies

Aflatoxins	LOD	LOQ	R	%RSD	Recovery	Real sample	Extraction method	Ref.
G1	0.04	0.1	No data	<5	97.7	Maize	HPLC	[24]
G2	0.02	0.06			95.7			
B1	0.04	0.1			98.1			
B2	0.02	0.06			97.8			
LOD and LOQ unit is µg/Kg -The highest recovery value is listed.								
G1	0.0012	No data	>0.955	6.7	99	Wine	HPLC-MS/MS	[25]
G2	0.003			7.1	100			
B1	0.0012			3.5	108			
B2	0.0012			5.2	90.9			
LOD unit is ng ml^{-1} - The recoveries mentioned are for example 1.								
G1	0.003	0.01	0.9965	(2.9-7.1)	103.8	Glycyrrhiza uralensis	HPLC-MS/MS	[26]
G2	0.005	0.015	0.997		88.2			
B1	0.007	0.02	09971		119.3			
B2	00.5	0.015	0.9973		119			
LOD and LOQ unit is µg/Kg - Intermediate recoveries are listed.								
G1	0.0109	0.0364	0.99993	5.7	69.9	Hazelnut	HPLC	[27]
G2	0.0059	0.020	0.99993	5.8	69.92			
B1	0.0029	0.0094	0.99927	17	75.72			
B2	0.0103	0.0333	0.99990	30	72.16			
LOD and LOQ unit is µg/Kg - The mentioned data are based on S/N calculations and first working.								
G1	10.6	21.1	0.998	<10	101.2	Arillus longan	HPLC-MS/MS	[28]
G2	5.8	13.69	0.995		98.8			
B1	5.2	10.4	0.999		106.2			
B2	6.3	12.6	0.995		98.2			
LOD and LOQ unit is ng l^{-1} -The highest recovery value is listed.								
G1	0.12	0.37	0.9920	5.1	87	Calf starter	HPLC	This study
G2	0.24	0.74	0.9918	5.3	95			
B1	0.17	0.52	0.9956	4.7	91			
B2	0.51	1.56	0.9903	3.9	80			

LOD and LOQ unit is ng ml^{-1}.

Scanning Electron Microscopy for Surface Morphology

Scanning electron micrographs of the uncoated surface of cellulose fabric substrate at ×200 magnification in Fig. S8 and sol-gel carbon dot coated cellulose fabric substrate at ×200 are presented in Fig. 5. The first images show the

