



A new ultrasound-assisted liquid-liquid microextraction method utilizing a switchable hydrophilicity solvent for spectrophotometric determination of nitrite in food samples

Adil Elik^a, Ayşenur Öztürk Altunay^a, Muhammad Farooque Lanjwani^{b,c}, Mustafa Tuzen^{b,*}

^a Sivas Cumhuriyet University, Faculty of Science, Department of Chemistry, Sivas, Turkey

^b Tokat Gaziosmanpaşa University, Faculty of Science and Arts, Chemistry Department, 60250 Tokat, Turkey

^c Dr M. A. Kazi Institute of Chemistry, University of Sindh, Jamshoro, Sindh, Pakistan

ARTICLE INFO

Keywords:

Green solvents
Heat-processed food samples
Central composite design
Trethylamine
Nitrite

ABSTRACT

A new and sustainable switchable hydrophilicity solvent-based ultrasound-assisted liquid-liquid microextraction (SHS-UA-LLME) procedure was proposed to extract and determine nitrite in heat processed foods using a spectrophotometer. Three switchable hydrophilicity solvents (SHS-1, SHS-2, and SHS-3) were prepared and used as an extraction solvent for nitrite by mixing N,N-dimethylcyclohexylamine (DMCA), trethylamine (TEA), and N,N-dimethylbenzylamine (DMBA) with water in a 1:1 molar ratio. Protonation of the SHSs were achieved by using dry ice (solid CO₂) to make it water soluble, and addition of NaOH converted it back to its original non-ionic state for phase separation and subsequent extraction of nitrite. Ultrasound was applied to increase the extraction capability of the nitrite in the samples. Various analytical parameters including the pH of the sample solution, SHS types and their volumes, extraction time, and sample volume were optimized. The effects of matrix ions were also studied. Limit of detection and limit of quantification were found 0.1 µg L⁻¹ and 0.3 µg L⁻¹, respectively. The linear range and preconcentration factor were 0.3–600 µg L⁻¹ and 250, respectively. The proposed SHS-UA-LLME was applied to the heat-processed foods with the standard addition method and recoveries were achieved in the range of 91–99%. The factorial design was also used and access the impact of factor and fitness of the model using response surface methodology. The innovation of the proposed SHS-UA-LLME was demonstrated the usability of SHSs for the selective determination and extraction of nitrite by multivariate statistical analysis. In addition, the proposed SHS-UA-LLME does not require heating and centrifugation steps, which provides superiority in terms of cost and time compared to other similar methods.

1. Introduction

Nitrite (NO₂) is a compound widely used in food production as a preservative, chemical fertilizer and decomposition inhibitor in the environment. (Mako et al., 2020; Xue et al., 2018; Jiao et al., 2015). However, studies have reported that inhaling low levels of nitrite can cause serious poisoning, and long-term ingestion can cause cancer (Adarsh et al., 2013; Ma et al., 2018). Further, the long-term or excessive accretion of NO₂ in humans may cause a sickness named “blue baby syndrome” (Ikhsan et al., 2015). However, high levels of nitrite speed up the conversion of regular hemoglobin to methemoglobin, which may cause hemoglobin to lose its ability to carry oxygen (Ahmad et al., 2018; Dou et al., 2021). Moreover, a nitrite may react with amides and amines

and change into cancer-causing N-nitrosamines that may cause stomach cancer (Zhe et al., 2019). The nitrite may destroy cells and help with acidification and hydrolysis, and it is toxic to many microorganisms (Hospital et al., 2017; Lu et al., 2020; Ma et al., 2015). Therefore, it is important to develop practical and efficient analytical techniques for the determination of nitrite in food samples.

The World Health Organization (WHO) guideline for nitrite in drinking waters is 3.0 mg L⁻¹ (WHO, 2017). European Regulation (EC) No 1333/2008 established the maximum permitted levels of addition of nitrite in meat products as 150 mg kg⁻¹ (European Commission, 2008). The Joint Expert Committee of the Food and Agriculture Organization of the United Nations/World Health Organization (WHO) (JECFA) has proposed an acceptable daily intake (ADI) from 0.00 to 0.06 mg nitrite

* Corresponding author.

E-mail address: mustafa.tuzen@gop.edu.tr (M. Tuzen).

<https://doi.org/10.1016/j.jfca.2023.105267>

Received 29 December 2022; Received in revised form 21 February 2023; Accepted 4 March 2023

Available online 7 March 2023

0889-1575/© 2023 Elsevier Inc. All rights reserved.

per kg of body weight (Joint FAO/WHO, 2002).

Electrochemical detection of nitrite was performed with a linearity range as 0.5–105 μM and limit of detection of 0.22 μM (Mehmeti et al., 2016). The electrochemical sensor was developed for the detection of nitrite with a linearity range of 5–1000 μM and a low detection limit of 0.094 μM (Zhang et al., 2021). Spectrophotometric method was developed for the determination of nitrite in the linear range of 0.091–1.47 $\mu\text{g mL}^{-1}$ with a detection limit of 0.053 $\mu\text{g mL}^{-1}$ (El hani et al., 2022). A microfluidic colorimetric sensor was developed for the quantitative detection of nitrite in aquatic environments in the linear range of 0.1–10 ppm with a 0.12 ppm detection limit (Rajasulochana et al., 2022). Voltammetric determination of nitrite was used in meat products in the two linear range from 6.2 to 125 $\mu\text{mol L}^{-1}$ and from 150 to 300 $\mu\text{mol L}^{-1}$ with a 1.89 $\mu\text{mol L}^{-1}$ detection limit (Berisha et al., 2020). A rapid smartphone based spectrophotometric method was developed for the detection of nitrite in environmental water samples in the linear range of 0.05–1.20 mg L^{-1} with 8.60×10^{-3} mg L^{-1} detection limit (Sargazi and Kaykhani, 2020).

Numerous analytical techniques including chromatography (Song et al., 2017), spectrophotometer (Chen et al., 2019), fluorescence (Zhan et al., 2019; Wang et al., 2020), Raman spectroscopy (Han et al., 2013; Correa-Duarte et al., 2015), chemiluminescence (Wang et al., 2013; Lin et al., 2015), colorimetric analysis (de Oliveira et al., 2013) and electrochemical approaches (Sahoo et al., 2020; Jian et al., 2018) have been published in recent years. Several extraction procedures such as cloud point extraction (CPE) (Pourreza et al., 2012), solid phase extraction (SPE) (Hamed et al., 2013), deep eutectic solvent extraction (DES) (Zhang et al., 2019), liquid liquid extraction (LLE) (Yamjala et al., 2016) and microwave assisted extraction (MAE) (Arabi et al., 2021) were reported for the extraction of nitrite in different samples. Switchable hydrophilicity solvents (SHSs) have some advantages such as green, cheap, high enhancement factor, sensitive and selective extraction of analytes. When using the SHSs as the extraction solvent, the centrifugation step is not required after the ultrasonic bath and sonication step.

