Deep eutectic solvent (DES) with silver nanoparticles (Ag-NPs) based assay for analysis of lead (II) in edible oils

Hameed Ul Haq[1], Rafiat Bibi[2], Muhammad Balal Arain[3], Faisal Safi[2], Sana Ullah[2], Roberto Castro-Muñoz[1,4], Grzegorz Boczkaj[1,5,*]

[1]*Gdansk University of Technology, Faculty of Chemistry, Department of Process Engineering and Chemical Technology, 80 – 233 Gdansk, G. Narutowicza St. 11/12, Poland. E-mail: grzegorz.boczkaj@pg.edu.pl / hameed.haq@pg.edu.pl

[2]Department of Chemistry, Abdul Wali Khan University, Mardan, 23200, Pakistan. E-mail: hulhaqchem@gmail.com / rafiatmohmand@hotmail.com / faisalsafi1993@gmail.com

[3]Department of Chemistry, University of Karachi, Karachi, Sindh, 75270 Pakistan. E-mail: bilal_ku2004@yahoo.com

[4]Tecnologico de Monterrey, Campus Toluca, Avenida Eduardo Monroy Cárdenas 2000 San Antonio Buenavista, 50110, Toluca de Lerdo, Mexico. Email: food.biotechnology88@gmail.com

[5]EkoTechCenter, Gdansk University of Technology, G.Narutowicza St. 11/12, 80-233 Gdansk, Poland

Corresponding author: Dr. Grzegorz Boczkaj, Assoc. Prof., PhD. Sc. Eng. Gdansk University of Technology, Faculty of Chemistry, Department of Process Engineering and Chemical Technology, 80 – 233 Gdansk, G. Narutowicza St. 11/12, Poland. Fax: (+48 58) 347-26-94; Tel: (+48) 697970303; e-mail: grzegorz.boczkaj@gmail.com or grzegorz.boczkaj@pg.edu.pl

Abstract

This paper presents an application of silver nanoparticles impregnated by Deep Eutectic Solvents (DES) as ultrasonication aided microextraction system for lead (II) determination in edible oils. The paper presents a systematic optimization of method parameters and examples of its application for analysis of real samples. Maximum recovery for lead (II) extraction was obtained for choline chloride and phenol with a 1:2 molar ratio. Optimum extraction conditions for 2g oil sample post-digested solution (10 mL, pH=2) require 1mL of Ag-nanoparticles solution (0,1mM) and per each 500 µL of DES and tetrahydrofurane. The limit of detection (LOD) and quantification (LOQ) were 0.28 µg/L and 0.94 µg/L. The developed method covers the entire range of expected levels of lead concentration in oil samples -parts per billion levels to higher ones. This method is many folds faster (only 6.5 minutes/sample are needed) as well as more sensitive comparing to already reported methods.

Keywords:

Food analysis; Green chemistry; Lead (II) extraction; Edible oils; FAAS; sample preparation; analytical chemistry; food contaminants.

1. Introduction

Edible oils are the major contributors in daily food intake serving as a rich source of vitamin E and mono-unsaturated fatty acids for human nutrition including the repair of worn out tissues and new cells formation as well as a useful source of energy (Musa et al., 2012). Olive oil is present in Mediterranean diet as its important component. Its presence in the diet was correlated with lowering of the probability of several helth issues such as coronary heart diseases and certain kinds of cancer, such as colon and breast cancer (Zeiner et al., 2005). Regarding their use, vegetable oils are used in different areas, such as kitchen and food processing, cosmetics, pharmaceuticals, and chemical industries. The use of edible oil in foods is also associated with trace elements, especially heavy metals since these oils are coming from natural resources (e.g., vegetable oils). Due to the metabolic role of metals and the possibility of identifying oil adulteration, the determination of the toxic elements in edible oils is important (Dugo et al., 2004). The metal content in vegetable oils is one of the most important quality criteria. In foods, several organoleptic properties, such as taste, color, and smell, are negatively influenced by the high levels of metal ions. Lead is a toxic element and WHO has identified lead (II) as 1 out of the 10 chemicals of major public health concerns. U.S. Public Health Service established a daily permissible lead intake for children of 300 µg/day from all sources. Additionally, WHO recommended a tolerable intake for adults of 600 µg/day accumulative in all foods and drinks (Sharrett et al., 1982). Since lead accumulates inside human tissues, even its low concentration in foods is of great interest. Lead can also be deposited in the bones disturbing calcium metabolism. For instance, inorganic lead (II) causes behavioral effects, mortality, worsening of renal role, hypertension, reduced fertility and adversarial consequences of pregnancy, delay in sexual maturation, dental impairments of cognitive development and intelligence, among others. Also, lead exposure is considered one of the most serious and cumulative environmental pollutants (Yusof & Ahmad, 2002). The dispersion of lead in the environment takes place through air and water, as well as accumulated forms in food making it possible to reach human organisms and cause extensive toxicity (Klaassen et al., 1986). Inorganic lead (II) ions can binds with the -SH group in their proteins and act as an inhibitor of enzymes (Pourrut et al., 2011). Lead can also be deposited in the bones disturbing calcium metabolism (Kovacs & Ward, 2020).

The determination of trace elements in edible oils is important because the metabolic role of metals (Zeiner et al., 2005) as well as possibilities for adulteration detection and oil characterization. Considering all the related drawbacks to lead exposure, there is a strong need for determining its content in food systems. Herein, one of the “hot topics” in sample preparation, which fulfills the requirements of green chemistry, is related to the application of deep eutectic solvents (DESs). Recently, DESs are widely used for the extraction of hydrocarbons, aromatic compounds, and other biomolecules (Haq et al., 2021a; Makoś, Fernandes, et al., 2018). As reported elsewhere, DESs have similar features as ionic liquids; however, DESs have some major advantages over ionic liquids including simplicity, low cost of synthesis, easy biodegradation, non-toxicity, and less use of raw material (Fernandes et al., 2019; Haq et al., 2021a). While various inorganic nanoparticles used in analytical methods have opened new avenues for sensing, purification, and quantitative analysis. Therefore, the present work presents a synergistic effect of the interaction of nanoparticles, thus silver nanoparticles (Ag-NPs), combined with the advantages of DESs as green solvents for sample preparation. In this way, a very sensitive and simple micro-extraction method based on Ag-NPs and an ultrasound-assisted DES system was developed. The method was implemented in the quantitative analysis of lead content in edible oils using flame atomic absorption spectroscopy (FAAS). To finalize, the developed method is compared with other methods reported in the literature.

2. Materials and methods

2.1. Chemicals and reagents

Analytical grade reagents were used in this study. All chemicals (if not indicated differently) were purchased from Sigma Aldrich. A stock standard (1000 mg/L) of lead (II) was prepared from lead acetate, Regarding DES synthesis, choline chloride (ChCl), a quaternary ammonium salt, was used as a hydrogen bond acceptor (HBA), while phenol (Ph) was used as hydrogen bond donor (HBD). The Ag-NPs were synthesized using sodium tetrahydroborate ($NaBH_4$) and silver nitrate ($AgNO_3$). The aprotic solvent, such as tetrahydrofuran (THF), was purchased from Bangkok 10330, Thailand. Deionized water was obtained from HX 7000 SD M-Q Merck.

2.2. Instrumentation

Lead analysis was done through PerkinElmer AAnalyst 700 Model (Norwalk, CT, USA). A centrifuge (model 2206A, China) was used for phase separation. A pH meter with a glass electrode (Arwa AD 8000) was used for the pH adjustment of the sample. For the synthesis of Ag-NPs, an ultrasonic bath (power sonic 405, China) was used.

2.3. Preparation of silver nanoparticles

The preparation of Ag-NPs was performed based on the procedure described by (Peng et al., 2013). Briefly, a 100 mL solution of 0.1 mM of silver nitrate was prepared in a flat bottom flask and then vibrantly shaken for mixing. After this, sodium borohydride (0.012 g) was added to the solution. The resulting mixture was shaken for the next 30 minutes over a magnetic stirrer. The prepared mixture remained in static conditions (without mixing) overnight in a dark place. The resulting Ag-NPs suspension was stable for several months at ordinary temperatures without any color change or aggregation. This suspension containing Ag-NPs was further used for the extraction of lead (II) ions in edible oil. For each experiment, freshly prepared Ag-NPs were used.

2.4. Preparation of DESs and buffers

Different combinations of DESs at different molar ratios were prepared using ChCl, like an HBA compound. On the other hand, five types of HBD were tested, including malonic acid, ethylene glycol, glycol, phenol, and urea, assaying their maximum metal recovery. These HBDs were mixed with ChCl at molar ratios of 1:1, 1:2, and 1:3. All the DES solvents were prepared under identical conditions. In a 50 mL polypropylene tube, the components of DES were mixed and then heated at 60 ºC in a water bath for 5 minutes. The mixtures were stirred with vortex for 2 min to make homogeneous mixtures and thus obtaining DESs. For each experiment, freshly prepared DES solvents were prepared and directly used without any dilution or purification.

Buffer systems ranged from pH 2 to 10 were prepared. The buffer, having pH 2, was prepared by dissolving phosphoric acid (purity 85%) and 3.118 g of disodium hydrogen phosphate in 100 mL of deionized water. Similarly, the buffer, having pH 4, was prepared by dissolving 5.76 mL acetic acid and 1.54 g sodium acetate in 100 mL deionized water; while the buffer of pH 6 was prepared by dissolving 0.5 mL acetic acid (purity > 99%) and 11.7 g ammonium acetate in 100 mL deionized water. For buffer pH 8, 0.8 mL ammonium hydroxide was dissolved in 100 mL deionized water with 10.7 g ammonium chloride, and the buffer pH 10 was prepared by dissolving 20 mL deionized water, containing 4.5 g ammonium chloride, with 35 mL of 10 M ammonia solution in 30 mL deionized water. Both solutions were previously mixed and diluted up to 100 mL. 2 mL of buffer solution in the reagents mixture was found enough for maintaining a stable pH value.

2.5. Samples collection and preparation

Three different commercial brands of edible oil were selected as a benchmark for determining lead (II) concentration and validate the method. Here, sesame, olive, and canola oils were acquired from Dalda oil, ghee industries Pvt limited, and Seasons oils Pvt limited, respectively. Each sample was prepared independently in triplicate. For metal analysis, an oil sample (2.0 g) was preliminarily digested by adding 2 mL HNO_3 and 1 mL H_2O_2 in a quartz tube and subsequently heated at 120 ºC. When the sample was near to dry, an additional 2 mL HNO_3 and 1 mL H_2O_2 were added to the sample. Finally, the sample was diluted with deionized water making a final solution of 10mL. The resulting final solution was used for metal extraction.