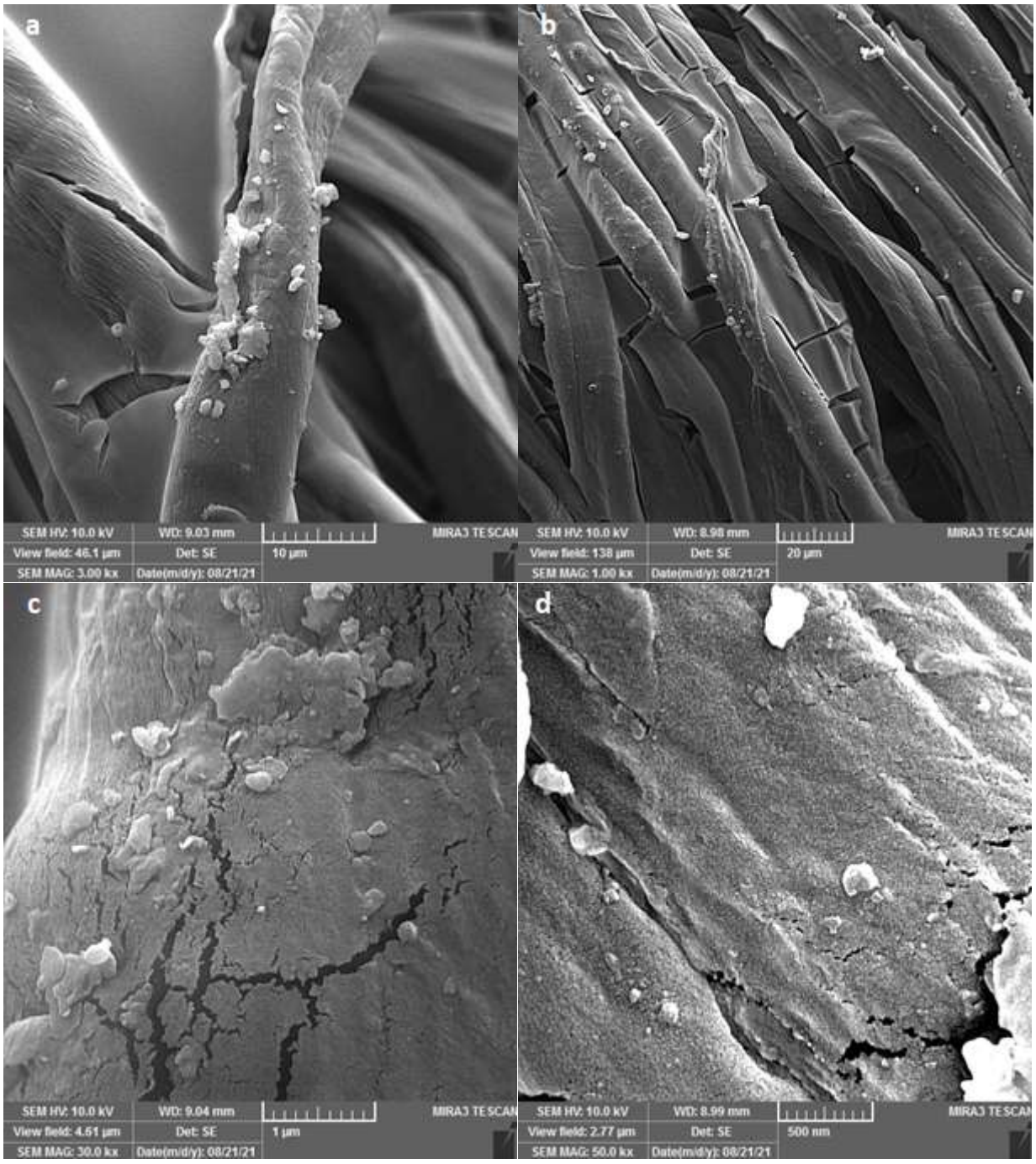

Fig. 5. Scanning electron micrographs of the coated surface of the cellulose fabric substrate, the image of 2a is on the scale of 10 µm, 2b image is on the scale of 20 µm, 2c image is on the scale of 1µm and 2d image is on the scale of 500 nm All images show the fibers being coated with nanoparticles.

surface of the fabric, which has a very permeable and well-organized structure. Then, using the sol-gel process, the nanoparticles were homogeneously distributed on the surface of the cellulose fabric and covered its surface to strengthen the FPSE sorbent. But the surface pores are perfectly healthy, so the aqueous sample matrix can easily pass through the sorbent and balance the extraction in a short time. SEM images also show good and homogeneous loading of the sorbent on the cellulose substrate.

FTIR Spectra Analysis

As was observed in Fig. 6a, Si-0-Si bonds (at 460, 1168, and 1200 cm^{-1}). IR bands located at 960 and 793 cm^{-1} showthe bands associated with Si-OH and Si-0, bonds in the gel.

The studies were carried out to understand the interaction between the sol-gel matrix and the CDs Fig. 6b. The intense broadband in the range3200-3500 cm^{-1} can be associated with the –OH stretching of hydroxyl groups present both in functionalized CDs and sol-gel. Carbon dots are a few nm scale small spherical particles of carbon with a high degree of oxygen modification of their surfaces. FTIR spectrum reveals the presence of a carbon framework well decorated with oxygen-rich functionalities. Figure 6b displays the FTIR spectrum of prepared CDsassisted sol-gel system. Detailed analysis reveals the presence of oxygen-rich functionalities like hydroxyl, carboxyl, ether or epoxy, and other oxygen-containing groups are attached to the surface of CDs during the synthesis process or due to intentional surface passivation treatments.