In this study, it was aimed to use SHSs in LLME for the sensitive and selective determination of nitrite in heat treated foods and to use multivariate statistical analysis in the optimization of the method. In this context, a new, simple, and sustainable switchable hydrophilicity solvent-based ultrasound-assisted liquid-liquid microextraction (SHS-UA-LLME)/spectrophotometric method was proposed for the extraction and determination of trace nitrite in the samples. The several parameters were also optimized and checked for their impact on the extraction recovery of nitrite. The multivariate statistical analysis was carried out like factorial design was utilized to investigate the significant level of factors and their impacts on extraction recovery of the nitrite in the samples. Overall, the proposed SHS-UA-LLME provides a simple, selective and rapid method for the determination of nitrite in heat-processed foods. In addition, the proposed SHS-UA-LLME does not require heating and centrifugation steps, which provides superiority in terms of cost and time compared to other similar methods.

2. Materials and methods

2.1. Reagents and solutions

All chemicals were of at least analytical purity. Stock nitrite (1000 mg L^{-1}) solution was prepared by dissolving an appropriate amount of analytical grade sodium nitrite (Sigma–Aldrich, Spruce, USA) in ultra-pure water. Added a drop of chloroform to preserve it. Working solutions of nitrite were prepared by sequential dilution of this stock solution before the experimental studies. Phthalate, Tris, borate and phosphate buffer solutions were utilized for pH adjust. 500 $\mu\text{mol L}^{-1}$ Fe(II) solution was prepared by FeCl_2 ($\geq 99.0\%$, Merck, Darmstadt, Germany) solid in the water. 5 mol L^{-1} NaOH solution was prepared by dissolving the appropriate amount of its solid ($\geq 97.0\%$, Merck) in water. N,N-dimethylcyclohexylamine (99%, Merck, DMCA), Trethylamine

($\geq 99.5\%$, Sigma, TEA), and N,N-dimethylbenzylamine ($\geq 99\%$, Sigma, DMBA) were utilized in preparation of the SHSs.

2.2. Apparatus

The equipment used in the current research was summarized below. First of all, the determination of nitrite was achieved with an Spectrophotometer (Shimadzu UV-1800 PC model, Tokyo, Japan). Ultrasonic bath (A SK5210LHC Kudos, Shanghai, China) was utilized for pretreatment of the collected samples and to allow the extraction solvent to disperse into the sample solution. The pH control of the extraction medium was achieved with a digital pH meter (Mettler Toledo FE28, Zurich, Switzerland). Milli-Q water purification system (Millipore, USA) was utilized to get ultra-pure water. Analytical balances were used in the weighing step before preparing the solutions and pretreatment of the samples.

2.3. Sampling and pre-treatment of samples

In this study, selective and accurate analysis of nitrite in heat-treated foods was aimed. In this context, all samples were obtained from local markets and restaurants in Sivas, Turkey. Chicken doner, meat doner, turkey salami, turkey sausage, chicken sausages, veal sausage, chicken salami, chicken dumplings and meatball were collected in March 2022. Pre-treatment of these samples was performed according to method (García-Robledo et al., 2014). Firstly samples were powdered with a laboratory mixer and their homogeneity was increased. Then, 10 g of the powdered samples were weighed and transferred to the beaker. Then, 5 mL of 5% borax solution and 150 mL of hot distilled water were added to the beakers, respectively. The beakers were sonicated in an ultrasonic bath at 80 °C for 30 min. After sonication, hexacyanoferrate 2 mL, 0.25 mol L^{-1} of potassium solution and 2 mL of 0.25 mol L^{-1} $\text{Zn}(\text{CH}_3\text{CO}_2)_2$ solution were added to the beakers, respectively, and shaken manually for 5 min. Finally, the obtained mixture was filtered using a membrane filter.

2.4. Preparation of switchable hydrophilicity solvents

The SHSs were performed according to the reported literature (Lasarte-Aragonés et al., 2019). First, amine-containing DMCA, TEA, and DMBA were added to three capped glass vials. Ultra-pure water was then added to the vessels in equimolar, and at this stage a two-phase system was formed as a hydrophobic form of SHS. Then, dry ice (10.0 g) was slowly added to the resulting mixture, avoiding excessive pressure. At this stage, the cloudy appearance was achieved, expressing its formation into protonated SHS, which was moderately dissolved through the reaction of CO_2 . Then, the mixture was vortexed to dissolve the carbon dioxide and to obtain a single-phase formation. In this study, SHS-1, SHS-2 and SHS-3 were obtained by mixing DMCA, TEA, and DMBA species with water in a 1:1 molar ratio, respectively.

2.5. The SHS-UA-LLME procedure

First, 5 mL of sample solution was added to test tubes containing 200 $\mu\text{g L}^{-1}$ nitrite. In the second step, pH of the obtained mixture was adjusted to 7.0 with borate buffer solution. In third step, 50 $\mu\text{mol L}^{-1}$ of Fe(II) solution was added to the obtained mixture to complex nitrite in the sample solution. In the fourth step, 800 μL of the prepared SHS-2 (contains a mixture of water and TEA at 1:1 molar ratio in the presence of carbon dioxide) was added in the obtained mixture (as extraction solvent), followed by the addition of 450 μL of 50 μM NaOH, instantly closing the SHS-2, resulting in a cloudy solution. In the fifth step, the obtained mixture was placed in ultrasonic bath and sonication for 12 min increased the extraction of nitrite into the SHS-2. The sonication step achieved phase separation resulting in a supernatant layer of sealed SHS-2 without the need for a centrifugation step. In the seventh step, the

aqueous portion was discarded by decantation, the remaining solution was taken into cuvettes and spectrophotometric analyses were performed at 407 nm. All experimental steps were continued with blank samples.