2.6. Analytical procedure for determination of lead in edible oil samples

2 mL of metal standard solution (100 mg/L) was added to edible oil samples and digested. After dilution, the total sample volume was 15 mL. For maintaining the pH, a buffer solution sample (2 mL) was added to 1 mL of Ag-NPs (0.1 mM). The mixture was later stirred for 2 min and then 500µL of DES was added. The mixture was again stirred using a vortex for 1.5 min. Afterward, THF (500 µL) was added to the sample solution; and the mixture was placed in an ultrasonic bath for 1 min. The mixture was centrifuged at 3500 rpm for 2 min to obtain a complete phase separation. After centrifugation, the DES layer was separated and obtained at the bottom. Such extracted layer was diluted with ethanol up to 5 mL. Finally, HNO_3 was added to the solution with a final concentration of 0.1M to avoid coagulation. Scheme of the developed procedure is provided as graphic abstract figure.

2.7. Quantitative analysis and quality assurance

For quantitative analysis and quality assurance, different analytical parameters were determined including the limit of detection (LOD) and limit of quantification (LOQ). Such parameters were calculated following the JRC technical report on estimation of LOD and LOQ in foods (Wenzl et al., 2016).

The LOD and LOD were determined using the blank samples. In other words, native samples were used without spiking (Pseudo-blank). These samples were analyzed in ten replicates under repeatability conditions. The variability of signal values, expressed as standard deviation, was used for the estimation of LOD and LOQ. Both parameters were calculated, as below:

$$\mathrm{LOD} = 3 \times \frac{\mathrm{SD}}{\mathrm{m}} \qquad (1)$$

$$\mathrm{LOQ} = 10 \times \frac{\mathrm{SD}}{\mathrm{m}} \qquad (2)$$

where LOD and LOQ are the limits of detection and quantification, respectively; SD is the standard deviation of blank signals, and m is the slope of the calibration curve.

For determining the accuracy of this new analytical method, the procedure M 1-92 from American Oil Chemists' Society (AOCS) was followed, which is an updated procedure prepared in collaboration with the International Union of Pure and Applied Chemistry (IUPAC) and the International Organization for Standardization (ISO)(Liao et al., 2006).

Reliability and accuracy of the method, as the relative standard deviation (RSD), was determined by applying six replicate determinations of 5 µg/L of analyte to deionized water, in which the % RSD value of the recovery was found to be 4.5%.

The linearity of this new method was determined by adding a series of standards solutions from the stock solution. Here, a defined amount of blank with a final concentration ranging from 5 to

150 µg/L was used according to the expected working range. Triplicate samples for each concentration were analyzed.

3. Results and discussion

To introduce a green method for lead (II) extraction in edible oil, the suitable DES solvent was preliminarily selected. It is reported in the literature that DES solvents are extensively used for the extraction of biomolecules from natural products (Corrêa et al., 2021; Dwamena, 2019); however, very few methods are available using DESs for the extraction of toxic metals in more complex samples, such as edible oil. On the other hand, Ag-NPs are used for heavy metal uptake (Sumesh et al., 2011). In this work, Ag-NPs along with adsorbing metal was extracted through DES, which is a new approach to exploring new aspects of DES with Ag-NPs.

3.1. Selection of DES system

The extraction efficiency of the target analyte is mainly affected by the composition and the nature of DES. The DES used for extraction must meet several requirements, such as high affinity for analytes, liquid state at RT, no interference in analytical signal, different density than that of water, high stability, and low solubility in an aqueous medium (Haq et al., 2021a; Makoś, Przyjazny, et al., 2018). The DES system usually consists of two components, its 1st part consists of quaternary ammonium salt while the second part consists of HBD. The mole ratio of the quaternary ammonium salt and HBD has a significant impact on the applications of DES for metal extraction. Therefore, different DES systems with different molar ratios were tested in this work. ChCl was separately mixed with different types, including phenol, ethylene glycol, urea, glycerol, and malonic acid, using different mole ratios. At this point, the optimization of the HBD and HBA ratio is crucial for the applications of DES for extraction procedures. The effectiveness of extraction generally decreases with an increase in HBD in the resulting DES. On the other hand, mass transfer during extraction is increased by decreasing the density and viscosity of DES (Hou et al., 2017; Razi Asrami et al., 2020). To determine the optimum molar ratio DES, different HBD and HBA ratios were tested evaluating their maximum recovery. In this way, a high recovery value was obtained with ChCl and phenol with a mole ratio of 1:2, which was selected for further experiments. The results are illustrated in **Figure 1**.

Figure 1. Evaluation and selection of DES for maximum recovery of lead (II) ions. ChCl: Chlorine chloride, M: Malonic acid, EG: Ethylene glycol, G: Glycerol, Ph: Phenol, U: Urea.

Phenol is an aromatic compound and capable of delocalizing negative charges across its entire ring system. Considering its pK a value of 9.99, it is capable to form stronger hydrogen bonding than other alcohols. The calculated interaction energies for clusters with similar hydrogen-bonding patterns reveal that intermolecular interaction in phenol clusters is slightly stronger than in water clusters. However, the fusion of phenol and water clusters leads to similar stability to that of H_2O clusters (Parthasarathi et al., 2005). It is clear from the above mentioned facts, that phenol presents a strong hydrogen bonding and fulfill the requirments of HBD in DES formation. To evaluate the efficiency of different DESs, different HBDs were tested for Pb (II) extraction. DES based on phenol as HBD was found advantageous for the extraction of Pb (II) from edible oil samples.

3.2. Optimization of extraction parameters

3.2.1. Volume optimization of DESs

DES prepared with ChCl and phenol with molar ratio (1:2) was directly used without any further purification or dilution. Since DES directly influences the extraction of lead (II), its optimum concentration was determined for this specific procedure. Under the optimum condition, the DES volume effect was studied ranging from 250 µL to 1250 µL for the recovery of lead (II). The results indicate that, with a DES volume of 500 µL, a maximum recovery was obtained. Thus, 500 µL volume of DES was selected for onward studies. The results of this study are illustrated in Figure S1.

3.2.2. pH effect

In adsorption/chelate formation and continuous extraction, pH plays a significant role (Haq et al., 2021a). The solution pH is one of the parameters having a strong influence on the heavy-metal ion uptake; this is due to the fact that the surface charge density of the adsorbent and the metallic species depend on the pH (Haq et al., 2021b; Kubilay et al., 2007). Here, the lead (II) recoveries were examined in the range of pH 2 to pH 8. The results revealed that a maximum recovery for lead (II) was obtained at pH 2, as shown in Figure S2, indicating that a regular drop in the recovery of lead (II) was found with a further increase after pH 2. Therefore, pH 2 was selected for further studies.

At higher pH, Ag-NPs are more stable but less capable of metal adsorption, while at lower pH, Ag-NPs are less stable but more capable of metal adsorption (Hosseini et al., 2016; Molleman & Hiemstra, 2017). As the pH increases, the prolongation of the adsorbent surface decreased, leading to a reduction in the electrostatic attraction between the metal ions species and the adsorbent surface (Fernando & Zhou, 2019; Molleman & Hiemstra, 2017); this results in a consequent decrease in the percentage removal of metal ions.

3.2.3. Optimization of THF addition

THF is an aprotic solvent, which was used in these studies to make phase separation easier. The outcomes of this paper revealed that, while it indeed enhances the phase separation, it also increases the % recovery of the analyte. These aprotic solvents tend to interact more with water than DES. After interaction with THF molecules, water molecules decline their interaction with DES, resulting in their self-aggregation and separation. In other words, THF acts as a dehydrating agent. The most plausible mechanism of DESs self-aggregation involves π–π overlap between the aromatic ring of phenol, followed by hydrogen bonding between functional groups of DES and other charge transfer interactions (Haq et al., 2021a; Khezeli et al., 2015). The volume effect of THF ranged from 250 µL to 1500µL was studied for the % recovery of lead (II) (see Figure S3), where the maximum recovery was obtained with a THF volume of 500 µL. For further studies, a THF volume of 500 µL was carefully used as an optimum value.

3.2.4. Effect of Ag-NPs concentration

For the separation of different metals, Ag-NPs have been used as a good adsorbent in the field of research. Here, the excess of Ag-NPs is needed to provide maximum surface area for metal adsorption. The mechanism of metal ion interaction with Ag-NPs is described and detailed in section 3.3. In this study, the effect of the amount of Ag-NPs (0.1mM) was studied in the range of 250 µL to 2000 µL. The results showed that the lead (II) signal response was increasing with the increase of the amount of Ag-NPs up to 500 µL and it remained constant. Therefore, 500 µL of Ag-NPs has been selected as the optimum value for lead (II) determination. The effect of Ag-NPs on lead (II) recovery is illustrated in figure S4.

3.2.5. Effect of ultra-sonication time

The efficiency of the extraction process was greatly affected by ultra-sonication time. Our study suggests that both THF amount and ultra-sonication waves caused the aggregation of the DES in the aqueous phase and the reduction in extraction time. In general, it was noticed that the conversion of these droplets of DES into tiny droplets contributed to reaching the equilibrium state, resulting in increased extraction efficiency. At constant experimental conditions, the effect of ultra-sonication time was studied from 1 to 5 min at 25 °C (see Figure S5). When the ultra-sonication time was extended to 2 min, the extraction efficiency was stabilized. Therefore, 2 minutes of ultra-sonication time was selected for the subsequent experiments.

