Magnetic hydrophobic deep eutectic solvents for orbital shaker-assisted dispersive liquid-liquid microextraction (MAGDES-OS-DLLME) - determination of nickel and copper in food and water samples by FAAS

Adil Elik[1], Hameed Ul Haq[2], Grzegorz Boczkaj[2], Seçkin Fesliyan[1], Özlem Ablak[1], and Nail Altunay[1,*]

[1] *Faculty of Science, Department of Chemistry, Sivas Cumhuriyet University, Sivas, Türkiye*
[2] *Gdansk University of Technology, Faculty of Civil and Environmental Engineering, Department of Sanitary Engineering, 80 – 233 Gdansk, G. Narutowicza St. 11/12, Poland.*

*Corresponding author: naltunay@cumhuriyet.edu.tr

Abstract

In this work, a cheap and widely applicable dispersive liquid-liquid microextraction (DLLME) method was developed for the extraction of Ni(II) and Cu(II) from water and food samples and analysis using flame atomic absorption spectrometry. DLLME was assisted by orbital shaker, while ferrofluid as an extractant was based on deep eutectic solvent (DES). This ferrofluid was made of hydrophobic DES (hDES), composed of lauric acid and menthol (molar ratio 1:2), and toner powder@aliquat 336 magnetic particles. The extraction procedure does not require any heating or centrifugation. The method limits of detection value were 0.15 µg L^{-1} and 0.03 µg L^{-1} for Ni(II) and Cu(II) respectively along with wide linearity range (0.4-250 µg L^{-1}). The validation of the method was performed using certified reference materials (CRMs). The studies revealed excellent accuracy between results obtained by the developed method and expected values for all CRMs. The relative recoveries of Ni(II) and Cu(II) ions ranged from 92.8% to 98.6%. The developed method was further used for the determination of Ni(II) and Cu(II) in real water and food samples and provided quantitative recoveries.

Keywords: Sample preparation; microextraction; food analysis; water and wastewater analysis; environmental analysis; miniaturization in analytical chemistry

Highlights

- ✓ Magnetic DES: Toner powder@aliquat 336 / lauric acid:menthol as extractant
- ✓ Robust and time saving miniaturized sample preparation method
- ✓ Validated method reliable for routine analysis of Ni and Cu in water and food
- ✓ Short extraction time (8 min) and high preconcentration factor (125) were obtained
- ✓ Sensitive (ppb level), selective and stable method towards complex sample matrix

1. Introduction

Copper is one of the heavy metals that has a red color and has been found in certain amounts in soil, water and air. As a result, its presence was confirmed also in plants and animal's bodies. Emission of this element from different industrial processes such as dyeing, oil, paper, plating, copper is released in amounts that can be dangerous for aquatic and terrestrial ecosystems (Seidi, & Alavi, 2019). Although copper is one of the essential elements for humans in terms of providing enzymatic activity and taking part in red blood cell formation, excessive copper levels can lead to diseases e.g epilepsy, dementia, depression and autistic disorder, (Shrivas, & Jaiswal, 2013). For this reason, there are some restrictions on copper intake and the recommended intake of copper by the World Health Organization is 0.5 mg Kg^{-1} per day body weight(Council, 2000, Olivares et al., 1998, Cross et al., 2005).

Nickel (Ni) is a metal used in various materials such as dental prosthesis, coatings, computer components, pigments, and ceramics. Release of nickel into water occurs as a result of the dissolution of soil and rocks, the biological functions of some living things and industrial activities (Kravkaz Kuşçu, Bayraktar, & Tunçer, 2022). Excessive nickel levels in the living system causes skin and respiratory system diseases (Elahi et al., 2022). Based on surveys performed on animals, it has been suggested that the daily intake of nickel for humans should be <100 µg/day (Nielsen, 2021). Considering the harmful effects of Ni and Cu and the related restrictions, the necessity of determining trace amounts of these metals from foodstuffs comes to the fore.

Different analytical methods are available for Cu(II) and Ni (II) determination including flame atomic absorption spectrometry (Jalbani, & Soylak, 2015), inductively coupled plasma-mass spectrometry (Wang et al., 2015), voltammetry (Primo, Buffon, & Stradiotto, 2021), UV-VIS spectrophotometry (Eshaghi, Vafaeinezhad, & Hooshmand, 2016), and liquid chromatography (De Oliveira Trinta et al., 2020) in different food and water samples. Flame atomic absorption spectrometry is very useful for the detection and quantification of heavy metals from various matrices such as environmental, food, and water samples because of its high sensitivity, low cost, and relative convenience compared to other methods (Tuzen et al., 2020). Despite of the above mentioned advantages, direct analysis of heavy metals from various samples is challenging with flame atomic absorption spectrometry due to possible interference of matrix, structure of the samples concerned and presence of interfering species. To overcome this problem, a reliable and highly selective separation and enrichment method should be used before the determination phase. Although traditional extraction techniques have an important place among the sample preparation methods, the interest of researchers has recently shifted to microextraction techniques as they offer various possibilities such as saving the time needed for effecitive extraction and minimization of organic solvents usage(Jayasinghe et al., 2022; Faraz et al., 2021).