2.6. Central composite design

Central composite design (CCD) was utilized to analyze the critical points and significant levels of the variables used in factorial design (Barreto et al., 2020). It is difficult task to select an appropriate experimental design that can clarify the response of variables. For such kinds of clarification, the CCD is best choice. The CCD is a very effective statistical method was chosen to evaluate its capability to assess the response and effects with a fairly small level of trials to express the interactions among the factors and find the best response. The CCD is intended through interaction among the parameters used in the design (Rind et al., 2023). The four variables such as pH, ultrasound time, SHS-2 solvent and sample volume were applied in factorial design (see Table S1) because of these parameters effects the recovery of analytes. The 30 experimental run was applied and access impact of every experimental run of the models using three coded level (see Table S2).

3. Results and discussion

3.1. Screening experimental design

The ANOVA was utilized to calculate the fitness of CCD models (see Table S3). If the p-value is less than 0.05, the CCD model, factors, and their interaction were significant and fit (Koosha et al., 2021). The P-values of variables including B, C, AA, BB, CC and DD were significant because their value was less than 0.05. The error was 14, pure error was found 4 and lack of fit was 10 and total was 29. The variables 2-way interaction were not fit and significant because of its P-value was higher than 0.05.

Three-dimensional response plots were verified in Fig. 1 (a-d). These plots were very beneficial to explore the interaction among the variables

and optimized level individual factor and these response plots demonstrate the impact of 2 factors on response (Koosha et al., 2021). The Figs. 1(a) and 1(b) display the response established for pH, ultrasound and sample volume. These figures shows that response was increased up to pH 7 and the graph revealed that neutral pH was more favorable for extraction of nitrite and also higher response was achieved at lower sample volume. The response surface plots (Figs. 1c and 1d) shows that interaction between ultrasound time and SHS-2 solvent and sample volume. These 3D plots shows that response was increase when increase the ultrasound time up to 12 min then decreased when increased the ultrasound time. The SHS-2 was also affect on the response and better response was observed at SHS-2 vol up to 800 μ L then decreased when increase the volume of SHS-2 solvent and lower level of sample volume was favourable of achieve the good extraction of nitrite in the food samples.

The Pareto chart was a horizontal bar graph demonstrating the evaluation of the influence of each variable and its fitness and significance level. The length or height of bar was related to the coefficient of factor and also vertical line indications the significant level and if bar line passes the vertical line shows as significant (Marrubini et al., 2020). The chart displays those factors including B, D, AA, BB, CC, and DD bar line passed the vertical line value 2.145 (see Fig. 2) and showed significant level of variable other variable did not pass the line and showed insignificant level of variables.

The estimate the significant level and fitness of factors by normal probability plot and estimate its significant level based on linear line (Nik et al., 2020). Normal probability plot (see Fig. S1) describes that variable B, D and joint variables AA, CC, DD and BB exhibited significant effects on the extraction of nitrite. Other variable did not show significant effects on extraction of the nitrite in food sample.

The residuals plots are applied to assess the quality of developed model and residuals must normally distributed, if model is significant (Matin et al. 2020). The residual plot histogram (see Fig. S2a) presented points randomly distributed in negative and positive sides and shows significant and insignificant level of the model. The Fig. S2b and S2c shows points were randomly distributed both sides and away from the

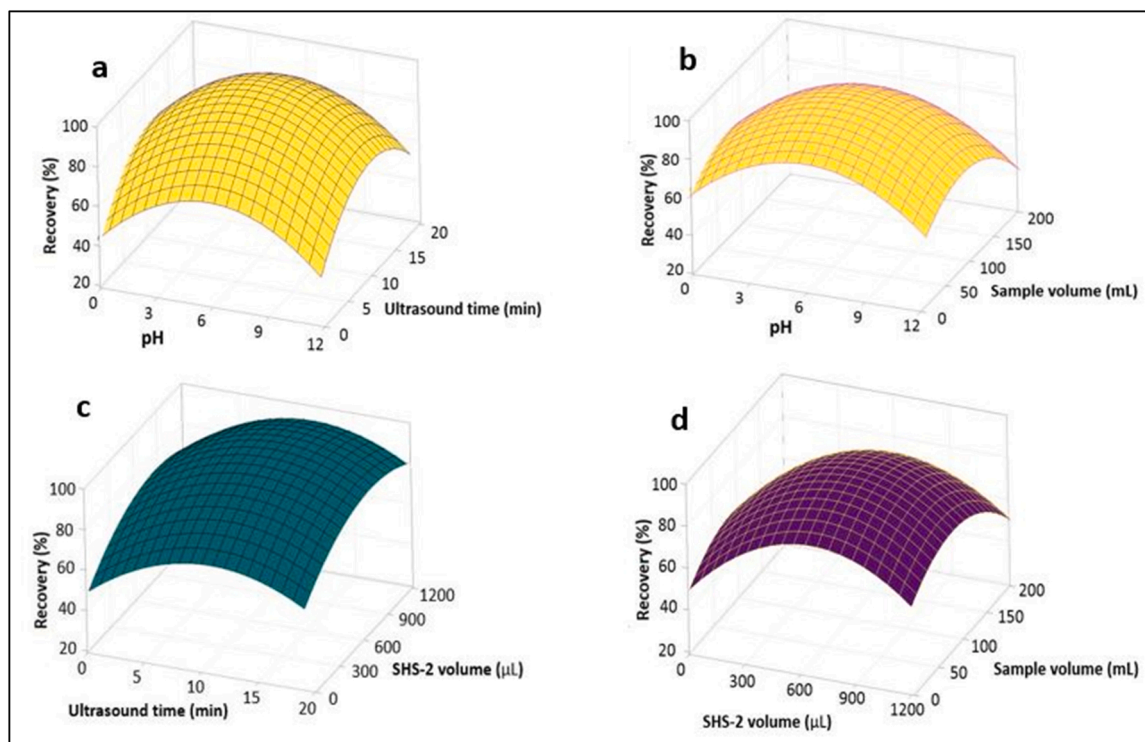


Fig. 1. Three-dimensional plots (a-d).

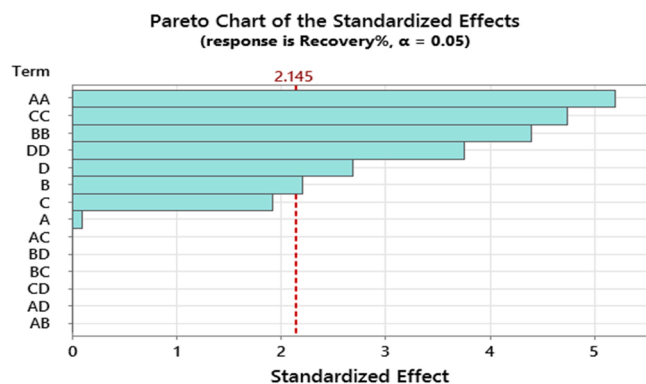


Fig. 2. Pareto chart.

vertical line and considered significant effects on the recovery of the NO_2 in food sample.