3.3. Development of quantitative method/validation

3.3.1. Interference study of different ions

To analyze lead content in food and environmental samples, an important issue relates to the presence of interfering ions which could affect to some extent the analytical signal. The goal during the development of this new method was to select the selective extraction conditions of (Ooi & Ng, 2018), back extraction methods (Arpa & Aridaşir, 2018), solid-phase extraction (Rahnama & Ghadiri, 2015), membrane techniques (Mesli & Belkhouche, 2018), adsorption methods (Mahmoud et al., 2010), flow-injection on-line adsorption(Salonia et al., 1999) and co-precipitation methods (Komjarova & Blust, 2006). The performance of the developed DES impregnated Ag-NPs method was compared with liquid-phase microextraction methods, which were used for lead (II) determination in edible oil, as shown in **Table 1**. Among all the reported methods, cloud point extraction is the most dominantly used technique for lead extraction due to its low cost, high enrichment factor, environment-friendly and minimal cost (Babaee et al., 2019). However, these methods are associated with some critical issues, such as a long time of the procedure related to heating and centrifugation stages. Even though these methods achieve good recovery after multi-cycle experiments (Galbeiro et al., 2014), while our proposed method is quicker and provides a very good recovery obtained in a single cycle. The time for the extraction procedure after digestion was estimated for the different reported methods in the literature, which is compiled in **Table 1**. The estimated time for our extraction procedure was as short as 6.5 minutes while estimated time values for other methods were 35 min (Coelho et al., 2008), 87 min (Blanchet-Chouinard & Larivière, 2018), 217 min (Gouda & Zordok, 2018), 35 min (Citak & Tuzen, 2012), 60 min (Kazi et al., 2012), 16 min (Rahnama & Ghadiri, 2015) and 40min (Citak & Tuzen, 2010). At this point, it is noted that the newly proposed method is many folds faster than the already reported methods. Application of AG-NPs with DES as extractant with addition of THF provides further improvement also in the field of lead extraction based on DESs in comparison to already published approaches (Karmini et al. 2016; Soylak & Koksal, 2019; Habila et al. 2020.). Preliminary studies for this paper indicated that in case of sole use of DES about 30 minutes of extraction is needed for acceptable recovery. Nanoparticle scale silver reveals to provide rapid extraction. It follows from very fast kinetics of sorption. The performance of this method is quite good due to its low LOD and LOQ values and high pre-concentration factor. This method opens a new aspect in the application of DES coupled with Ag-NPs for lead extraction.

Table 1. Comparative study of the new method with reported methods in the literature.

3.3.4. Application of the method for the analysis of real samples

Since edible oils are an essential part of the human diet, the developed procedure was applied to the analysis of several categories of edible oils, such as sesame, olive, and canola oil obtained from commercial brands of the district Mardan, Pakistan. In this approach, the standard addition method, which is commonly used for AAS, was used. Known concentrations of lead (II) were gradually added to real samples and percent recovery was determined in each case. The results of the recovery performance are presented in **Table 2**. The recovery for real samples was between 97-107%. Since the concentration in real samples was revealed to be much higher than the limit established by this method, the samples were diluted with methanol right after extraction. In these experiments, three different types of edible oils were tested for lead (II) concentration, exhibiting a lead (II) concentration of 1.21 mg/L, 1.26 mg/L and1.19 mg/L for olive, sesame, and canola oil, respectively. This method was sensitive and valid for determining very low concentrations. To summarize, it can be stated that the performed experiments confirmed that the developed procedure is reliable for the determination of lead (II) in different commercial edible oil samples.

lead from the matrix, which was fully obtained making this method highly selective. DESs are highly selective in extraction procedures (Rad et al., 2019); however, in this case, preliminary studies revealed that additional selectivity must be provided. Thus, the application of Ag-NPs was further studied to assure adequate accuracy of the quantitative analysis; for this, a standard addition method was used.

To determine the effect of external ions on lead (II) extraction, known concentrations of different ions were added in separate sets of the experiment. The interference of Na^+($NaNO_3$), K^+(KCl), Mg^{2+}($Mg(NO_3)_2$), Cd^{2+}($CdCl_2$), Zn^{2+}($ZnNO_3)_2$, Co^{2+}($CoCl_2$), Ni^{2+}($NiCl_2$), SO_4^{2-}(K_2SO_4),

$Pb^{2+}(PbCl_2)$ was investigated. The recovery of the lead (II) in presence of interfering ions was found to be higher than 90%. These results are compiled in supporting information (Table S1). Importantly, it was proved that the interfering ions have no significant effect on the lead (II) extraction/analysis and the proposed microextraction method fits the purpose.

3.3.2. Analytical performance of the method

According to the methodology described in section 2.7, LOD and LOQ values were calculated as 0.28 and 0.94 µg/L, respectively. **Figure 2** shows the calibration curve, which has an equation as follows: $Y= 0.0037x + 0.0026$, displaying an acceptable coefficient of determination (R^2) of 0.9931. The linearity range was established from 5 to 140 µg/L. The relative standard deviation calculated for six repetitions of lead (II) analysis (at a concentration level of 5 µg/L) was 4.5%, showing the repeatability and reproducibility of the developed method.

Figure 2. Calibration curve of lead (II) at different concentrations.

3.3.3. Comparison with other methods

Many methods have been reported in the literature for lead extraction and analysis, including cloud point extraction (Blanchet-Chouinard & Larivière, 2018), liquid-liquid microextraction

Table 2. Determination of lead in edible oil samples.

Several papers recently reported presence of lead ions in oil samples. For example, it was reported as lead (II) concentrations of 0.06-0.21 mg/Kg, 0.08-1.12 mg/Kg, and 0.06-0.08 mg/Kg for rapeseed, soybean, and linseed oil, respectively (Szyczewski et al., 2016). In other study lead (II) concentrations of 6-15 µg/Kg, 7.3-21 µg/Kg, and 3.4-16 µg/Kg were reported for canola, corn, and soybean oil, respectively (Allen et al., 1998). In vegetable hydrogenated oil, a lead concentration of 15.92 µg/L was reported by (Abbasi et al., 2009), while (Ng, 2010) documented 0.71 mg/Kg of lead in black olive oil and 0.75 mg/Kg in green olive oil. In more recent work, (Zhuravlev et al., 2015) evaluated and determined a lead concentration in different types of oil, e.g., 0.006 mg/Kg in corn oil, 0.014 mg/Kg in olive oil, 0.016 mg/Kg in refined sunflower oil, 0.062 mg/Kg sunflower oil, and 0.027 mg/Kg in soybean oil. It can be seen that the range of reported values strongly differs in the type and origin of oil samples. It is worth mentioning that this developed method covers the entire range of expected levels of lead concentration from low parts per billion levels to higher ones after dilution. The modern instruments are equipped with an automatic module for sample dilution; therefore, this approach fully fits the purpose of lead determination in edible oils.

4. Conclusions

In this new method, lead (II) ions were efficiently extracted and determined from different commercial edible oil products. Deep eutectic solvents based on ChCl and phenol with a 1:2 molar ratio demonstrated a maximum recovery for lead (II) extraction. It was concluded that Ag nano particles can adsorb Pb (II) and facilitate the Pb (II) mobility from aqueouse phase to DES phase. This stage is very fast, as nanoparticles ensure very good kinetics of sorption. The sonication process accelerates the formation of nano-sized fine droplets and as a result increases the contact surface area between the extracting solvent (DES) and Pb(II). The optimized method implies simplicity, ease of operation, short extraction times, and high enrichment factor. Quantitative recovery (97-105%) from spiked samples demonstrated the suitability of the optimized method for the quality control of the analyzed samples. The LOD and LOQ were 0.28 µg/L and 0.94 µg/L, respectively with an RSD value of 4.5% and a pre-concentration factor of 25 which are comparatively improved comparing other methods reported in the literature. The optimized procedure is green, simple and requires a small volume of extraction solvent. The comparison of the developed method with already-existing analytical procedures confirmed its advantages, such as shortening the time of analysis and sensitivity. To finalize, the outcomes of this new assay are comparable to those obtained by the ICP-MS technique with more simplicity, better accuracy, high enrichment factor, less time-consuming, and environment-friendly. Further improvements in method development, also in green chemistry aspects, should focus on minization of the scale of procedure. This studies confirmed that the optimized procedure is perfectly useful for routine analysis of food especialy edible oil samples containing traces of Pb(II) ions.

Acknowledgment

Prof. Grzegorz Boczkaj and Hameed Ul Haq gratefully acknowledge the financial support from the National Science Centre, Warsaw, Poland – decision no. UMO-2018/30/E/ST8/ 00642.

Prof. Muhammad Balal Arain and Hameed Ul Haq are thankful to the PAK-US Science and Technology coopration program No.6-4/PAK-US/HEC/2015/04 for financial aid and AWKUM Mardan, Pakistan for providing us the conveniences and opportunity. R. Castro-Muñoz acknowledges the financial support from Polish National Agency for Academic Exchange (NAWA) under Ulam Programme (Agreement No. PPN/ULM/2020/1/00005/U/00001).

Conflict of interest

The authors declare no conflict of interest.

References:

Abbasi, S., Allahyari, M., Taherimaslak, Z., Nematollahi, D., & Abbasi, F. (2009). New determination of lead in edible oil and water samples by high selective adsorptive stripping voltammetry with SPADNS. *International Journal of Electrochemical Science*, *4*, 602-613.

Allen, L. B., Siitonen, P. H., & Thompson Jr, H. C. (1998). Determination of copper, lead, and nickel in edible oils by plasma and furnace atomic spectroscopies. *Journal of the American Oil Chemists' Society*, *75*(4), 477-481.

Arpa, Ç., & Aridaşir, I. (2018). A Method to Determination of Lead Ions in Aqueous Samples: Ultrasound-Assisted Dispersive Liquid-Liquid Microextraction Method Based on Solidification of Floating Organic Drop and Back-Extraction Followed by FAAS. *Journal of analytical methods in chemistry*, *2018*.

Babaee, S., Hosseini, S. G., & Mirzaei, M. (2019). Rapid and selective extraction of trace amount of Pb (II) in aqueous samples using a magnetic ion-imprinted polymer and detection by flame atomic absorption spectrometry. *Eclética Química Journal*, *44*(4), 73-84.

Blanchet-Chouinard, G., & Larivière, D. (2018). Determination of Pb in environmental samples after cloud point extraction using crown ether. *Talanta*, *179*, 300-306.

Citak, D., & Tuzen, M. (2010). A novel preconcentration procedure using cloud point extraction for determination of lead, cobalt and copper in water and food samples using flame atomic absorption spectrometry. *Food and Chemical toxicology*, *48*(5), 1399-1404.

Citak, D., & Tuzen, M. (2012). Cloud point extraction of copper, lead, cadmium, and iron using 2, 6-diamino-4-phenyl-1, 3, 5-triazine and nonionic surfactant, and their flame atomic absorption spectrometric determination in water and canned food samples. *Journal of AOAC International*, *95*(4), 1170-1175.

Coelho, L. M., Bezerra, M. A., Arruda, M. A. Z., Bruns, R. E., & Ferreira, S. L. C. (2008). Determination of Cd, Cu, and Pb after cloud point extraction using multielemental sequential determination by thermospray flame furnace atomic absorption spectrometry (TS-FF-AAS). *Separation Science and Technology*, *43*(4), 815-827.

Corrêa, P. S., Morais Júnior, W. G., Martins, A. A., Caetano, N. S., & Mata, T. M. (2021). Microalgae biomolecules: Extraction, separation and purification methods. *Processes*, *9*(1), 10.