DLLME is important as it is a simple and cost-effective microextraction technique that allows simultaneous analysis of multiple analytes (Elik et al., 2023b, Haq et al., 2022)). Although deep eutectic solvents (DESs) have comparable properties with ionic liquids in terms of their stability, and tenability, they are superior to ionic liquids as they are cheaper, and easier to synthesizes (Makoś et al., 2018;Mbous et al., 2017;Haq et al., 2023a). DESs provide unique intermolecular interactions with specific analytes, thus ‘‘tuning’’ the selectivity of the designed separation system (Momotko et al., 2022, Momotko et al., 2021, Khajavian et al., 2022;Elik et al., 2023a, Faraz et al., 2021, Haq et al., 2021). Their superior properties were proved in several analytical procedures (Haq et al., 2023b, Ullah et al., 2022). Recently, researchers have combined extraction solvents with magnetic materials to increase extraction efficiency (Chisvert et al., 2017; An, Rahn, & Anderson, 2017). One of the important examples of magnetic materials used in microextraction techniques are ferrofluids (Altunay et al., 2023). Ferrofluids combined with the DLLME are rapidly injected into the sample, while dispersive solvent is dissolved in the aqueous phase, resulting in the formation of small droplets of extractant. It causes a large surface area for mass transfer between sample and extractant. In this case, the extraction rate is accelerated and the extraction time is minimized. Another advantage is that they are easily separated from the sample after extraction due to their magnetic properties and there is no need to use complex devices and methods for their separation (Nayebi, & Shemirani, 2021).

This study aimto prepare and test ferrofluid based on DESs for selective extraction and analysis of Ni(II) and Cu(II) ions in different water and food samples. As a result of the ongoing studies, a fast, simple and economical analytical method was developed. This method does not include either a heating step or a centrifugation step. Effective separation of the extractant was achieved using a magnetic stir bar. Important microextraction parameters of the sample preparation procedure were investigated and optimized in details. The method was validated by using certified reference materials (CRMs) such as GBW10015 Spinach, GBW10016 Tea and GBW10019 Apple.

2. Experimental

2.1. Apparatus

The pH of the extraction solution was measured using a Metrohm 691 pH meter (Herisau, Switzerland). A magnetic heating plate was used for the preparation of ferrofluid based DESs. An orbital shaker (Multi Bio RS-24, BioSan, Berlin, Germany) was used dispersion of the ferrofluid-based DES in the sample solution. A neodymium magnet was used to accelerate the separation of magnetic DES from the aqueous solution. Ultrapure water was obtained using a Milli-Q system (ICW-3000, Merck, Darmstadt, Germany). The digestion step was carried out by microwave system (Milestone Ethos D model, Sorisole-Bg, Italy). The analysis step was carried out with a flame atomic absorption spectrometry instrument (AAS-6300, Shimadzu, Kyoto, Japan) with D_2 background correction.. The measurements were performed at two wavelengths (Ni-232nm and Cu-324.8nm). The lamp current used for these wavelengths was 10mA and 3.0mA, respectively. The spectral bandwidths for the two measurements were 0.2nm and 0.5nm. The flow rates of acetylene and air during the experiment were set at 1.8 and 8.0 mL min^{-1}, respectively.

2.1. Chemicals and reagents

The chemicals utilized in the research were of analytical grade and were employed without any additional purification procedures. Standard solutions of Ni(II) and Cu(II) ions, each with a concentration of 500 mg L^{-1}, were prepared by dissolving their nitrate salts in deionized water. The experimental solutions and calibration standards were created by employing a sequential dilution formula. Once prepared, these solutions were stored in a refrigerator at a temperature of 4 °C. Acetone, tetrahydrofuran, acetonitrile, methanol, ethanol, and methyl violet were obtained from Sigma-Aldrich (Barcelona, Spain). Borate, phthalate, citrate and TRİS buffer solutions were used to fix the pH in the range from 3 to 9. Aliquat-336 (95.0 %, Merck, Germany), butyric acid (99%, Merck), thymol, lactic acid (≥ 95.0 %, Sigma Aldrich), lauric acid (≥98%, Sigma Aldrich), menthol (≥99%, Sigma Aldrich) and *o*-xylene (≥ 98, Merck) were used in the preparation of ferrofluid based DESs. Toner powder was purchased from Thermo Scientific (Norway).