3.2. Effects of parameters

The optimization pH of sample supports to improve extraction recovery and sensitivity of the developed method (Li et al., 2020). The optimization of pH was necessary to know its effect on recovery of any sample and different pH was used 3–12 (see Fig. S3). The pH results show that the good recovery was achieved in pH 7. According to the results obtained, the pH of the sample solutions was adjusted to pH 7 with borate buffer solution for further experiments. The best recovery of nitrite was found at pH 7 because of nitrite (NO_2) has own pH 7.3 therefore its best recovery was also achieved at neutral pH 7. The different Fe(II) concentration μM were applied and access their effects of the recovery of nitrite in the food samples. The Fe(II) 10–90 μM concentration were applied and results designated that extraction of NO_2 was improved when increase the concentration of Fe(II) upto 60 μM then decreased (see Fig. S4). The best recovery of nitrite was observed at 60 μM and selected for further experiment. The ultrasound is used to break the extraction sample or solvent into small droplet, to rise the interaction area among the water and droplets and then increase the equilibrium that depends on the ultrasound time and speed (see Fig. S5). The ultrasound time 1–20 min was applied for recovery of nitrite and results indicated that ultrasound time 12 min showed better recovery of nitrite because equilibrium was achieved at 12 min ultrasound time and selected for further experiment. In microextraction process, solvent showed very important role to get better recovery of any sample therefore selection of solvent is very critical. The present study switchable hydrophilic solvent was used as solvent for the recovery of nitrite (see Fig. S6). There are three types of switchable SHS-1, SHS-2 and SHS-3 solvent were used in extraction process and SHS-2 shows good extraction recovery of nitrite and selected for experimental work. The volume of solvent is a main factor and play vital role in extraction process. To know the optimum volume of solvent, volume of SHS-2 solvent was used in range 200–1100 μL (see Fig. S7). The obtained results indicated that good recovery of nitrite was achieved at 800 μL volume of SHS-2 solvent because higher volume of solvent may be interacted with sample and then increase the recovery%, therefore 800 μL of solvent was optimized for further experiment. The NaOH concentration (5 mol L^{-1}) was used and check their impact on recovery of nitrite from food samples (see Fig. S8). The NaOH volume 150–750 mL (5 mol L^{-1}) was applied and obtained results indicated that NaOH volume 450 mL (5 mol L^{-1}) showed better recovery of nitrite and selected for further experiment. Volume of sample may affect and reduce its recovery of applied sample. Therefore, to observe the probable extraction factors, effect the volume of sample on recovery process was examined. For this purpose, volume of sample was used in ranged from 25 to 200 mL (see Fig. S9). From the related figure, it can be seen that

the extraction recovery of nitrite is quantitative in the sample volume range of 20–120 mL and the recovery of nitrite decreases after 120 mL sample volume. In addition, the sample volume of 120 mL was chosen to ensure a high preconcentration factor.

3.3. Analytical figures

The different validity parameters such as limit of quantification (LOQ), and limit of detection (LOD), linear dynamic range, relative standard deviation (RSD%), extraction time, recovery%, inter-day and intra-day study were analyzed to validate the proposed SHS-UA-LLME. Analytical characteristics was obtained before and after the proposed SHS-UA-LLME method. After the proposed SHS-UA-LLME, the LOD ($3S_b/m$) was 0.1 $\mu\text{g L}^{-1}$, while before the SHS-UA-LLME was 151.5 $\mu\text{g L}^{-1}$. After the proposed SHS-UA-LLME, the LOQ ($10S_b/m$) was found 0.3 $\mu\text{g L}^{-1}$ while before the proposed SHS-UA-LLME was found 500 $\mu\text{g L}^{-1}$. After the proposed SHS-UA-LLME, the linear range was 0.3–600 $\mu\text{g L}^{-1}$. The preconcentration factor (PF, 250) was calculated from the ratio of the sample volume to the final volume. Detailed results were given in Table 1. The 12 min extraction time was used for this method and recovery of nitrite was achieved as 95%. The intra-day and inter-day precision/accuracy was also determined using 3 different concentrations of nitrite (10, 200 and 400 $\mu\text{g L}^{-1}$, see Table 2). The intra-day recovery of nitrite was found 95% in 10 $\mu\text{g L}^{-1}$, 96% in 200 $\mu\text{g L}^{-1}$ and 98% in 400 $\mu\text{g L}^{-1}$ with RSD% value was in ranged 2.6–3.9%. The inter-day recovery of nitrite was 93% in 10 $\mu\text{g L}^{-1}$, 92% in 200 $\mu\text{g L}^{-1}$ and 94% in 400 $\mu\text{g L}^{-1}$. RSD% values were found in the range of 2.9–4.4%.

3.4. Matrix effect

The matrix ions were added to estimate the accuracy and selectivity of the proposed SHS-UA-LLME. The different ions such as Na^+ , NH_4^+ , CH_3COO^- , CO_3^{2-} , Mg^{2+} , PO_4^{3-} , Cl^- , K^+ , SO_4^{2-} , Cd^{2+} , citrate, SO_3^{2-} , iodate, Cr^{3+} , Ni^{2+} , NO_3^- , IO_3^- , Cu^{2+} and iodide were investigated for matrix effect. In this context, the different dosages of these ions 10–5000 mg L^{-1} were added in the samples and checked for their interference effect on the recovery of nitrite. The results showed that 90–99% recovery of nitrite was found after addition of these ions. The results revealed the after addition of ions no particular interference was found (see Table S4). The little interference was found after addition of iodide, IO_3^- , Cu^{2+} and NO_3^- . The high tolerance limit obtained indicates the selectivity of the optimized extraction medium for nitrite.

3.5. Determination of nitrite in food samples

Several heat-processed foods including chicken doner, meat doner, Turkey salami, Turkey sausage, chicken sausages, veal sausage, chicken salami, chicken dumplings and meatball were analyzed of their nitrite contents after application of the proposed SHS-UA-LLME. The 30 and

Table 1

The analytical characteristics obtained before and after the SHS-UA-LLME method.