Dugo, G., La Pera, L., La Torre, G. L., & Giuffrida, D. (2004). Determination of Cd (II), Cu (II), Pb (II), and Zn (II) content in commercial vegetable oils using derivative potentiometric stripping analysis. *Food chemistry*, *87*(4), 639-645.

Dwamena, A. K. (2019). Recent advances in hydrophobic deep eutectic solvents for extraction. *Separations*, *6*(1), 9.

Fernandes, A., Makoś, P., Khan, J. A., & Boczkaj, G. (2019). Pilot scale degradation study of 16 selected volatile organic compounds by hydroxyl and sulfate radical based advanced oxidation processes. *Journal of cleaner production*, *208*, 54-64.

Fernando, I., & Zhou, Y. (2019). Impact of pH on the stability, dissolution and aggregation kinetics of silver nanoparticles. *Chemosphere*, *216*, 297-305.

Galbeiro, R., Garcia, S., & Gaubeur, I. (2014). A green and efficient procedure for the preconcentration and determination of cadmium, nickel and zinc from freshwater, hemodialysis solutions and tuna fish samples by cloud point extraction and flame atomic absorption spectrometry. *Journal of Trace Elements in Medicine and Biology*, *28*(2), 160-165.

Gouda, A. A., & Zordok, W. A. (2018). Solid-phase extraction method for preconcentration of cadmium and lead in environmental samples using multiwalled carbon nanotubes. *Turkish Journal of Chemistry*, *42*(4), 1018-1031.

Haq, H. U., Balal, M., Castro-Muñoz, R., Hussain, Z., Safi, F., Ullah, S., & Boczkaj, G. (2021a). Deep eutectic solvents based assay for extraction and determination of zinc in fish and eel samples using FAAS. *Journal of Molecular Liquids*, 115930.

Haq, H. U., Balal, M., Castro-Muñoz, R., Hussain, Z., Safi, F., Ullah, S., & Boczkaj, G. (2021b). Deep eutectic solvents based assay for extraction and determination of zinc in fish and eel samples using FAAS. *Journal of Molecular Liquids*, *333*, 115930.

Hosseini, S., Aghaie, H., Ghaedi, M., Dashtian, K., & Purkait, M. (2016). Lead (II) adsorption from aqueous solutions onto modified ag nanoparticles: Modeling and optimization. *Environmental Progress & Sustainable Energy*, *35*(3), 743-749.

Hou, Y., Kong, J., Ren, Y., Ren, S., & Wu, W. (2017). Mass transfer dynamics in the separation of phenol from model oil with quaternary ammonium salts via forming deep eutectic solvents. *Separation and Purification Technology*, *174*, 554-560.

Kazi, T. G., Shah, F., Afridi, H. I., Khan, S., Arian, S. S., & Brahman, K. D. (2012). A green preconcentration method for determination of cobalt and lead in fresh surface and waste water samples prior to flame atomic absorption spectrometry. *Journal of analytical methods in chemistry*, *2012*.

Khezeli, T., Daneshfar, A., & Sahraei, R. (2015). Emulsification liquid–liquid microextraction based on deep eutectic solvent: an extraction method for the determination of benzene, toluene, ethylbenzene and seven polycyclic aromatic hydrocarbons from water samples. *Journal of Chromatography A*, *1425*, 25-33.

Klaassen, C. D., Casarett, L. J., & Amdur, M. O. (1986). *Toxicology: the basic science of poisons*. Macmillan.

Komjarova, I., & Blust, R. (2006). Comparison of liquid–liquid extraction, solid-phase extraction and co-precipitation preconcentration methods for the determination of cadmium, copper, nickel, lead and zinc in seawater. *Analytica chimica acta*, *576*(2), 221-228.

Kovacs, C. S., & Ward, L. M. (2020). Physiology of calcium, phosphorus, and bone metabolism during fetal and neonatal development. In *Maternal-Fetal and Neonatal Endocrinology* (pp. 573-586). Elsevier.

Kubilay, Ş., Gürkan, R., Savran, A., & Şahan, T. (2007). Removal of Cu (II), Zn (II) and Co (II) ions from aqueous solutions by adsorption onto natural bentonite. *Adsorption*, *13*(1), 41-51.

Liao, J. J., Capen, R. C., & Schofield, T. L. (2006). Assessing the reproducibility of an analytical method. *Journal of chromatographic science*, *44*(3), 119-122.

Mahmoud, M. E., Osman, M. M., Hafez, O. F., Hegazi, A. H., & Elmelegy, E. (2010). Removal and preconcentration of lead (II) and other heavy metals from water by alumina adsorbents developed by surface-adsorbed-dithizone. *Desalination*, *251*(1-3), 123-130.

Makoś, P., Fernandes, A., Przyjazny, A., & Boczkaj, G. (2018). Sample preparation procedure using extraction and derivatization of carboxylic acids from aqueous samples by means of deep eutectic solvents for gas chromatographic-mass spectrometric analysis. *Journal of Chromatography A*, *1555*, 10-19.

Makoś, P., Przyjazny, A., & Boczkaj, G. (2018). Hydrophobic deep eutectic solvents as “green” extraction media for polycyclic aromatic hydrocarbons in aqueous samples. *Journal of Chromatography A*, *1570*, 28-37.

Mesli, M., & Belkhouche, N.-E. (2018). Extraction and pre-concentration of lead from copper by emulsion liquid membrane technique using an ionic liquid. *Euro-Mediterranean Journal for Environmental Integration*, *3*(1), 14.

Molleman, B., & Hiemstra, T. (2017). Time, pH, and size dependency of silver nanoparticle dissolution: the road to equilibrium. *Environmental Science: Nano*, *4*(6), 1314-1327.

Musa, M., Sulaiman, A., Bello, I., Itumoh, J., Bello, K., Bello, A., & Arzika, A. (2012). Physicochemical properties of some commercial groundnut oil products sold in Sokoto metropolis. Northwest Nigeria. *Journal of Biological Science and Bioconservation*, *4*, 17-24.

Ng, K. L. (2010). *Determination of lead, cadmium, zinc and nickel in edible oil products by stripping voltammetry/Ng Khan Loon* University of Malaya].

Ooi, E., & Ng, Y. S. (2018). Removal of Lead from Water by Liquid-Liquid Extraction using trihexyltetradecylphosphonium Chloride in Different Diluents. E3S Web of Conferences,

Parthasarathi, R., Subramanian, V., & Sathyamurthy, N. (2005). Hydrogen bonding in phenol, water, and phenol− water clusters. *The Journal of Physical Chemistry A*, *109*(5), 843-850.

Peng, S., Li, L., Tan, H., Wu, Y., Cai, R., Yu, H., . . . Srinivasan, M. (2013). Monodispersed Ag nanoparticles loaded on the PVP-assisted synthetic Bi 2 O 2 CO 3 microspheres with enhanced photocatalytic and supercapacitive performances. *Journal of Materials Chemistry A*, *1*(26), 7630-7638.

Pourrut, B., Shahid, M., Dumat, C., Winterton, P., & Pinelli, E. (2011). Lead uptake, toxicity, and detoxification in plants. *Reviews of environmental contamination and toxicology volume 213*, 113-136.

Rad, A. S., Rahnama, R., Zakeri, M., & Jamali, M. R. (2019). Dispersive liquid–liquid microextraction based on green type solvents—" deep eutectic solvents"—for highly selective separation and efficient preconcentration of nickel in water samples. *Journal of the Iranian Chemical Society*, *16*(8), 1715-1722.

Rahnama, R., & Ghadiri, R. (2015). Separation and preconcentration of trace amounts of lead from water samples using solvent-assisted dispersive solid phase extraction. *Journal of the Brazilian Chemical Society*, *26*(8), 1642-1647.

Razi Asrami, M., Tran, N. N., Saien, J., & Hessel, V. (2020). Mass Transfer Characterization of Ionic Liquid Solvents for Extracting Phenol from Aqueous Phase in a Microscale Coiled Flow Inverter. *Industrial & Engineering Chemistry Research*, *59*(37), 16427-16436.

Salonia, J., Wuilloud, R., Gáquez, J., Olsina, R., & Martinez, L. (1999). Determination of lead in tap water by ICP-AES with flow-injection on-line adsorption preconcentration using a knotted reactor and ultrasonic nebulization. *Journal of Analytical Atomic Spectrometry*, *14*(8), 1239-1243.

Sharrett, A. R., Carter, A. P., Orheimt, R. M., & Feinleib, M. (1982). Daily intake of lead, cadmium, copper, and zinc from drinking water: the Seattle study of trace metal exposure. *Environmental research*, *28*(2), 456-475.

Sumesh, E., Bootharaju, M., & Pradeep, T. (2011). A practical silver nanoparticle-based adsorbent for the removal of Hg2+ from water. *Journal of hazardous materials*, *189*(1-2), 450-457.

Szyczewski, P., Frankowski, M., Zioła-Frankowska, A., Siepak, J., Szyczewski, T., & Piotrowski, P. (2016). A comparative study of the content of heavy metals in oils: linseed oil, rapeseed oil and soybean oil in technological production processes. *Archives of Environmental Protection*, *42*(3), 37-40.

Wenzl, T., Haedrich, J., Schaechtele, A., Piotr, R., Stroka, J., Eppe, G., & Scholl, G. (2016). *Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Food and Feed*.

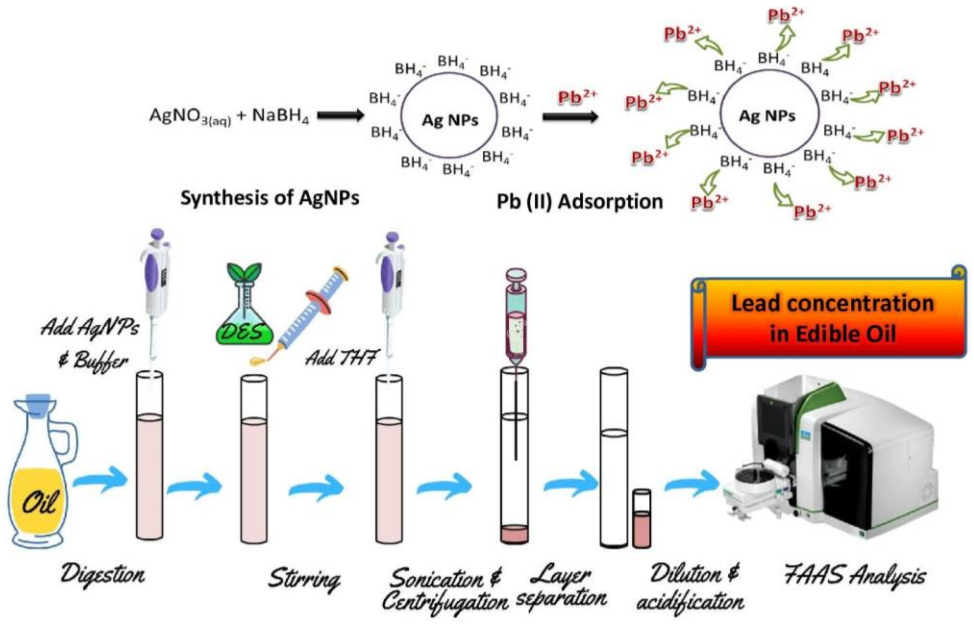
Yusof, N. A., & Ahmad, M. (2002). A flow cell optosensor for lead based on immobilized gallocynin in chitosan membrane. *Talanta*, *58*(3), 459-466.