2.3. Sampling

Bottled waters were bought from local markets in Sivas/Türkiye. Waste water samples were collected from the industrial zone in Sivas/Türkiye. Well water was collected from an agricultural region located in Sivas, Turkey. The river water was obtained from Kızılırmak passing through Sivas. The spring waters were collected from the hot spring area in in Sivas/Türkiye. The collected water samples were first filtered through a 0.45 µm pore size membrane filter (cellulose membrane filter, Whatman®, USA) and then stored in the refrigerator at 4 °C. All of the food samples including black tea, rice flour, wheat, green pepper, spinach, apple, eggplant, pomegranate, parsley, mint, tomato, mushroom and potato were purchased from local grocery stores and markets in Sivas/Türkiye. The collected food samples were first washed with distilled water, the edible parts were cut with the help of a knife, and then dried in the oven. The dried samples were homogenized with a laboratory blender. Then, microwave digestion method was applied to these powdered samples.

2.4. Microwave based sample digestion

Food samples were digested according to the recommended methods in the literature (Abdulkhaliq et al., 2012, Elahi et al., 2022). The microwave digestion steps used for food samples are summarized below. First 1 g of the food samples was transferred into Teflon tubes containing concentrated HNO_3 (20 mL) and concentrated H_2O_2 (5mL). Next, microwave digestion was carried out with gradual change in temperature. For first 2 min the temperature was 60 °C, with microwave power 250 W. In the second step temperature was 100 °C for 5 min with microwave power 250 W. In third step 150 °C for 2 min with microwave power 500 W. In the fourthstep temperature was 200 °C for 3 min with microwave power 600 W. The residue obtained after microwave digestion was diluted to 10 mL with the water. All samples were prepared in triplicate with sample blanks. In case ofwater samples analysis, a 100 mL of the collected water samples were heated on heating plate. Volume of water sample was reduced to a 10 mL. The remaining 10 mL sample was used for the recommended procedure.

2.5. Preparation of ferrofluid based DESs

In this study, the ferrofluid based DESs were prepared using the previously reported method in literature (Mohebbi et al., 2021; Zarei, Nedaei, & Ghorbanian, 2018). Four different types of DESs were prepared. Menthol was used as the hydrogen bond acceptor (HBA), while butyric acid, thymol, lactic acid, and lauric acid served as the hydrogen bond donors (HBD). These HBA and HBD compounds were added to beakers with their respective mixed molar ratios, as specified in Table 1. The beakers were then heated on a magnetic stirrer heating plate at 80°C until a clear liquid was formed. This resulting liquid was employed as a supporting solvent for the production of a ferrofluid. To accomplish this, 1 g of toner powder was mixed with a 10 mL of o-xylene in a tube, followed by sonication at room temperature for 10 min. The mixture was subsequently heated to approximately 70°C for 2 h . Afterward, magnets were employed to separate the magnetic particles from the aqueous solution, which were then properly washed with ultrapure water and ethanol. The particles were subsequently dried in an oven and heated up to 80°C. To prevent the agglomeration of the magnetic particles, 1.0 mL of aliquat-336 was added. Then, a 150 mg of the obtained magnetic particles were introduced into a glass vial. A 1.0 mL portion of the prepared DES was added to the mixture, followed by stirring at 50°C for 3 h. Finally, the resulting ferrofluid was tested as the extracting solvent for the DLLME procedure. After the ferrofluid-based DES-4 was separated from the aqueous solution, different solvents such as ethanol, methanol, tetrahydrofuran, acetonitrile and acetone were investigated to both dilute the viscous liquid formed and to strip the analytes from the magnetic phase. All solvents were tested in equal volume.

2.6. MAGDES-OS-DLLME method

The magnetic deep eutectic solvent based orbital shaker-assisted dispersive liquid-liquid microextraction (MAGDES-OS-DLLME) method was applied to the digested samples by following the experimental steps belowInitially, a 10 mL of the digested samples were transferred to vial that already contained a solution of Ni(II) and Cu(II) ions each with a concentration of 25 µg L^{-1}. Secondly, a 0.1 M citrate buffer solution was added to adjust desired pH (6.0). In third step, a methyl violet was added (final solution was 75 µmol L^{-1}) to ensure the complexation. Fourth, a 0.8 mL ferrofluid-based DES-4 was added to the sample solution to separate the metal-ligand complex. In fifth step, conical tubes were shaken at 1200 rpm for 8 min on orbital shaker and for dispersion of the ferrofluid-based DES-4 in the sample solution. Six, a magnetic stir bar was dipped into the tube and the ferrofluid containing the analytes was transferred along with the magnetic stir bar to other tubes. Seven, tetrahydrofuran (300 µL) was added followed by vortexing for 30 seconds. Finally, the resulting solution (approximately 1.0 mL) was analyzed by flame atomic absorption spectrometry. The experimental steps of MAGDES-OS-DLLME are presented in Figure 1.