Analytical factors	After the SHS-UA-LLME	Before the SHS-UA-LLME
Linear dynamic range ($\mu\text{g L}^{-1}$)	0.3–600	500–10000
R^2	0.9918	0.9947
LOD ($\mu\text{g L}^{-1}$)	0.10	151.5
LOQ ($\mu\text{g L}^{-1}$)	0.33	500
PF	250	-
RSD% for 50 $\mu\text{g L}^{-1}$ of nitrite, N = 3	2.9	-
Recovery% for 50 $\mu\text{g L}^{-1}$ of nitrite, N = 3	95 ± 4	-
Extraction time (min)	12	-

Table 2

Evaluation of the precision and accuracy of the SHS-UA-LLME method with intraday and interday studies.

Tested concentration of nitrite ($\mu\text{g L}^{-1}$)	Intra-day repeatability (N = 5)		Inter-day reproducibility (N = 5 × 5)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
10	95 ± 2	2.6	93 ± 4	2.9
200	96 ± 4	3.4	92 ± 2	3.7
400	98 ± 1	3.9	94 ± 5	4.4

150 $\mu\text{g g}^{-1}$ dosage of nitrite was added to the samples and estimated their recovery. The proposed SHS-UA-LLME for nitrite in the food samples were also compared with AOAC reference method. The recovery values were found nearly quantitative for analysed the samples (see Table 3). The critical value of t_{10} for $p = 0.05$ was 2.23. The results revealed that proposed SHS-UA-LLME showed excellent accuracy of nitrite in different food samples.

3.6. Comparison of nitrite determination by different methods

The proposed SHS-UA-LLME was compared with other reported methods and check their ability for extraction of nitrite (see Table 4). Tamen et al. reported the extraction of nitrite by IV-HS-LPME method using spectrophotometer and 17 min extraction time was optimized for the recovery of nitrite. Altunay and Elik reported the extraction of nitrite from meat samples using spectrophotometer. The LOD and LOQ values were in the range of 0.035 $\mu\text{g L}^{-1}$ -0.1 $\mu\text{g L}^{-1}$ and linearity was 0.1–300 $\mu\text{g mL}^{-1}$ (Altunay and Elik, 2020). Zhang et al. investigated the extraction of nitrite from water samples by VA-DLLME method using HPLC and the method was successfully utilized for extraction of nitrite from three environmental samples and recovery was found in range of 90.5–115.2% at the linearity range of 1–300 $\mu\text{g L}^{-1}$. Present proposed method showed excellent recovery of nitrite with a good linearity range at lower LOQ and LOD values. The most methods compared involve excessive use of organic reagents, time-consuming and purification steps that do not fit the concept of green chemistry. In conclusion, the SHS-based microextraction procedure for the determination of trace levels of nitrite with the developed method in this study showed high recovery, low extraction time and sensitivity and was more compatible with the idea of "green chemistry" than other commonly used methods.

4. Conclusions

In the current study, a green and novel switchable hydrophilicity solvent-based ultrasound-assisted liquid-liquid microextraction (SHS-UA-LLME) process was applied for the determination of nitrite using a spectrophotometer. The SHS solvents are easily synthesized, accessible, inexpensive and variable to solubility against several compounds and it has low melting point. The different parameters were optimized like pH of solution, selection of solvent type and their volume, ultrasound time and samples volume and access their impact on recovery of nitrite from food samples. The LOD and LOQ value was found in the range of 0.1–151.5 $\mu\text{g L}^{-1}$ and 0.3–5000 $\mu\text{g L}^{-1}$. The linear range was found to be 0.3–10000 $\mu\text{g L}^{-1}$, PF was 250 and RSD% was 2.9. The proposed method was also compared with other reported process for extraction of NO_2 from different samples. In addition, the proposed SHS-UA-LLME does not require heating and centrifugation steps, which provides superiority in terms of cost and time compared to other similar methods. The factorial design method was also drawn and investigated the effects of factors and the significant level of the model. Tolerance limit of matrix ions were found high. The proposed SHS-UA-LLME for the determination of nitrite that may occur in processed foods can be applied without harmful effects on human health or the environment. Therefore, the

Table 3

Determination of nitrite in food samples to check accuracy and applicability of the SHS-UA-LLME method.

Heat treated samples	SHS-UA-LLME method			AOAC reference method (AOAC, 1995)	t-test ^b
	Added ($\mu\text{g g}^{-1}$)	Measured ($\mu\text{g g}^{-1}$)	Recovery (%)		
Chicken doner	-	61.9 ± 0.4 ^a	-	63.8 ± 0.6	1.15
	30	91.0 ± 2.1	97 ± 3		
	150	210.4 ± 4.3	99 ± 2		
Meat Doner	-	25.5 ± 0.9	-	24.1 ± 1.1	0.67
	30	54.0 ± 1.8	95 ± 4		
	150	171.0 ± 3.6	97 ± 2		
Turkey salami	-	88.3 ± 2.8	-	91.8 ± 3.2	0.89
	30	115.9 ± 3.9	92 ± 5		
	150	232.3 ± 4.5	96 ± 4		
Turkey Sausage	-	97.8 ± 3.7	-	95.1 ± 3.4	1.25
	30	126.9 ± 5.6	93 ± 3		
	150	240.3 ± 7.9	95 ± 2		
Chicken sausages	-	36.2 ± 0.8	-	33.9 ± 1.0	1.49
	30	63.2 ± 2.6	90 ± 6		
	150	177.2 ± 4.3	94 ± 3		
Veal Sausage	-	52.6 ± 1.5	-	50.1 ± 1.2	0.92
	30	81.4 ± 3.0	96 ± 5		
	150	199.6 ± 8.3	98 ± 4		
Chicken salami	-	102.9 ± 4.6	-	108.2 ± 4.1	1.17
	30	130.9 ± 6.7	93 ± 3		
	150	246.9 ± 9.3	96 ± 5		
Chicken dumplings	-	46.7 ± 1.7	-	43.9 ± 1.5	1.68
	30	75.2 ± 3.1	95 ± 3		
	150	193.7 ± 8.3	98 ± 2		
Meatball	-	23.8 ± 0.8	-	20.3 ± 0.6	0.73
	30	51.1 ± 2.9	91 ± 6		
	150	166.3 ± 7.3	95 ± 4		

^a Mean ± standard deviation. ^bThe critical value of t_{10} for $p = 0.05$ was 2.23.

proposed SHS-UA-LLME has the potential to be a useful tool for the sustainable green extraction and spectrophotometric analysis of nitrite in heat-processed foods, and future experimental approaches can also be extended to a wider range of analytes and matrices.