The peak at 1475 cm^{-1} was assumed to originate from carbon-carbon vibrations of the carbogenic core of C-dots. Broadening the band between 1168 and 1200 cm^{-1} which was designated Si-O-Si bonds, indicates the interaction between carbon dot and sol structure. The spectra show changes, like the addition of a new peak due to new –CH bends, as well as shift peaks because of the possible interaction of active groups on the sol with the –OH groups on CDs.

REAL SAMPLE ANALYSIS

In order to evaluate this method and its effectiveness in identifying aflatoxins, we used real and common examples in the food industry. We tested rice and calf starter samples as the real samples in which the presence of aflatoxins was proven by the Research Institute of Food Science and Technology, Mashhad, Iran.

The assessment of accuracy and relative recovery is basically equivalent to the research performed. Relative recovery is also an expression of matrix effects. Likewise, the relative recovery is defined as the following equation:

$$RR\% = (C_{found} - C_{real}/C_{added}) \times 100$$

Where C_{found}, $C_{real,}$ and C_{added} are the concentration of analyte after the addition of a known amount of standard in the real sample, the concentration of analyte in the real sample, and the concentration of the known amount of standard which was spiked to the real sample, respectively. The closer the relative recovery value to 100, the greater the accuracy of the method, and the lesser the effects of the matrix in the analysis process. For measurement of the relative recovery of aflatoxins from real samples, they spiked at the 5 ng l^{-1} concentration level. The relative recovery rates for aflatoxins G2, G1, B2, and B1 were 95%, 87%, 80%, and 91% in the calf starter sample, respectively, and in the rice, a sample containing only aflatoxin B1, the calculated relative recovery was 87% (See Table 5).

CONCLUSIONS

As noted, aflatoxins are one of the most important and most commonly used cancer materials in food, which is why identifying and measuring them in food is very important and vital. There are many methods to calculate the concentration of aflatoxins that are present under different conditions and one of the most effective and important steps in this analysis is the sample preparation stage. The new FPSE method is fast and practical and significantly simplifies the sample preparation process and significantly reduces the consumption of hazardous and toxic organic solvents, which is in agreement with the criteria of green chemistry. This method was developed based on the coupling of HPLC and NFPSE to study the measurement of aflatoxins in food. Parameters that could increase the efficiency of our analysis were reviewed and optimized. The use of nanoparticles in fabric coating was investigated as a modifier and improver of the FPSE method.