2.7. Method optimization and calculation of validation parameters

The method was optimized to obtain the highest recovery of the Ni(II) and Cu(II) ions. The main extraction factors including pH, concentration of complexing agent, ferrofluid-based DESs type and volume, mixing type, shaking time, type and volume of desorption solvent, KCl amount and volume of sample were investigated in details. The extraction recovery % of the DLLME was calculated by the following Equation.

$$\text{Extraction recovery (\%)}=[C_{final}\, V_{final} / C_o\, V_o] \times 100$$

Where C_{final} - concentration of Ni(II) and Cu(II) ions determined by the developed method, C_o- concentration in the sample before applying the method, V_{final} - the final volume and V_o - the initial volume.

Detection limit (LOD) and quantification limit (LOQ) for the method were calculated by using the formulas [$3S_{blank}/m$] and [$10S_{blank}/m$] respectively. Where S_{blank} is the standard deviation for the blank solution and m is the slope of the standard curve.

The precision of the method was calculated as relative standard deviation (RSD%) and calculated as [$\text{RSD\%}=\frac{SD}{Mean\ value} \times 100$]. Where SD is the standard deviation based on three replicate determinations.

Enrichment factor (EF) was calculated as [$\text{EF}=\frac{m_f}{m_i}$], where m_i is the slope of the calibration graph obtained before the MAGDES-OS-DLLME and m_f is the slope of the calibration graph obtained after the MAGDES-OS-DLLME.

The pre-concentration factor (PF) was evaluated by using the equation [PF=$\frac{V_f}{V_i}$], where V_f is the final volume of sample and V_i is the initial volume of the sample.

The tolerance limit for the interfering species on the extraction and determination was calculated using the formula [Matrix species amount, μg L^{-1}] / [Analyte amount, μg L^{-1}] for highest concentration of matrix species that didn’t cause error at determination step.

In CRM the t_{exp} was calculated using the following formula [t_{exp}=$\frac{I\mu-\bar{x}I\sqrt{N}}{s}$]. Where t_{exp}, s, N, $\bar{x}$ and μ were statistical values, the standard deviation, the number of independent determinations, the experimental mean value, and the certified value, respectively.

3. Results and discussion

3.1. Optimization of parameters

A selective and sensitive microextraction step should be applied in order to quantify Ni(II) and Cu(II) in food and water samples. To increase the extraction efficiency of the MAGDES-DLLME procedure, a univariate optimization strategy was optimized. All runs were performed in triplicate and the percent relative standard deviation was calculated for each experiment run.

3.1.1. Effect of pH

In case of extraction process from aqueous solutions, it is desirable to provide selective interaction of the target analytes with the extractant added to the sample. In this context, a one of the most significant factor affecting selective interaction is the pH of the aqueous solution (Haq et al., 2021). Chemical species in aqueous solution may exist in different forms such as neutral form or anions, cations, hydroxides, depends on the pH of sample medium. As a result, the interaction between extractant and target analytes will be strongly varied depending on pH. Taking these factors into consideration, the potential impact of the pH of the aqueous medium on the recovery percentage of Ni(II) and Cu(II) ions was examined within the pH range of 2-

10. Figure S1 illustrates that the highest extraction recovery was achieved at pH 6. The decrease in extraction recovery, especially in the basic region, is attributed to the increased hydroxide formation of these metals. Due to the increase in the hydroxide concentration, the complexation of the analyte ions cannot be completed due to the attack of hydroxide ions on the cationic region of the chelating agent (methyl violet). As a result, for subsequent microextraction studies, the pH of the medium was adjusted to 6.0 using a 0.1 M citrate buffer solution.

3.1.2. Effect of concentration of complexing agent

The extraction process was facilitated when the metal ions could be complexed with a suitable chelating agent. In light of these facts, methyl violet was tested for complexation of Ni(II) and Cu(II) ions. Another factor affectingeffective complexation is the concentration of the complexing agent. Methyl violet should be added to the aqueous solution in sufficient quantity to quantitatively complex the metal ions in the medium. Therefore, the effect of concentration of methyl violet on the recovery of Ni(II) and Cu(II) ions was investigated by changing the concentration of methyl violet from 10 μmol L^{-1} to 150 μmol L^{-1}. The results in Figure S2 reveal that 75 μmol L^{-1} of methyl violet was sufficient to achieve quantitative recoveries of both analytes. It is worth to mention, that, the extraction recovery of the analytes was almost stable at higher methyl violet concentration values. Based on these considerations, a concentration of 75 μmol L^{-1} of methyl violet was selected as the optimal concentration for the DLLME procedure

3.1.3. Effect of ferrofluid-based DES type and volume

The most important parameter to achieve the desired goal in microextraction studies was the type of extraction solvent. The extraction solvent should be specific (selective) and have high extraction efficiency. Furthermore, the extraction solvent should not be miscible with the sample solution. Based on these expectations, four ferrofluid-based DESs were prepared and tested for the extraction of Ni(II) and Cu(II). The results of this part of the study were presented in Figure S3a. The maximum extraction recovery for both analytes was obtained using the ferrofluid-based DES-4 (toner powder@aliquat 336@lauric acid:menthol). Therefore, the ferrofluid-based DES-4 was selected as extraction solvent DLLME studies.