CRediT authorship contribution statement

Adil Elik: Investigation, Validation, Writing – original draft, Writing – review & editing, Software. **Ayşenur Öztürk Altunay:** Investigation, Writing – original draft, Writing – review & editing, Software. **Muhammad Farooque Lanjwani:** Investigation, Validation, Writing –

Table 4

Comparison of the present method with reported methods for the determination of nitrite.

Instruments	Method	LOD $\mu\text{g L}^{-1}$	LOQ $\mu\text{g L}^{-1}$	LR $\mu\text{g L}^{-1}$	ET (time min)	PF, EF	RSD%	References
$\mu\text{PD-OES}$	HS-SPME	0.1	-	1–100	5.0	10	4.1	Yang et al., 2021
Spectrophotometer	IV-HS-LPME	1.0	-	3–120	17		2.0	Tamen et al., 2021
Spectrophotometer	VA-SUPRAS-LPME	0.035	0.1	0.1–300	2.0	200	4.8	Altunay and Elik., 2020
HPLC	DLLME	9.1	31	31–50000	10		1.9	Khodaei et al., 2020
HPLC	VA-DLLME	0.2	1.0	1–300	2.0	100	6.0	Zhang et al., 2019
Spectrophotometer	3HF-SSBME	1.6	5.3	5.3–1000	8.0	282	4.0	Badiee et al., 2019
HPLC	DLLME	0.2	0.6	1–1000	1.0	94	5.0	Saraji and Ghambari., 2018
ET AAS	In-syringe-LME	0.013	0.043	0.05–10	3.0	79.5	4.8	Roohparvar et al., 2018
Spectrophotometer	DLLME-SFO	9.04	314.0	770–1760	1.0	52	5.0	Zahedi et al., 2015
HPLC	ILDLLME	0.05	0.4	0.4–500	5.0	430	4.1	He et al., 2011
Spectrophotometer	VALLME	0.5	-	1–100	2	-	3.9	(Jiao et al., 2017)
Spectrophotometer	CPE	6.0	8.0	8–120	25	50	3.4	(Pourreza et al., 2012)
Spectrophotometer	CPE	0.42	1.40	1.5–150	22	67	4.8	(Gürkan & Altunay, 2018)
Spectrophotometer	HF-SSBME	1.6	5.30	5.3–1000	40	282	4.0	(Badiee et al., 2019)
ETAAS	SLME	0.013	0.043	0.05–10	5	82.6	5.1	(Roohparvar et al., 2018)
Spectrophotometer	SHS-UA-LLME	0.10	0.33	0.3–600	12	250	2.9–4.4	Present work

Headspace solid-phase microextraction (HS-SPME), miniaturized point discharge optical emission spectrometry ($\mu\text{PD-OES}$), in-vessel headspace liquid-phase microextraction (IV-HS-LPME), Dispersive liquid liquid microextraction (DLLME), vortex-assisted supramolecular solvent-based liquid phase microextraction (VASUPRAS-LPME), High performance liquid chromatography (HPLC), Vortex-assisted dispersive liquid–liquid microextraction (VA-DLLME), Solvent stir bar microextraction system with three-hollow fiber configuration (3HF-SSBME), In-syringe liquid microextraction (In-syringe-LME), Electrothermal-atomic absorption spectroscopy (ET-AAS), dispersive liquid–liquid microextraction based on solidification offloating organic droplets (DLLME-SFO), ionic liquid dispersive liquid–liquid microextraction (ILDLLME), Vortex-assisted liquid–liquid microextraction (VALLME), cloud point extraction (CPE), Solvent stir bar microextraction system with three-hollow fiber (HF-SSBME) Syringe liquid microextraction (SLME), Limit of detection (LOD), Limit of quantification (LOQ), Linear range (LR), Extraction recovery time (ER), Pre-concentration factor (PF), Enrichment factor (EF), Relative standard deviation (RSD).

original draft, Writing – review & editing, Software. **Mustafa Tuzen:** Investigation, Validation, Writing – original draft, Writing – review & editing, Software.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The data that has been used is confidential.

Acknowledgments

Dr. M. Tuzen thanks to Turkish Academy of Sciences (TUBA) for partial supports.

Funding

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jfca.2023.105267](https://doi.org/10.1016/j.jfca.2023.105267).

References

- Adarsh, N., Shanmugasundaram, M., Ramaiah, D., 2013. Efficient reaction based colorimetric probe for sensitive detection, quantification, and on-site analysis of nitrite ions in natural water resources. *Anal. Chem.* 85 (21), 10008–10012.
- Ahmad, R., Mahmoudi, T., Ahn, M.S., Yoo, J.Y., Hahn, Y.B., 2018. Fabrication of sensitive non-enzymatic nitrite sensor using silver-reduced graphene oxide nanocomposite. *J. Colloid Interface Sci.* 516, 67–75.
- Altunay, N., Elik, A., 2020. A green and efficient vortex-assisted liquid-phase microextraction based on supramolecular solvent for UV–VIS determination of nitrite in processed meat and chicken products. *Food Chem.* 332, 127395.
- AOAC, (1995) Official methods of analysis, Method 36.1.2116th ed.
- Badiee, H., Zanjanchi, M.A., Zamani, A., Fashi, A., 2019. Solvent stir bar microextraction technique with three-hollow fiber configuration for trace determination of nitrite in river water samples. *Environ. Sci. Pollut. Res.* 26 (32), 32967–32976.
- Barreto, J.A., de Assis, R.D.S., Santos, L.B., Cassella, R.J., Lemos, V.A., 2020. Pressure variation in-syringe dispersive liquid-liquid microextraction associated with digital image colorimetry: determination of cobalt in food samples. *Microchem. J.* 157, 105064.
- Berisha, L., Maloku, A., Haliti, M., Jashari, G., Ukmata, A., Sýs, M., 2020. Voltammetric determination of nitrites in meat products after reaction with ranitidine producing 2-methylfuran cation. *Microchem. J.* 159, 105403.
- Chen, H., Yang, T., Liu, F., Li, W., 2019. Electrodeposition of gold nanoparticles on Cu-based metal-organic framework for the electrochemical detection of nitrite. *Sens. Actuators B: Chem.* 286, 401–407.
- Correa-Duarte, M.A., Pazos Perez, N., Guerrini, L., Giannini, V., Alvarez-Puebla, R.A., 2015. Boosting the quantitative inorganic surface-enhanced Raman scattering sensing to the limit: the case of nitrite/nitrate detection. *J. Phys. Chem. Lett.* 6 (5), 868–874.
- Dou, B., Yan, J., Chen, Q., Han, X., Feng, Q., Miao, X., Wang, P., 2021. Development of an innovative nitrite sensing platform based on the construction of carbon-layer-coated In₂O₃ porous tubes. *Sens. Actuators B: Chem.* 328, 129082.
- El hani, O., Karrat, A., Digua, K., Amine, A., 2022. Development of a simplified spectrophotometric method for nitrite determination in water samples. *Spectrochim. Acta Part A: Mol. Biomol. Spectrosc.* 267, 120574.
- , 2008European Commission (2008). European Commission Regulation (EC) No 1333/2008 of the European parliament and of the council of 16 December 2008 on food additives Official Journal of the European Union, L354 (2008), 16–33.
- García-Robledo, E., Corzo, A., Papaspyrou, S., 2014. A fast and direct spectrophotometric method for the sequential determination of nitrate and nitrite at low concentrations in small volumes. *Mar. Chem.* 162, 30–36.
- Gürkan, R., Altunay, N., 2018. Preconcentration and indirect quantification of trace nitrite, nitrate and total nitrite in selected beverage and milk samples using ion-pairing cloud-point extraction with acridine orange. *J. Food Compos. Anal.* 69, 129–139.
- Hamed, M.M., Yakout, S.M., Hassan, H.S., 2013. Solid phase extraction of nitrate and nitrite anions using naturally and available sorbent. *J. Radioanal. Nucl. Chem.* 295 (1), 697–708.
- Han, X.X., Schmidt, A.M., Marten, G., Fischer, A., Weidinger, I.M., Hildebrandt, P., 2013. Magnetic silver hybrid nanoparticles for surface-enhanced resonance Raman