Zeiner, M., Steffan, I., & Cindric, I. J. (2005). Determination of trace elements in olive oil by ICP-AES and ETA-AAS: A pilot study on the geographical characterization. *Microchemical Journal*, *81*(2), 171-176.

Zhuravlev, A., Zacharia, A., Gucer, S., Chebotarev, A., Arabadji, M., & Dobrynin, A. (2015). Direct atomic absorption spectrometry determination of arsenic, cadmium, copper, manganese, lead and zinc in vegetable oil and fat samples with graphite filter furnace atomizer. *Journal of Food Composition and Analysis*, *38*, 62-68.

Graphic abstract

New version:



Figures

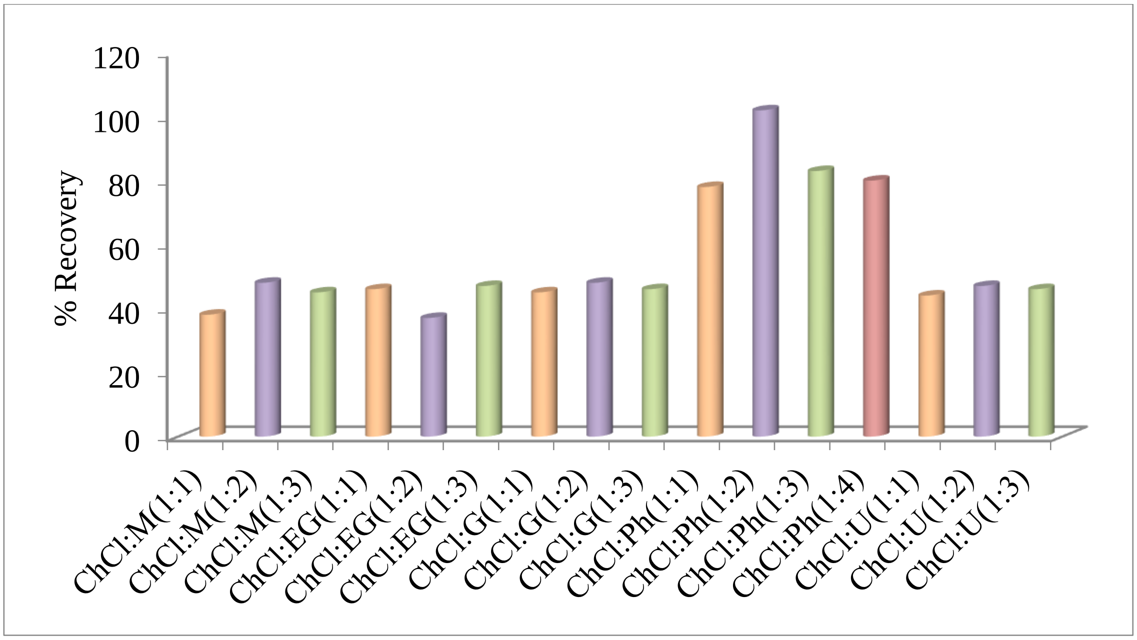


Figure 1. Evaluation and selection of DES for maximum recovery of lead (II) ions. ChCl: Chlorine chloride, M: Malonic acid, EG: Ethylene glycol, G: Glycerol, Ph: Phenol, U: Urea.

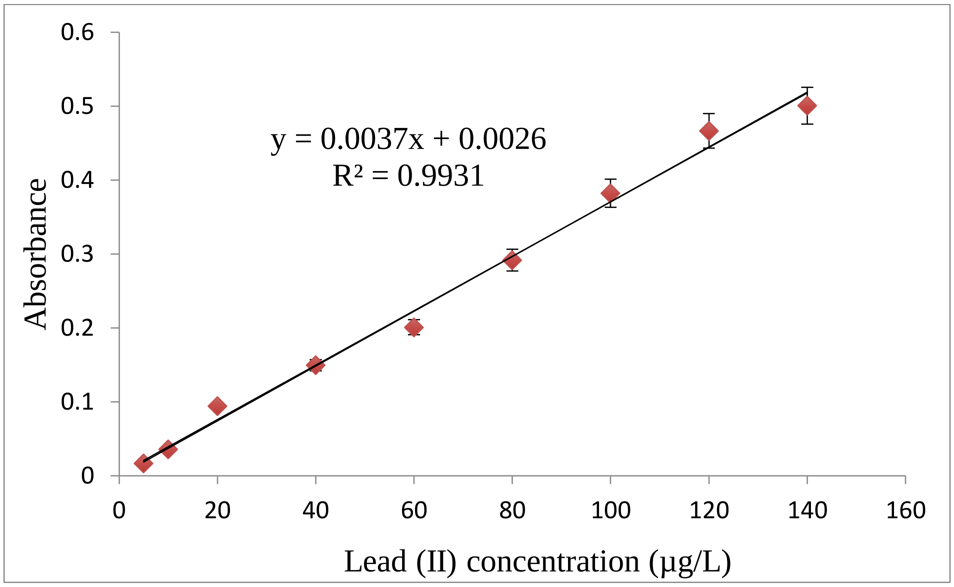


Figure 2. Calibration curve of lead (II) at different concentrations.

Tables

Table 1. Comparative study of the new method with reported methods in the literature.

Method	Detection techniques	LOD (µg/L)	LOQ (µg/L)	Linearity (µg/L)	RSD (%)	Estimated time of sample preparation* (minutes)	References
Cloud point extraction	TS-FF-AAS	0.43	1.44	5-50	8.7	>35	(Coelho et al., 2008)
Cloud point extraction	ICP-OES	0.8	2.60	---	13	>67	(Blanchet-Chouinard & Larivière, 2018)
Solid phase extraction	FAAS	0.2	1	1-60	3.2	>180	(Gouda & Zordok, 2018)
Cloud point extraction	FAAS	1.33	6.65	20-320	3.06	>35	(Citak & Tuzen, 2012)
Cloud point extraction	FAAS	0.26	0.86	20-100	1.88	>60	(Kazi et al., 2012)
SADSPE	FAAS	1.3	4.30	4-100	5	>21	(Rahnama & Ghadiri, 2015)
Cloud point extraction	FAAS	3.42	11.31	5-10	4.8	>45	(Citak & Tuzen, 2010)
DES-based extraction	FAAS	2.4	7.9	5-60	0.9-4.3	37	(Soylak & Koksal, 2019)
DES-based extraction with Fe_3O_4 sorbent	FAAS	0.4	2	2-250	1.8	>75	(Karmini et al. 2016)
DES-based extraction with Ag-NPs	FAAS	0.28	0.92	5-140	4.5	6.5	*Present work*

*after digestion

SADSPE; Solvent-assisted dispersive solid-phase extraction, FAAS; Flame atomic absorption spectroscopy, ICP-OES; Inductively coupled plasma optical emission spectroscopy, TS-FF-AAS; Thermo spray flame furnace atomic absorption, DES; Deep eutectic solvent

Table 2. Determination of lead (II) in edible oil samples.

Samples	Lead spiked (µg/mL)	Lead found (µg/mL)	% Recovery
Olive oil	--	1.21± 0.01	-------
	2.5	3.90± 0.02	105.12
	5.0	6.64± 0.02	106.92
Sesame oil	--	1.26± 0.03	-------
	2.5	3.65± 0.017	97.07
	5.0	6.11± 0.04	97.60
Canola oil	--	1.19± 0.01	-------
	2.5	3.62± 0.01	98.10
	5.0	6.19± 0.04	100.00

Deep eutectic solvent (DES) with silver nanoparticles (Ag-NPs) based assay for analysis of lead (II) in edible oils

Hameed Ul Haq[1], Rafiat Bibi[2], Muhammad Balal Arain[3], Faisal Safi[2], Sana Ullah[2], Roberto Castro-Muñoz[1,4], Grzegorz Boczkaj[1,5,*]

[1]*Gdansk University of Technology, Faculty of Chemistry, Department of Process Engineering and Chemical Technology, 80 – 233 Gdansk, G. Narutowicza St. 11/12, Poland. E-mail: grzegorz.boczkaj@pg.edu.pl / hameed.haq@pg.edu.pl

[2]Department of Chemistry, Abdul Wali Khan University, Mardan, 23200, Pakistan. E-mail: hulhaqchem@gmail.com / rafiatmohmand@hotmail.com / faisalsafi1993@gmail.com

[3]Department of Chemistry, University of Karachi, Karachi, Sindh, 75270 Pakistan. E-mail: bilal_ku2004@yahoo.com

[4]Tecnologico de Monterrey, Campus Toluca, Avenida Eduardo Monroy Cárdenas 2000 San Antonio Buenavista, 50110, Toluca de Lerdo, Mexico. Email: food.biotechnology88@gmail.com

[5]EkoTechCenter, Gdansk University of Technology, G.Narutowicza St. 11/12, 80-233 Gdansk, Poland

Corresponding author: Dr. Grzegorz Boczkaj, Assoc. Prof., PhD. Sc. Eng. Gdansk University of Technology, Faculty of Chemistry, Department of Process Engineering and Chemical Technology, 80 – 233 Gdansk, G. Narutowicza St. 11/12, Poland. Fax: (+48 58) 347-26-94; Tel: (+48) 697970303; e-mail: grzegorz.boczkaj@gmail.com or grzegorz.boczkaj@pg.edu.pl

Abstract

This paper presents an application of silver nanoparticles impregnated by Deep Eutectic Solvents (DES) as ultrasonication aided microextraction system for lead (II) determination in edible oils. The paper presents a systematic optimization of method parameters and examples of its application for analysis of real samples. Maximum recovery for lead (II) extraction was obtained for choline chloride and phenol with a 1:2 molar ratio. Optimum extraction conditions for 2g oil sample post-digested solution (10 mL, pH=2) require 1mL of Ag-nanoparticles solution (0,1mM) and per each 500 µL of DES and tetrahydrofurane. The limit of detection (LOD) and quantification (LOQ) were 0.28 µg/L and 0.94 µg/L. The developed method covers the entire range of expected levels of lead concentration in oil samples -parts per billion levels to higher ones. This method is many folds faster (only 6.5 minutes/sample are needed) as well as more sensitive comparing to already reported methods.