Next, the volume of ferrofluid-based DES-4 extractant was optimised.. The addition of excessive extraction solvent causes an increase in the magnetic phase in the final volume and thus decrease the concentration of the analytes. The increase in the magnetic phase volume causes excessive use of dispersive solvents. In this section, the effect of the volume of ferrofluid-based DES-4 on the extraction recovery of Ni(II) and Cu(II) ions was investigated in the volume range of 0.2-1.5 mL. Analysis of the results presented on Figure S3b reveals that the best extraction recovery for both analytes was obtained when 0.8 mL of ferrofluid-based DES-4 was used. Thus, this optimized value was used in the further studies..

3.1.4. Effect of mixing type and time

In order to to obtain effective extraction, i.e. effectively complex the Ni(II) and Cu(II) from the sample, the ferrofluid-based DES-4 must be completely dispersed in the medium. Therefore, after adding ferrofluid-based DES-4 to the aqueous solution, mixing steps including oribtal shaking, vortexing, hand mixing and sonication were compared. According to the results in Figure S4a, the maximum recovery for both analytes was achieved when orbital shaking was applied. Another parameter that affects the effective distribution of the ferrofluid-based DES-4 in the aqueous solution is the shaking time. The effect of orbital shaking time on the extraction recovery of Ni(II) and Cu(II) ions was studied from 1 min to 15 min. Figure S4b shows that the 8 min shaking time was enough for quantitative recovery of analytes. Therefore, 8 min of orbital shaking was used in the further studies..

3.1.5. Effect of desorption solvent type and volume

After the ferrofluid-based DES-4 was separated from the aqueous solution, different solvents such as ethanol (EtOH), methanol (MeOH), tetrahydrofuran (THF), acetonitrile (ACN) and acetone were investigated to both dilute the viscous liquid formed and to strip the analytes from the magnetic phase. All solvents were tested in equal volume. Based on the results presented in Figure S5a, THF was chosen as the suitable desorption solvent. Next, the THF volume was optimized. While increasing the volume of solvent, firstly the desorbed amount of analyte increases up to full recovery, but further excess volume of the solvent causes decrease of analyte concentration. On the other side, too low volume of desorption solvent makes the analytes recovery difficult. The impact of varying THF volume between 0 and 750 µL was investigated. Figure S5b reveals that 300 µL of THF was sufficient to achieve maximum recoveries. Thus, 300 µL of THF was used in the further studies..

3.1.6. Effect of KCl amount

In DES-based microextraction studies, the ionic strength of the aqueous solution can have different effect on the recovery of analyte. The addition of salt increases the ionic strenght of the aqueous phase and causes the salting effect of the extraction solvents to shift the dispersion balance towards the anhydrous phases, thus maximizing the extraction recovery and phase separation. Consequently, the influence of different concentrations of KCl ranging from 0 to 15 (w/v%) on the retrieval of analytes was examined (see Figure S6). The samples spiked with KCl don’t have any significant effect on the extraction efficiency of analytes. Thus, KCl solution was not used in the further studies..

3.1.7. Effect of sample volume

To maximize the PF of the method, it was necessary to optimize the volume of sample used in microextration stage. The PF was determined by comparing the initial volume of the sample with the final volume after extraction. The extraction recoveries (approximately 95%) for both analytes were stable from 25 mL to 125 mL (see Figure S7). Furthermore, it was observed that at higher sample volumes, there was a significant decrease in the extraction recoveries for both analytes. For 125 mL sample volume, 95% recovery was achieved so this value was used in further studies..

3.2. Validation studies

For routine application of DLLME method for real samples, validation parameters such as linearity range, LOD, LOQ, EF, PF, matrix effect, precision, accuracy and sensitivity of the MAGDES-OS-DLLME method must be evaluated under optimized conditions.

3.2.1. Aspects of quantitative analysis

Different concentrations of Ni(II) and Cu(II) ions standard were added to the sample solutions and linearity studies were performed for each analyte. As a result of the study, linearity for Ni(II) and Cu(II) ions were 0.1-330 μg L^{-1} and 0.5-250 μg L^{-1}, respectively. LOD and LOQ calculated were 0.03 μg L^{-1} and 0.10 μg L^{-1} for Ni(II) ions and 0.15 μg L^{-1} and 0.46 μg L^{-1} for Cu(II) ions.. In this context, the PF of the method was 125. Moreover, the EF for Ni(II) and Cu(II) were 108 and 97, respectively. Detailed information and comprehensive data can be found in Table12.