- spectroscopic detection and decontamination of small toxic molecules. *ACS Nano* 7 (4), 3212–3220.
- He, L., Zhang, K., Wang, C., Luo, X., Zhang, S., 2011. Effective indirect enrichment and determination of nitrite ion in water and biological samples using ionic liquid-dispersive liquid-liquid microextraction combined with high-performance liquid chromatography. *J. Chromatogr. A* 1218 (23), 3595–3600.
- Hospital, X.F., Hierro, E., Arnau, J., Carballo, J., Aguirre, J.S., Gratacós-Cubarsí, M., Fernández, M., 2017. Effect of nitrate and nitrite on *Listeria* and selected spoilage bacteria inoculated in dry-cured ham. *Food Res. Int.* 101, 82–87.
- Iksan, N.I., Rameshkumar, P., Pandikumar, A., Shahid, M.M., Huang, N.M., Kumar, S. V., Lim, H.N., 2015. Facile synthesis of graphene oxide-silver nanocomposite and its modified electrode for enhanced electrochemical detection of nitrite ions. *Talanta* 144, 908–914.
- Jian, J.M., Fu, L., Ji, J., Lin, L., Guo, X., Ren, T.L., 2018. Electrochemically reduced graphene oxide/gold nanoparticles composite modified screen-printed carbon electrode for effective electrocatalytic analysis of nitrite in foods. *Sens. Actuators B: Chem.* 262, 125–136.
- Jiao, S., Jin, J., Wang, L., 2015. One-pot preparation of Au-RGO/PDDA nanocomposites and their application for nitrite sensing. *Sens. Actuators B: Chem.* 208, 36–42.
- Jiao, Y., Yu, J., Yang, Y., 2017. Vortex-assisted liquid-liquid microextraction combined with spectrophotometry for the determination of trace nitrite in water samples. *Water Sci. Technol.: Water Supply* 17 (5), 1225–1231.
- Joint FAO/WHO (2002). **Expert Committee on Food Additives Summary and Conclusions of the Fifty-ninth Meeting; 2002 Jun 4–13; Geneva World Health Organization, Geneva (2002).**
- Khodaei, H., Afshar Mogaddam, M.R., Hamidi, A.A., Farajzadeh, M.A., Bavili Tabrizi, A., Ansarin, K., Nemat, M., 2020. Determination and validation of simultaneous derivatization and dispersive liquid-liquid microextraction method for analysis of nitrate and nitrite contents as nitrate ions in onion and potato samples. *Sep. Sci.* 3 (6), 225–234.
- Koosha, E., Shamsipur, M., Salimi, F., Ramezani, M., 2021. A microextraction method based on precipitation for the simultaneous separation and preconcentration of cadmium and lead before their determination by FAAS: experimental design methodology. *Sep. Sci. Technol.* 56 (10), 1721–1729.
- Lasarte-Aragón, G., Álvarez-Lueje, A., Salazar, R., Toledo-Neira, C., 2019. Application of switchable hydrophobic solvents for extraction of emerging contaminants in wastewater samples. *Molecules* 25 (1), 86.
- Li, T., Song, Y., Li, J., Zhang, M., Shi, Y., Fan, J., 2020. New low viscous hydrophobic deep eutectic solvents in vortex-assisted liquid-liquid microextraction for the determination of phthalate esters from food-contacted plastics. *Food Chem.* 309, 125752.
- Lin, Z., Dou, X., Li, H., Ma, Y., Lin, J.M., 2015. Nitrite sensing based on the carbon dots-enhanced chemiluminescence from peroxy-nitrous acid and carbonate. *Talanta* 132, 457–462.
- Lu, Y., Xu, Y., Chen, S., Dong, B., Dai, X., 2020. Effect of nitrite addition on the two-phase anaerobic digestion of waste activated sludge: Optimization of the acidogenic phase and influence mechanisms. *Environ. Pollut.* 261, 114085.
- Ma, B., Peng, Y., Wei, Y., Li, B., Bao, P., Wang, Y., 2015. Free nitrous acid pretreatment of wasted activated sludge to exploit internal carbon source for enhanced denitrification. *Bioresour. Technol.* 179, 20–25.
- Ma, Y., Wang, Y., Xie, D., Gu, Y., Zhang, H., Wang, G., Wong, P.K., 2018. NiFe-layered double hydroxide nanosheet arrays supported on carbon cloth for highly sensitive detection of nitrite. *ACS Appl. Mater. Interfaces* 10 (7), 6541–6551.
- Mako, T.L., Levenson, A.M., Levine, M., 2020. Ultrasensitive detection of nitrite through implementation of N-(1-naphthyl) ethylenediamine-grafted cellulose into a paper-based device. *ACS Sens.* 5 (4), 1207–1215.
- Marrubini, G., Dugheri, S., Cappelli, G., Arcangeli, G., Mucci, N., Appelblad, P., Speltini, A., 2020. Experimental designs for solid-phase microextraction method development in bioanalysis: a review. *Anal. Chim. Acta* 1119, 77–100.
- Matin, P., Ayazi, Z., Jamshidi-Ghaleh, K., 2020. Montmorillonite reinforced polystyrene nanocomposite supported on cellulose as a novel layered sorbent for microextraction by packed sorbent for determination of fluoxetine followed by spectrofluorimetry based on multivariate optimisation. *Int. J. Environ. Anal. Chem.* 1–16.
- Mehmeti, E., Stanković, D.M., Hajrizi, A., Kalcher, K., 2016. The use of graphene nanoribbons as efficient electrochemical sensing material for nitrite determination. *Talanta* 159, 34–39.