Keywords:

Food analysis; Green chemistry; Lead (II) extraction; Edible oils; FAAS; sample preparation; analytical chemistry; food contaminants.

1. Introduction

Edible oils are the major contributors in daily food intake serving as a rich source of vitamin E and mono-unsaturated fatty acids for human nutrition including the repair of worn out tissues and new cells formation as well as a useful source of energy (Musa et al., 2012). Olive oil is present in Mediterranean diet as its important component. Its presence in the diet was correlated with lowering of the probability of several helth issues such as coronary heart diseases and certain kinds of cancer, such as colon and breast cancer (Zeiner et al., 2005). Regarding their use, vegetable oils are used in different areas, such as kitchen and food processing, cosmetics, pharmaceuticals, and chemical industries. The use of edible oil in foods is also associated with trace elements, especially heavy metals since these oils are coming from natural resources (e.g., vegetable oils). Due to the metabolic role of metals and the possibility of identifying oil adulteration, the determination of the toxic elements in edible oils is important (Dugo et al., 2004). The metal content in vegetable oils is one of the most important quality criteria. In foods, several organoleptic properties, such as taste, color, and smell, are negatively influenced by the high levels of metal ions. Lead is a toxic element and WHO has identified lead (II) as 1 out of the 10 chemicals of major public health concerns. U.S. Public Health Service established a daily permissible lead intake for children of 300 µg/day from all sources. Additionally, WHO recommended a tolerable intake for adults of 600 µg/day accumulative in all foods and drinks (Sharrett et al., 1982). Since lead accumulates inside human tissues, even its low concentration in foods is of great interest. Lead can also be deposited in the bones disturbing calcium metabolism. For instance, inorganic lead (II) causes behavioral effects, mortality, worsening of renal role, hypertension, reduced fertility and adversarial consequences of pregnancy, delay in sexual maturation, dental impairments of cognitive development and intelligence, among others. Also, lead exposure is considered one of the most serious and cumulative environmental pollutants (Yusof & Ahmad, 2002). The dispersion of lead in the environment takes place through air and water, as well as accumulated forms in food making it possible to reach human organisms and cause extensive toxicity (Klaassen et al., 1986). Inorganic lead (II) ions can binds with the -SH group in their proteins and act as an inhibitor of enzymes (Pourrut et al., 2011). Lead can also be deposited in the bones disturbing calcium metabolism (Kovacs & Ward, 2020).

The determination of trace elements in edible oils is important because the metabolic role of metals (Zeiner et al., 2005) as well as possibilities for adulteration detection and oil characterization. Considering all the related drawbacks to lead exposure, there is a strong need for determining its content in food systems. Herein, one of the "hot topics" in sample preparation, which fulfills the requirements of green chemistry, is related to the application of deep eutectic solvents (DESs). Recently, DESs are widely used for the extraction of hydrocarbons, aromatic compounds, and other biomolecules (Haq et al., 2021a; Makoś, Fernandes, et al., 2018). As reported elsewhere, DESs have similar features as ionic liquids; however, DESs have some major advantages over ionic liquids including simplicity, low cost of synthesis, easy biodegradation, non-toxicity, and less use of raw material (Fernandes et al., 2019; Haq et al., 2021a). While various inorganic nanoparticles used in analytical methods have opened new avenues for sensing, purification, and quantitative analysis. Therefore, the present work presents a synergistic effect of the interaction of nanoparticles, thus silver nanoparticles (Ag-NPs), combined with the advantages of DESs as green solvents for sample preparation. In this way, a very sensitive and simple micro-extraction method based on Ag-NPs and an ultrasound-assisted DES system was developed. The method was implemented in the quantitative analysis of lead content in edible oils using flame atomic absorption spectroscopy (FAAS). To finalize, the developed method is compared with other methods reported in the literature.

2. Materials and methods

2.1. Chemicals and reagents

Analytical grade reagents were used in this study. All chemicals (if not indicated differently) were purchased from Sigma Aldrich. A stock standard (1000 mg/L) of lead (II) was prepared from lead acetate, Regarding DES synthesis, choline chloride (ChCl), a quaternary ammonium salt, was used as a hydrogen bond acceptor (HBA), while phenol (Ph) was used as hydrogen bond donor (HBD). The Ag-NPs were synthesized using sodium tetrahydroborate ($NaBH_4$) and silver nitrate ($AgNO_3$). The aprotic solvent, such as tetrahydrofuran (THF), was purchased from Bangkok 10330, Thailand. Deionized water was obtained from HX 7000 SD M-Q Merck.

2.2. Instrumentation

Lead analysis was done through PerkinElmer AAnalyst 700 Model (Norwalk, CT, USA). A centrifuge (model 2206A, China) was used for phase separation. A pH meter with a glass electrode (Arwa AD 8000) was used for the pH adjustment of the sample. For the synthesis of Ag-NPs, an ultrasonic bath (power sonic 405, China) was used.

2.3. Preparation of silver nanoparticles

The preparation of Ag-NPs was performed based on the procedure described by (Peng et al., 2013). Briefly, a 100 mL solution of 0.1 mM of silver nitrate was prepared in a flat bottom flask and then vibrantly shaken for mixing. After this, sodium borohydride (0.012 g) was added to the solution. The resulting mixture was shaken for the next 30 minutes over a magnetic stirrer. The prepared mixture remained in static conditions (without mixing) overnight in a dark place. The resulting Ag-NPs suspension was stable for several months at ordinary temperatures without any color change or aggregation. This suspension containing Ag-NPs was further used for the extraction of lead (II) ions in edible oil. For each experiment, freshly prepared Ag-NPs were used.

2.4. Preparation of DESs and buffers

Different combinations of DESs at different molar ratios were prepared using ChCl, like an HBA compound. On the other hand, five types of HBD were tested, including malonic acid, ethylene glycol, glycol, phenol, and urea, assaying their maximum metal recovery. These HBDs were mixed with ChCl at molar ratios of 1:1, 1:2, and 1:3. All the DES solvents were prepared under identical conditions. In a 50 mL polypropylene tube, the components of DES were mixed and then heated at 60 ºC in a water bath for 5 minutes. The mixtures were stirred with vortex for 2 min to make homogeneous mixtures and thus obtaining DESs. For each experiment, freshly prepared DES solvents were prepared and directly used without any dilution or purification.

Buffer systems ranged from pH 2 to 10 were prepared. The buffer, having pH 2, was prepared by dissolving phosphoric acid (purity 85%) and 3.118 g of disodium hydrogen phosphate in 100 mL of deionized water. Similarly, the buffer, having pH 4, was prepared by dissolving 5.76 mL acetic acid and 1.54 g sodium acetate in 100 mL deionized water; while the buffer of pH 6 was prepared by dissolving 0.5 mL acetic acid (purity > 99%) and 11.7 g ammonium acetate in 100 mL deionized water. For buffer pH 8, 0.8 mL ammonium hydroxide was dissolved in 100 mL deionized water with 10.7 g ammonium chloride, and the buffer pH 10 was prepared by dissolving 20 mL deionized water, containing 4.5 g ammonium chloride, with 35 mL of 10 M ammonia solution in 30 mL deionized water. Both solutions were previously mixed and diluted up to 100 mL. 2 mL of buffer solution in the reagents mixture was found enough for maintaining a stable pH value.

2.5. Samples collection and preparation

Three different commercial brands of edible oil were selected as a benchmark for determining lead (II) concentration and validate the method. Here, sesame, olive, and canola oils were acquired from Dalda oil, ghee industries Pvt limited, and Seasons oils Pvt limited, respectively. Each sample was prepared independently in triplicate. For metal analysis, an oil sample (2.0 g) was preliminarily digested by adding 2 mL HNO_3 and 1 mL H_2O_2 in a quartz tube and subsequently heated at 120 ºC. When the sample was near to dry, an additional 2 mL HNO_3 and 1 mL H_2O_2 were added to the sample. Finally, the sample was diluted with deionized water making a final solution of 10mL. The resulting final solution was used for metal extraction.

2.6. Analytical procedure for determination of lead in edible oil samples

2 mL of metal standard solution (100 mg/L) was added to edible oil samples and digested. After dilution, the total sample volume was 15 mL. For maintaining the pH, a buffer solution sample (2 mL) was added to 1 mL of Ag-NPs (0.1 mM). The mixture was later stirred for 2 min and then 500µL of DES was added. The mixture was again stirred using a vortex for 1.5 min. Afterward, THF (500 µL) was added to the sample solution; and the mixture was placed in an ultrasonic bath for 1 min. The mixture was centrifuged at 3500 rpm for 2 min to obtain a complete phase separation. After centrifugation, the DES layer was separated and obtained at the bottom. Such extracted layer was diluted with ethanol up to 5 mL. Finally, HNO_3 was added to the solution with a final concentration of 0.1M to avoid coagulation. Scheme of the developed procedure is provided as graphic abstract figure.

2.7. Quantitative analysis and quality assurance

For quantitative analysis and quality assurance, different analytical parameters were determined including the limit of detection (LOD) and limit of quantification (LOQ). Such parameters were calculated following the JRC technical report on estimation of LOD and LOQ in foods (Wenzl et al., 2016).

The LOD and LOD were determined using the blank samples. In other words, native samples were used without spiking (Pseudo-blank). These samples were analyzed in ten replicates under repeatability conditions. The variability of signal values, expressed as standard deviation, was used for the estimation of LOD and LOQ. Both parameters were calculated, as below:

$$\mathrm{LOD} = 3 \times \frac{\mathrm{SD}}{\mathrm{m}} \qquad (1)$$

$$\mathrm{LOQ} = 10 \times \frac{\mathrm{SD}}{\mathrm{m}} \qquad (2)$$

where LOD and LOQ are the limits of detection and quantification, respectively; SD is the standard deviation of blank signals, and m is the slope of the calibration curve.

For determining the accuracy of this new analytical method, the procedure M 1-92 from American Oil Chemists' Society (AOCS) was followed, which is an updated procedure prepared in collaboration with the International Union of Pure and Applied Chemistry (IUPAC) and the International Organization for Standardization (ISO)(Liao et al., 2006).

Reliability and accuracy of the method, as the relative standard deviation (RSD), was determined by applying six replicate determinations of 5 µg/L of analyte to deionized water, in which the % RSD value of the recovery was found to be 4.5%.