The method was validated using CRM. GBW10015-Spinach, GBW10016-Tea, and GBW10019-Apple were analyzed for Ni (II) and Cu (II). In GBW10015 Spinach (Ni), the calculated Ni concentration in Spinach was 0.86 mg kg^{-1}, and the reference value was 0.92 mg kg^{-1}. The calculated percent recovery was 93.4%, indicating that the method was quite accurate for this element in this reference material. The t-exp value was 0.84, which suggests a good agreement between the experimental mean and the certified value. In GBW10015 Spinach (Cu), for Cu in Spinach, the calculated value was 8.5 mg kg^{-1}, while the reference value was 8.9 mg kg^{-1}. Thus, the percent recovery was 95.0%, indicating a high level of accuracy. The t-exp value was 1.12, which confirms good agreement. The results of GBW10016 Tea and GBW10019 Apple for both Ni and Cu gave high percent recovery% and t-exp values close to 1 indicate that the method was providing accurate results, confirming high applicability of the method in routine analysis of real samples..

3.2.2. Precision

The MAGDES-OS-DLLME method was examined for both intraday and inter-day variations at different concentrations of analytes within the specified working range. The concentrations tested included low (5 µg L^{-1}), medium (100 µg L^{-1}), and high (200 µg L^{-1}) levels. In the intraday study, the added concentrations of analyte ions were investigated with five repetitive extractions on the single day. In the interday study, the same concentrations were studied with five repetitive extractions on three consecutive days. In addition, the accuracy of the MAGDES-OS-DLLME method was tested by performing a recovery study for the concentrations added in these studies. The RSD% for Ni (II) ion in the intraday and interday study were in the range of 2.8-3.3% and 3.1-3.6%, respectively. In addition, the RSD for Cu (II) ion in the intraday and interday study were in the range of 2.4-3.5% and 2.9-3.9%, respectively. Furthermore, it provided quantitative recoveries (see Table 1) for both analytes.

3.2.3. Accuracy

Some certified reference materials (GBW10015 spinach, GBW10016 tea and GBW10019 apple) were analyzed in this study with the MAGDES-OS-DLLME method. Five replicates of each reference material were analyzed and the results averaged. Experimental results from analysis of GBW10015 spinach, GBW10016 tea and GBW10019 apple were 0.86±0.16 mg Kg^{-1} for Ni/8.9±0.4 mg Kg^{-1} for Cu, 3.3±0.18 mg Kg^{-1} for Ni/18.6±0.7 mg Kg^{-1} for Cu and 0.13±0.04 mg Kg^{-1} for Ni/2.5±0.2 mg Kg^{-1} for Cu, respectively. At a 95% confidence level, the obtained results were in good agreement with the reference values. Also, the t-exp (0.56-1.24) of all studies was smaller than the t-tabulate (2.31). These findings suggest that the observed results exhibit no statistically significant difference when compared to the expected values. In this particular aspect of the research, the recovery rates for Ni (II) ranged from 92.9% to 97.1%, while the recovery rates for Cu (II) ranged from 95.0% to 97.8%. Detailed results were given in Table 2.

3.2.4. Matrix effect

The matrix effect on the MAGDES-OS-DLLME method under optimized conditions was investigated in the following part of the study. This study was carried out on the food samples because it can create stronger matrix effects comparing to relatively simple water samples. On the other hand, the mineralization step of the procedure, significantly simplifies the matrix proceeded to extraction. First, the anions and cations listed in Table S2 were added to the food sample in different proportions. Then, the recovery, RSD% and tolerance limit of the related species were estimated for the target analytes. According to Table S2, the RSD% for both analytes was lower than 2.6%. Quantitative recoveries (92-99%) were also obtained for both analytes. High tolerable limits (up to 10000) were obtained in the presence of studied anions and cations. Results shows that the MAGDES-OS-DLLME is highly selective and stable for matrix interferences for both analytes.

3.3. Application of method- real samples analysis

The MAGDES-OS-DLLME method was employed to determine the targeted analytes in water and food samples. To assess the method's accuracy in analyzing water samples, a two-level standard addition approach was employed, involving additions of 10 and 100 µg L^{-1} concentrations. Both Ni and Cu were not detected in bottled water, spring water-1 and mineral water. The highest Ni (14.2±1.6 µg L^{-1}) and Cu (9.2±0.2 µg L^{-1}) contents were detected in spring water-2 and well-water, respectively. Furthermore, recoveries for both analytes were in the range of 92.5-98.3% and 93.0-98.6%, respectively (Table 5a). Using the MAGDES-OS-DLLME method, the Ni could not be detected in food samples including rice flour, wheat and, and pomegranate. In addition, the highest Ni content (5.12 ± 0.09 μg g^{-1}) was detected in spinach. The Cu was detected in all food samples. In addition, the highest Cu content (44.2 ± 0.8 μg g^{-1}) was detected in green pepper. Finally, all results were within WHO's acceptable limits.