- Nik, V.M., Konoz, E., Feizbakhsh, A., Sharif, A.A.M., 2020. Simultaneous extraction of chromium and cadmium from bean samples by SrFe₂O₇@CTAB magnetic nanoparticles and determination by ETAAS: an experimental design methodology. *Microchem. J.* 159, 105588.
- Pourreza, N., Fat'hi, M.R., Hatami, A., 2012. Indirect cloud point extraction and spectrophotometric determination of nitrite in water and meat products. *Microchem. J.* 104, 22–25.
- Rajasulochana, P., Ganesan, Y., Kumar, P.S., Mahalaxmi, S., Tasneem, F., Ponnuchamy, M., Koor, A., 2022. Paper-based microfluidic colorimetric sensor on a 3D printed support for quantitative detection of nitrite in aquatic environments. *Environ. Res.* 208, 112745.
- Rind, I., Sari, A., Tuzen, M., Lanjwani, M., Karaman, I., Saleh, T., 2023. Influential biosorption of lead from aquatic solution using *Escherichia coli*/carbon nanofibers. *Environ. Nanotechnol. Monit. Manag.* 19.
- Roohparvar, R., Shamspur, T., Mostafavi, A., Bagheri, H., 2018. Indirect ultra-trace determination of nitrate and nitrite in food samples by in-syringe liquid microextraction and electrothermal atomic absorption spectrometry. *Microchem. J.* 142, 135–139.
- Sahoo, S., Sahoo, P.K., Sharma, A., Satpati, A.K., 2020. Interfacial polymerized RGO/MnFe₂O₄/polyaniline fibrous nanocomposite supported glassy carbon electrode for selective and ultrasensitive detection of nitrite. *Sens. Actuators B: Chem.* 309, 127763.
- Saraji, M., Ghambari, H., 2018. Dispersive liquid-liquid microextraction based on liquid anion exchanger for the direct extraction of inorganic anions. *J. Chromatogr. A* 1574, 27–35.
- Sargazi, M., Kaykhaii, M., 2020. Application of a smartphone based spectrophotometer for rapid in-field determination of nitrite and chlorine in environmental water samples. *Spectrochim. Acta Part A: Mol. Biomol. Spectrosc.* 227, 117672.
- Song, X., Gao, L., Li, Y., Mao, L., Yang, J.H., 2017. A sensitive and selective electrochemical nitrite sensor based on a glassy carbon electrode modified with cobalt phthalocyanine-supported Pd nanoparticles. *Anal. Methods* 9 (21), 3166–3171.
- Wang, Q., Ye, L., Jiang, G., Jensen, P.D., Batstone, D.J., Yuan, Z., 2013. Free nitrous acid (FNA)-based pretreatment enhances methane production from waste activated sludge. *Environ. Sci. Technol.* 47 (20), 11897–11904.
- Wang, X., Hou, J., Shen, X., He, Q., Hou, C., Huo, D., 2020. Fluorescence-based measurements for the determination of nitrite using a coumarin derivative sensor based on inner filter effect. *Anal. Methods* 12 (8), 1107–1114.
- Xue, Z., Fu, X., Rao, H., Zhou, X., Liu, X., Lu, X., 2018. A new electron transfer mediator actuated non-enzymatic nitrite sensor based on the voltammetry synthetic composites of 1-(2-pyridylazo)-2-naphthol nanostructures coated electrochemical reduced graphene oxide nanosheets. *Electrochim. Acta* 260, 623–629.
- Yamjala, K., Nainar, M.S., Ramiseti, N.R., 2016. Methods for the analysis of azo dyes employed in food industry—a review. *Food Chem.* 192, 813–824.
- Yang, R., Lin, Y., Yang, J., He, L., Tian, Y., Hou, X., Zheng, C., 2021. Headspace solid-phase microextraction following chemical vapor generation for ultrasensitive, matrix effect-free detection of nitrite by microplasma optical emission spectrometry. *Anal. Chem.* 93 (18), 6972–6979.
- Zahedi, M.M., Amiri, A.H., Nasiri, M., 2015. Spectrophotometric monitoring of nitrite in seawater after liquid microextraction of its derivative with 2,3-diaminonaphthalene. *Water Qual. Res. J. Sci.* 52 (1), 11–17.
- Zhan, Y., Zeng, Y., Li, L., Luo, F., Qiu, B., Lin, Z., Guo, L., 2019. Ratiometric fluorescent hydrogel test kit for on-spot visual detection of nitrite. *ACS Sens.* 4 (5), 1252–1260.
- Zhang, K., Li, S., Liu, C., Wang, Q., Wang, Y., Fan, J., 2019. A hydrophobic deep eutectic solvent-based vortex-assisted dispersive liquid-liquid microextraction combined with HPLC for the determination of nitrite in water and biological samples. *J. Sep. Sci.* 42 (2), 574–581.
- Zhang, W., Ge, C.Y., Jin, L., Yoon, S., Kim, W., Xu, G.R., Jang, H., 2021. Nickel nanoparticles incorporated Co, N co-doped carbon polyhedron derived from core-shell ZIF-8@ZIF-67 for electrochemical sensing of nitrite. *J. Electroanal. Chem.* 887, 115163.
- Zhe, T., Sun, X., Wang, Q., Liu, Y., Li, R., Li, F., Wang, L., 2019. A screen printed carbon electrode modified with a lamellar nanocomposite containing dendritic silver nanostructures, reduced graphene oxide, and β -cyclodextrin for voltammetric sensing of nitrite. *Microchim. Acta* 186 (5), 1–8.