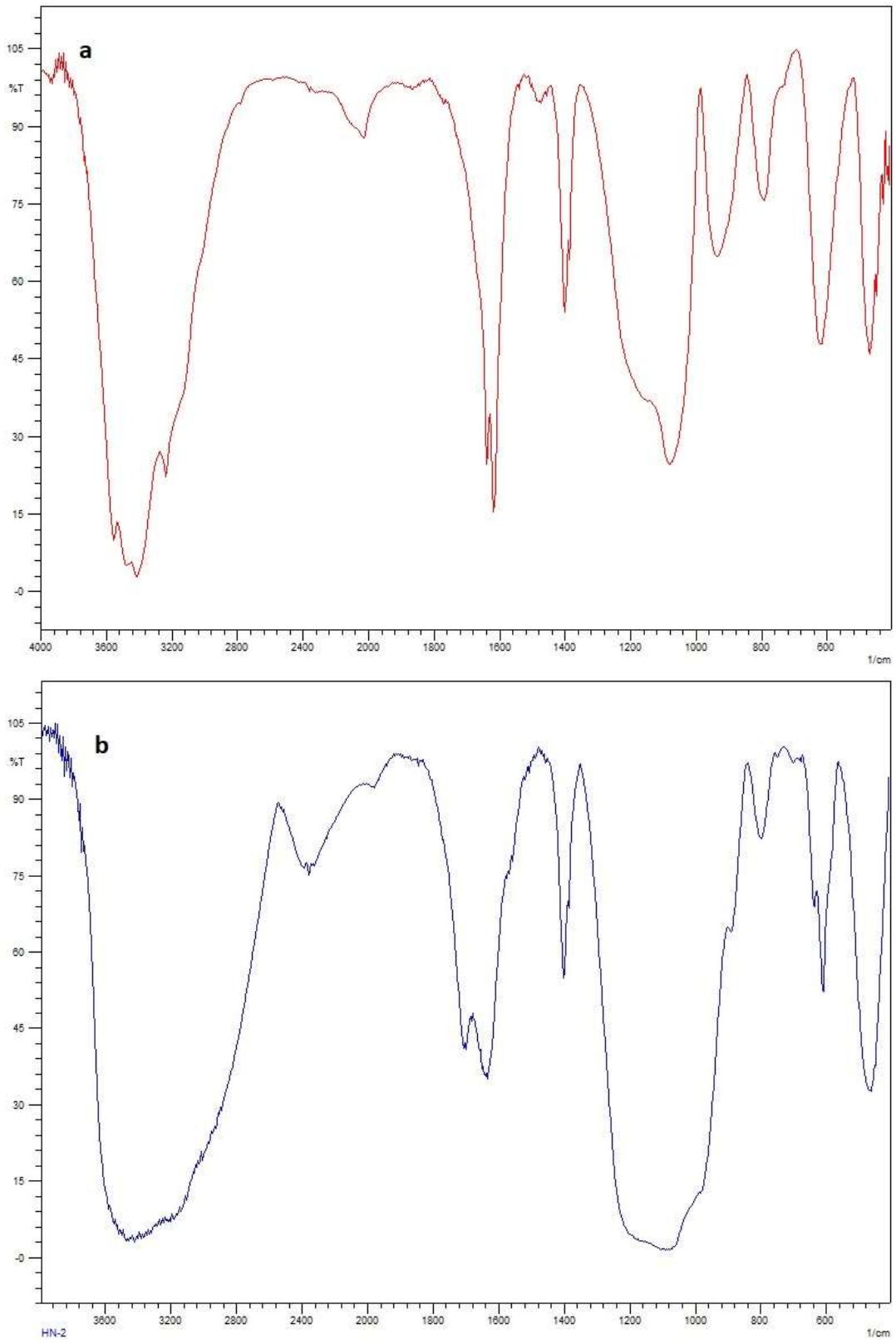

Fig. 6. FTIR Spectra of (a) TEOS-based sol-gel; (b) carbon dot assisted sol-gel.

Table 5. Determination of Aflatoxins in Real Samples

Sample		Spiked (ng l^{-1})	Found (ng l^{-1})	Recovery (%)	RSD (%)
	G1	0	4.35 ± 0.25	-	5.1
Calf starter[a]	G1	5	8.70 ± 0.11	87	4.2
	G2	0	4.75± 0.19	-	3.9
	G2	5	9.50 ± 0.08	95	3.7
	B1	0	4.55 ± 0.26	-	5.3
	B1	5	9.10 ± 0.24	91	4.6
	B2	0	4.00 ±0.21	-	4.2
	B2	5	8.04 ± 0.33	80	5.4
Rice[b]	B1	0	4.35 ± 0.20	-	4.0
	B1	5	8.72 ± 0.16	87	3.8

[a]Calf starter, Mashhad, Iran. [b]Rice, Mashhad, Iran.

The NFPSE gave us acceptable results and was developed in a fast and practical way to analyze all aflatoxins B1, B2, G1, and G2 in food. In future research, it is suggested that we look for challenges in finding new coatings in this method to better respond to complex matrices.

It is estimated that the major drawbacks of FPSE and NFPSE techniques are due to the contact surface area of the fabric and the coating technology that is applied to immobilize the sorbent on the fabric surface. The augmentation in contact surface area offers higher sorbent loading. Therefore, more target analytes are adsorbed by the sorbent, and a reduction of the extraction equilibrium time is achieved. Additionally, sorbent coating technology is very important. Of all the alternative ones that have been developed, the sol-gel coating technology is the most flexible and convenient. There is a strong chemical bond between the nanoparticle-assisted sol-gel coated sorbent and the fabric, which leads to high chemical stability. In summary, both the coating technology and the surface area have to be increased, which results in a sensitive and fast sample preparation technique.

Also, the strength of the adsorbent on the fabric pores is one of the factors that need to be modified. The not-so-perfect repeatability of the method is considered one of the weak points of this method due to the non-uniformity of the fabric structure.

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