3.4. Comparative study

The important analytical parameters of the MAGDES-OS-DLLME method were compared with different micro-extraction studies using a similar technique for quantitative analysis. In terms of linearity, LOD, RSD%, EF/PF, and estimated extraction procedure time, the developed method underwent assessment and was compared to other reported methods. A detailed comparison of the data can be found in Table S3. The extraction time of the developed method (only 8 mins) was lower than all the compared methods. Also, the linearity of the method was comparable to other methods. In addition, the LOD of the method was lower than other procedures. The PF obtained for both analytes was higher in most microextraction methods.

4. Conclusions.

The MAGDES-OS-DLLME method was employed for extraction of Ni(II) and Cu(II) from water and food samples. Among the studied ferrofluid-based DESs, the one made of toner powder@aliquat 336 andlauric acid:menthol (molar ratio 1:2) was selected as extraction solvent. The obtained magnetic DES extract is collected by magnetic stir bar, followed by analytes desorption by 300μL of THF. Final determination step is performed by means of FAAS. To the best of our knowledge, this study is the first ferrofluid DES-based analytical method reported so far for the simultaneous determination of Ni(II) and Cu(II). In addition, negligible matrix effect and short extraction time were obtained with the developed method. Extensive validation studies, proved the usefulness of the MAGDES-DLLME-FAAS method in routine analysis of water and food samples.. Based on the obtained results, it has been shown that the MAGDES-DLLME-FAAS provide high reproducibility, low LODs, high PF and low matrix effect. Analysis of real samples revealed presence of controlled metals, but in all cases reported values were below WHO limits.

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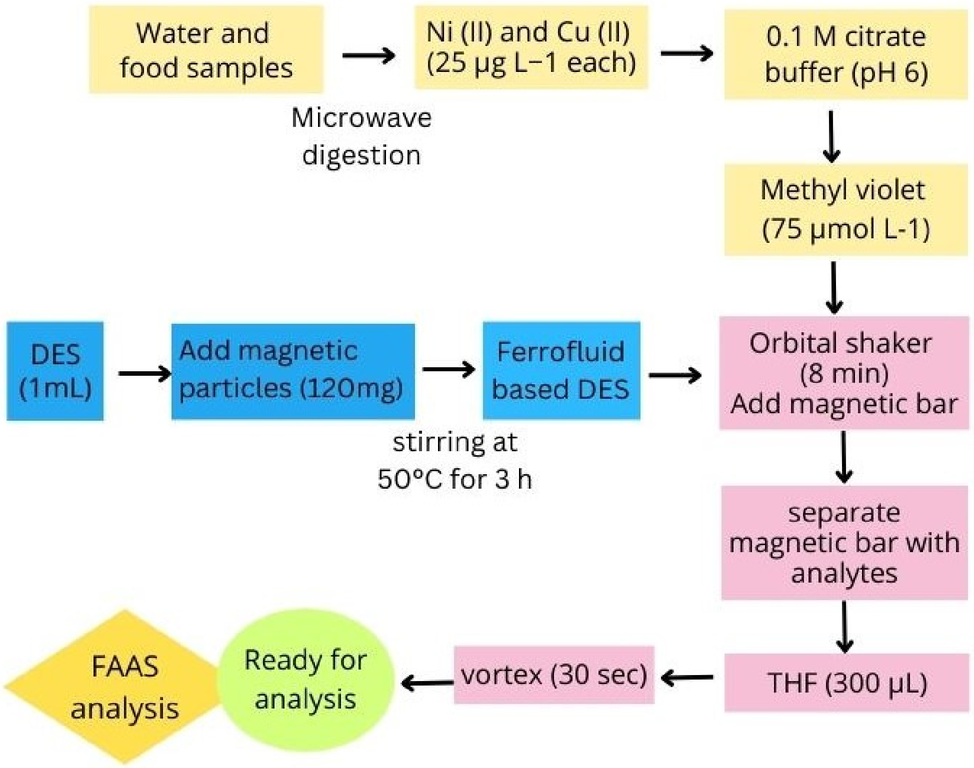

Figure 1. Schematic diagram for the MAGDES-OS-DLLME method

Table 1. Analytical characteristics of the MAGDES-OS-DLLME method.