The linearity of this new method was determined by adding a series of standards solutions from the stock solution. Here, a defined amount of blank with a final concentration ranging from 5 to

150 μg/L was used according to the expected working range. Triplicate samples for each concentration were analyzed.

3. Results and discussion

To introduce a green method for lead (II) extraction in edible oil, the suitable DES solvent was preliminarily selected. It is reported in the literature that DES solvents are extensively used for the extraction of biomolecules from natural products (Corrêa et al., 2021; Dwamena, 2019); however, very few methods are available using DESs for the extraction of toxic metals in more complex samples, such as edible oil. On the other hand, Ag-NPs are used for heavy metal uptake (Sumesh et al., 2011). In this work, Ag-NPs along with adsorbing metal was extracted through DES, which is a new approach to exploring new aspects of DES with Ag-NPs.

3.1. Selection of DES system

The extraction efficiency of the target analyte is mainly affected by the composition and the nature of DES. The DES used for extraction must meet several requirements, such as high affinity for analytes, liquid state at RT, no interference in analytical signal, different density than that of water, high stability, and low solubility in an aqueous medium (Haq et al., 2021a; Makoś, Przyjazny, et al., 2018). The DES system usually consists of two components, its 1st part consists of quaternary ammonium salt while the second part consists of HBD. The mole ratio of the quaternary ammonium salt and HBD has a significant impact on the applications of DES for metal extraction. Therefore, different DES systems with different molar ratios were tested in this work. ChCl was separately mixed with different types, including phenol, ethylene glycol, urea, glycerol, and malonic acid, using different mole ratios. At this point, the optimization of the HBD and HBA ratio is crucial for the applications of DES for extraction procedures. The effectiveness of extraction generally decreases with an increase in HBD in the resulting DES. On the other hand, mass transfer during extraction is increased by decreasing the density and viscosity of DES (Hou et al., 2017; Razi Asrami et al., 2020). To determine the optimum molar ratio DES, different HBD and HBA ratios were tested evaluating their maximum recovery. In this way, a high recovery value was obtained with ChCl and phenol with a mole ratio of 1:2, which was selected for further experiments. The results are illustrated in **Figure 1**.

Figure 1. Evaluation and selection of DES for maximum recovery of lead (II) ions. ChCl: Chlorine chloride, M: Malonic acid, EG: Ethylene glycol, G: Glycerol, Ph: Phenol, U: Urea.

Phenol is an aromatic compound and capable of delocalizing negative charges across its entire ring system. Considering its pK a value of 9.99, it is capable to form stronger hydrogen bonding than other alcohols. The calculated interaction energies for clusters with similar hydrogen-bonding patterns reveal that intermolecular interaction in phenol clusters is slightly stronger than in water clusters. However, the fusion of phenol and water clusters leads to similar stability to that of H_2O clusters (Parthasarathi et al., 2005). It is clear from the above mentioned facts, that phenol presents a strong hydrogen bonding and fulfill the requirments of HBD in DES formation. To evaluate the efficiency of different DESs, different HBDs were tested for Pb (II) extraction. DES based on phenol as HBD was found advantageous for the extraction of Pb (II) from edible oil samples.

3.2. Optimization of extraction parameters

3.2.1. Volume optimization of DESs

DES prepared with ChCl and phenol with molar ratio (1:2) was directly used without any further purification or dilution. Since DES directly influences the extraction of lead (II), its optimum concentration was determined for this specific procedure. Under the optimum condition, the DES volume effect was studied ranging from 250 µL to 1250 µL for the recovery of lead (II). The results indicate that, with a DES volume of 500 µL, a maximum recovery was obtained. Thus, 500 µL volume of DES was selected for onward studies. The results of this study are illustrated in Figure S1.

3.2.2. pH effect

In adsorption/chelate formation and continuous extraction, pH plays a significant role (Haq et al., 2021a). The solution pH is one of the parameters having a strong influence on the heavy-metal ion uptake; this is due to the fact that the surface charge density of the adsorbent and the metallic species depend on the pH (Haq et al., 2021b; Kubilay et al., 2007). Here, the lead (II) recoveries were examined in the range of pH 2 to pH 8. The results revealed that a maximum recovery for lead (II) was obtained at pH 2, as shown in Figure S2, indicating that a regular drop in the recovery of lead (II) was found with a further increase after pH 2. Therefore, pH 2 was selected for further studies.

At higher pH, Ag-NPs are more stable but less capable of metal adsorption, while at lower pH, Ag-NPs are less stable but more capable of metal adsorption (Hosseini et al., 2016; Molleman & Hiemstra, 2017). As the pH increases, the prolongation of the adsorbent surface decreased, leading to a reduction in the electrostatic attraction between the metal ions species and the adsorbent surface (Fernando & Zhou, 2019; Molleman & Hiemstra, 2017); this results in a consequent decrease in the percentage removal of metal ions.

3.2.3. Optimization of THF addition

THF is an aprotic solvent, which was used in these studies to make phase separation easier. The outcomes of this paper revealed that, while it indeed enhances the phase separation, it also increases the % recovery of the analyte. These aprotic solvents tend to interact more with water than DES. After interaction with THF molecules, water molecules decline their interaction with DES, resulting in their self-aggregation and separation. In other words, THF acts as a dehydrating agent. The most plausible mechanism of DESs self-aggregation involves π–π overlap between the aromatic ring of phenol, followed by hydrogen bonding between functional groups of DES and other charge transfer interactions (Haq et al., 2021a; Khezeli et al., 2015). The volume effect of THF ranged from 250 µL to 1500µL was studied for the % recovery of lead (II) (see Figure S3), where the maximum recovery was obtained with a THF volume of 500 µL. For further studies, a THF volume of 500 µL was carefully used as an optimum value.

3.2.4. Effect of Ag-NPs concentration

For the separation of different metals, Ag-NPs have been used as a good adsorbent in the field of research. Here, the excess of Ag-NPs is needed to provide maximum surface area for metal adsorption. The mechanism of metal ion interaction with Ag-NPs is described and detailed in section 3.3. In this study, the effect of the amount of Ag-NPs (0.1mM) was studied in the range of 250 µL to 2000 µL. The results showed that the lead (II) signal response was increasing with the increase of the amount of Ag-NPs up to 500 µL and it remained constant. Therefore, 500 µL of Ag-NPs has been selected as the optimum value for lead (II) determination. The effect of Ag-NPs on lead (II) recovery is illustrated in figure S4.

3.2.5. Effect of ultra-sonication time

The efficiency of the extraction process was greatly affected by ultra-sonication time. Our study suggests that both THF amount and ultra-sonication waves caused the aggregation of the DES in the aqueous phase and the reduction in extraction time. In general, it was noticed that the conversion of these droplets of DES into tiny droplets contributed to reaching the equilibrium state, resulting in increased extraction efficiency. At constant experimental conditions, the effect of ultra-sonication time was studied from 1 to 5 min at 25 °C (see Figure S5). When the ultra-sonication time was extended to 2 min, the extraction efficiency was stabilized. Therefore, 2 minutes of ultra-sonication time was selected for the subsequent experiments.

3.3. Development of quantitative method/validation

3.3.1. Interference study of different ions

To analyze lead content in food and environmental samples, an important issue relates to the presence of interfering ions which could affect to some extent the analytical signal. The goal during the development of this new method was to select the selective extraction conditions of (Ooi & Ng, 2018), back extraction methods (Arpa & Aridaşir, 2018), solid-phase extraction (Rahnama & Ghadiri, 2015), membrane techniques (Mesli & Belkhouche, 2018), adsorption methods (Mahmoud et al., 2010), flow-injection on-line adsorption(Salonia et al., 1999) and co-precipitation methods (Komjarova & Blust, 2006). The performance of the developed DES impregnated Ag-NPs method was compared with liquid-phase microextraction methods, which were used for lead (II) determination in edible oil, as shown in **Table 1**. Among all the reported methods, cloud point extraction is the most dominantly used technique for lead extraction due to its low cost, high enrichment factor, environment-friendly and minimal cost (Babaee et al., 2019). However, these methods are associated with some critical issues, such as a long time of the procedure related to heating and centrifugation stages. Even though these methods achieve good recovery after multi-cycle experiments (Galbeiro et al., 2014), while our proposed method is quicker and provides a very good recovery obtained in a single cycle. The time for the extraction procedure after digestion was estimated for the different reported methods in the literature, which is compiled in **Table 1**. The estimated time for our extraction procedure was as short as 6.5 minutes while estimated time values for other methods were 35 min (Coelho et al., 2008), 87 min (Blanchet-Chouinard & Larivière, 2018), 217 min (Gouda & Zordok, 2018), 35 min (Citak & Tuzen, 2012), 60 min (Kazi et al., 2012), 16 min (Rahnama & Ghadiri, 2015) and 40min (Citak & Tuzen, 2010). At this point, it is noted that the newly proposed method is many folds faster than the already reported methods. Application of AG-NPs with DES as extractant with addition of THF provides further improvement also in the field of lead extraction based on DESs in comparison to already published approaches (Karmini et al. 2016; Soylak & Koksal, 2019; Habila et al. 2020.). Preliminary studies for this paper indicated that in case of sole use of DES about 30 minutes of extraction is needed for acceptable recovery. Nanoparticle scale silver reveals to provide rapid extraction. It follows from very fast kinetics of sorption. The performance of this method is quite good due to its low LOD and LOQ values and high pre-concentration factor. This method opens a new aspect in the application of DES coupled with Ag-NPs for lead extraction.

Table 1. Comparative study of the new method with reported methods in the literature.

3.3.4. Application of the method for the analysis of real samples

Since edible oils are an essential part of the human diet, the developed procedure was applied to the analysis of several categories of edible oils, such as sesame, olive, and canola oil obtained from commercial brands of the district Mardan, Pakistan. In this approach, the standard addition method, which is commonly used for AAS, was used. Known concentrations of lead (II) were gradually added to real samples and percent recovery was determined in each case. The results of the recovery performance are presented in **Table 2**. The recovery for real samples was between 97-107%. Since the concentration in real samples was revealed to be much higher than the limit established by this method, the samples were diluted with methanol right after extraction. In these experiments, three different types of edible oils were tested for lead (II) concentration, exhibiting a lead (II) concentration of 1.21 mg/L, 1.26 mg/L and1.19 mg/L for olive, sesame, and canola oil, respectively. This method was sensitive and valid for determining very low concentrations. To summarize, it can be stated that the performed experiments confirmed that the developed procedure is reliable for the determination of lead (II) in different commercial edible oil samples.

lead from the matrix, which was fully obtained making this method highly selective. DESs are highly selective in extraction procedures (Rad et al., 2019); however, in this case, preliminary studies revealed that additional selectivity must be provided. Thus, the application of Ag-NPs was further studied to assure adequate accuracy of the quantitative analysis; for this, a standard addition method was used.