Analytes	Linearity (μg L^{-1})	LOD (μg L^{-1})	LOQ (μg L^{-1})	EF	PF	^{a,b} Recovery (%)			^{a,b} RSD (%)		
						5 μg L^{-1}	100 μg L^{-1}	200 μg L^{-1}	5 μg L^{-1}	100 μg L^{-1}	200 μg L^{-1}
Ni(II)	0.1-330	0.03	0.1	135.9	125	96.4^{a} (93.1)^{b}	97.3(95.2)	97.8 (96.1)	3.1^{a} (3.4)^{b}	2.8 (3.1)	3.3 (3.6)
Cu(II)	0.5-250	0.15	0.46	112.5	125	94.1 (94.8)	95.4 (95.5)	97.2 (96.7)	2.4 (2.9)	2.8 (3.3)	3.5 (3.9)

a: intraday studies (N=5) b: interday studies (N=5x3)
LOD: limit of detection
LOQ: Limit of quantification
EF: Enrichment factor
PF: Preconcentration factor
RSD: Relative standard deviations

Table 2. Analysis of reference materials using the MAGDES-OS-DLLME method (N=5).

Reference material	Ni				Cu			
	Reference value (mg kg^{-1})	Calculated (mg kg^{-1})	Recovery (%)	*t-exp	Reference value (mg kg^{-1})	Calculated (mg kg^{-1})	Recovery (%)	*t-exp
GBW10015 Spinach	0.92±0.12	0.86±0.16	93.4	0.84	8.9±0.4	8.5±0.8	95.0	1.12
GBW10016 Tea	3.4±0.30	3.3±0.18	97.1	1.24	18.6±0.7	18.2±1.2	97.8	0.74
GBW10019 Apple	0.14±0.05	0.13±0.04	92.9	0.56	2.5±0.2	2.4±0.2	96.2	1.15

*$t_{exp}=\frac{|\mu-\bar{x}|\sqrt{N}}{s}$, where t_{exp}, s, N, $\bar{x}$ and μ were statistical value, the standard deviation, number of independent determinations, the experimental mean value, and the certified value, respectively.

Table 3. Results from the analysis of the water samples using the MAGDES-OS-DLLME method (N=3)

Water samples	Spiked,	Ni		Cu	
	Spiked, Ni(II), Cu(II), µg L^{-1}	Calculated, µg L^{-1}	Recovery, %	Calculated, µg L^{-1}	Recovery, %
Bottled water	-	≤LOD	-	≤LOD	-
	10	9.7±0.5*	97.0	9.5±0.5	95.0
	100	98.3±3.4	98.3	97.2±4.1	97.2
Wastewater	-	5.4±0.2	-	2.6±0.2	-
	10	15.0±1.1	96.1	12.0±0.9	94.0
	100	102.9±4.8	97.6	99.3±4.4	96.7
River water	-	11.8±1.6	-	3.3±0.8	-
	10	21.5±2.3	96.7	12.6±1.2	93.0
	100	107.7±5.2	95.9	100.2±3.6	96.9
Spring water-1	-	≤LOD	-	≤LOD	-
	10	9.7±0.8	97.0	9.6±0.7	96.0
	100	96.3±4.1	96.3	98.5±4.2	98.5
Spring water-2	-	14.2±1.6	-	4.7±0.3	-
	10	23.7±2.8	95.0	14.4±0.8	97.0
	100	11.9±4.9	97.7	103.1±5.2	98.4
Mineral water	-	≤LOD	-	≤LOD	-
	10	9.6±0.4	96.0	9.3±0.1	93.0
	100	98.1±3.7	98.1	95.7±3.8	95.7
Well-water	-	8.2±0.9	-	9.2±0.2	-
	10	17.5±1.3	93.0	18.8±1.1	96.0
	100	103.8±4.6	95.6	107.4±4.6	98.2

*Mean value ± standard deviation based on three replicate determinations

Table 4. Results from the analysis of the food samples using the MAGDES-OS-DLLME method (N=3)

Food samples	Ni		Cu	
	Calculated, μg g^{-1}	RSD, %	Calculated, μg g^{-1}	RSD, %
Black tea	0.75 ± 0.01*	1.3	24.4 ± 0.6	2.5
Rice flour	≤LOD	-	28.7 ± 0.9	3.1
Wheat	≤LOD	-	5.9 ± 0.2	3.4
Green pepper	2.26 ± 0.03	1.3	44.2 ± 0.8	1.8
Spinach	5.12 ± 0.09	1.8	10.3 ± 0.3	2.9
Apple	0.26 ± 0.01	3.8	3.9 ± 0.1	2.6
Eggplant	0.38 ± 0.01	2.6	6.2 ± 0.2	3.2
Pomegranate	≤LOD	-	11.3 ± 0.5	4.4
Parsley	1.95 ± 0.05	2.7	26.5 ± 0.6	2.2
Mint	2.04 ± 0.01	2.6	11.6 ± 0.4	3.5
Tomato	0.33 ± 0.01	3.0	6.9 ± 0.1	1.5
Mushroom	1.58 ± 0.03	2.2	2.3 ± 0.1	4.3
Potato	1.92 ± 0.04	2.1	81.3 ± 2.9	3.6

*Mean value ± standard deviation based on three replicate determination