To determine the effect of external ions on lead (II) extraction, known concentrations of different ions were added in separate sets of the experiment. The interference of Na^+($NaNO_3$), K^+(KCl), Mg^{2+}($Mg(NO_3)_2$), Cd^{2+}($CdCl_2$), Zn^{2+}($(ZnNO_3)_2$), Co^{2+}($CoCl_2$), Ni^{2+}($NiCl_2$), SO_4^{2-}(K_2SO_4),

$Pb^{2+}(PbCl_2)$ was investigated. The recovery of the lead (II) in presence of interfering ions was found to be higher than 90%. These results are compiled in supporting information (Table S1). Importantly, it was proved that the interfering ions have no significant effect on the lead (II) extraction/analysis and the proposed microextraction method fits the purpose.

3.3.2. Analytical performance of the method

According to the methodology described in section 2.7, LOD and LOQ values were calculated as 0.28 and 0.94 µg/L, respectively. **Figure 2** shows the calibration curve, which has an equation as follows: Y= 0.0037x + 0.0026, displaying an acceptable coefficient of determination (R^2) of 0.9931. The linearity range was established from 5 to 140 µg/L. The relative standard deviation calculated for six repetitions of lead (II) analysis (at a concentration level of 5 µg/L) was 4.5%, showing the repeatability and reproducibility of the developed method.

Figure 2. Calibration curve of lead (II) at different concentrations.

3.3.3. Comparison with other methods

Many methods have been reported in the literature for lead extraction and analysis, including cloud point extraction (Blanchet-Chouinard & Larivière, 2018), liquid-liquid microextraction

Table 2. Determination of lead in edible oil samples.

Several papers recently reported presence of lead ions in oil samples. For example, it was reported as lead (II) concentrations of 0.06-0.21 mg/Kg, 0.08-1.12 mg/Kg, and 0.06-0.08 mg/Kg for rapeseed, soybean, and linseed oil, respectively (Szyczewski et al., 2016). In other study lead (II) concentrations of 6-15 µg/Kg, 7.3-21 µg/Kg, and 3.4-16 µg/Kg were reported for canola, corn, and soybean oil, respectively (Allen et al., 1998). In vegetable hydrogenated oil, a lead concentration of 15.92 µg/L was reported by (Abbasi et al., 2009), while (Ng, 2010) documented 0.71 mg/Kg of lead in black olive oil and 0.75 mg/Kg in green olive oil. In more recent work, (Zhuravlev et al., 2015) evaluated and determined a lead concentration in different types of oil, e.g., 0.006 mg/Kg in corn oil, 0.014 mg/Kg in olive oil, 0.016 mg/Kg in refined sunflower oil, 0.062 mg/Kg sunflower oil, and 0.027 mg/Kg in soybean oil. It can be seen that the range of reported values strongly differs in the type and origin of oil samples. It is worth mentioning that this developed method covers the entire range of expected levels of lead concentration from low parts per billion levels to higher ones after dilution. The modern instruments are equipped with an automatic module for sample dilution; therefore, this approach fully fits the purpose of lead determination in edible oils.

4. Conclusions

In this new method, lead (II) ions were efficiently extracted and determined from different commercial edible oil products. Deep eutectic solvents based on ChCl and phenol with a 1:2 molar ratio demonstrated a maximum recovery for lead (II) extraction. It was concluded that Ag nano particles can adsorb Pb (II) and facilitate the Pb (II) mobility from aqueouse phase to DES phase. This stage is very fast, as nanoparticles ensure very good kinetics of sorption. The sonication process accelerates the formation of nano-sized fine droplets and as a result increases the contact surface area between the extracting solvent (DES) and Pb(II). The optimized method implies simplicity, ease of operation, short extraction times, and high enrichment factor. Quantitative recovery (97-105%) from spiked samples demonstrated the suitability of the optimized method for the quality control of the analyzed samples. The LOD and LOQ were 0.28 µg/L and 0.94 µg/L, respectively with an RSD value of 4.5% and a pre-concentration factor of 25 which are comparatively improved comparing other methods reported in the literature. The optimized procedure is green, simple and requires a small volume of extraction solvent. The comparison of the developed method with already-existing analytical procedures confirmed its advantages, such as shortening the time of analysis and sensitivity. To finalize, the outcomes of this new assay are comparable to those obtained by the ICP-MS technique with more simplicity, better accuracy, high enrichment factor, less time-consuming, and environment-friendly. Further improvements in method development, also in green chemistry aspects, should focus on minization of the scale of procedure. This studies confirmed that the optimized procedure is perfectly useful for routine analysis of food especialy edible oil samples containing traces of Pb(II) ions.

Acknowledgment

Prof. Grzegorz Boczkaj and Hameed Ul Haq gratefully acknowledge the financial support from the National Science Centre, Warsaw, Poland – decision no. UMO-2018/30/E/ST8/ 00642.

Prof. Muhammad Balal Arain and Hameed Ul Haq are thankful to the PAK-US Science and Technology coopration program No.6-4/PAK-US/HEC/2015/04 for financial aid and AWKUM Mardan, Pakistan for providing us the conveniences and opportunity. R. Castro-Muñoz acknowledges the financial support from Polish National Agency for Academic Exchange (NAWA) under Ulam Programme (Agreement No. PPN/ULM/2020/1/00005/U/00001).

Conflict of interest

The authors declare no conflict of interest.

References:

Abbasi, S., Allahyari, M., Taherimaslak, Z., Nematollahi, D., & Abbasi, F. (2009). New determination of lead in edible oil and water samples by high selective adsorptive stripping voltammetry with SPADNS. *International Journal of Electrochemical Science*, *4*, 602-613.

Allen, L. B., Siitonen, P. H., & Thompson Jr, H. C. (1998). Determination of copper, lead, and nickel in edible oils by plasma and furnace atomic spectroscopies. *Journal of the American Oil Chemists' Society*, *75*(4), 477-481.

Arpa, Ç., & Aridaşir, I. (2018). A Method to Determination of Lead Ions in Aqueous Samples: Ultrasound-Assisted Dispersive Liquid-Liquid Microextraction Method Based on Solidification of Floating Organic Drop and Back-Extraction Followed by FAAS. *Journal of analytical methods in chemistry*, *2018*.

Babaee, S., Hosseini, S. G., & Mirzaei, M. (2019). Rapid and selective extraction of trace amount of Pb (II) in aqueous samples using a magnetic ion-imprinted polymer and detection by flame atomic absorption spectrometry. *Eclética Química Journal*, *44*(4), 73-84.

Blanchet-Chouinard, G., & Larivière, D. (2018). Determination of Pb in environmental samples after cloud point extraction using crown ether. *Talanta*, *179*, 300-306.

Citak, D., & Tuzen, M. (2010). A novel preconcentration procedure using cloud point extraction for determination of lead, cobalt and copper in water and food samples using flame atomic absorption spectrometry. *Food and Chemical toxicology*, *48*(5), 1399-1404.

Citak, D., & Tuzen, M. (2012). Cloud point extraction of copper, lead, cadmium, and iron using 2, 6-diamino-4-phenyl-1, 3, 5-triazine and nonionic surfactant, and their flame atomic absorption spectrometric determination in water and canned food samples. *Journal of AOAC International*, *95*(4), 1170-1175.

Coelho, L. M., Bezerra, M. A., Arruda, M. A. Z., Bruns, R. E., & Ferreira, S. L. C. (2008). Determination of Cd, Cu, and Pb after cloud point extraction using multielemental sequential determination by thermospray flame furnace atomic absorption spectrometry (TS-FF-AAS). *Separation Science and Technology*, *43*(4), 815-827.

Corrêa, P. S., Morais Júnior, W. G., Martins, A. A., Caetano, N. S., & Mata, T. M. (2021). Microalgae biomolecules: Extraction, separation and purification methods. *Processes*, *9*(1), 10.

Dugo, G., La Pera, L., La Torre, G. L., & Giuffrida, D. (2004). Determination of Cd (II), Cu (II), Pb (II), and Zn (II) content in commercial vegetable oils using derivative potentiometric stripping analysis. *Food chemistry*, *87*(4), 639-645.

Dwamena, A. K. (2019). Recent advances in hydrophobic deep eutectic solvents for extraction. *Separations*, *6*(1), 9.

Fernandes, A., Makoś, P., Khan, J. A., & Boczkaj, G. (2019). Pilot scale degradation study of 16 selected volatile organic compounds by hydroxyl and sulfate radical based advanced oxidation processes. *Journal of cleaner production*, *208*, 54-64.

Fernando, I., & Zhou, Y. (2019). Impact of pH on the stability, dissolution and aggregation kinetics of silver nanoparticles. *Chemosphere*, *216*, 297-305.

Galbeiro, R., Garcia, S., & Gaubeur, I. (2014). A green and efficient procedure for the preconcentration and determination of cadmium, nickel and zinc from freshwater, hemodialysis solutions and tuna fish samples by cloud point extraction and flame atomic absorption spectrometry. *Journal of Trace Elements in Medicine and Biology*, *28*(2), 160-165.

Gouda, A. A., & Zordok, W. A. (2018). Solid-phase extraction method for preconcentration of cadmium and lead in environmental samples using multiwalled carbon nanotubes. *Turkish Journal of Chemistry*, *42*(4), 1018-1031.

Haq, H. U., Balal, M., Castro-Muñoz, R., Hussain, Z., Safi, F., Ullah, S., & Boczkaj, G. (2021a). Deep eutectic solvents based assay for extraction and determination of zinc in fish and eel samples using FAAS. *Journal of Molecular Liquids*, 115930.

Haq, H. U., Balal, M., Castro-Muñoz, R., Hussain, Z., Safi, F., Ullah, S., & Boczkaj, G. (2021b). Deep eutectic solvents based assay for extraction and determination of zinc in fish and eel samples using FAAS. *Journal of Molecular Liquids*, *333*, 115930.

Hosseini, S., Aghaie, H., Ghaedi, M., Dashtian, K., & Purkait, M. (2016). Lead (II) adsorption from aqueous solutions onto modified ag nanoparticles: Modeling and optimization. *Environmental Progress & Sustainable Energy*, *35*(3), 743-749.

Hou, Y., Kong, J., Ren, Y., Ren, S., & Wu, W. (2017). Mass transfer dynamics in the separation of phenol from model oil with quaternary ammonium salts via forming deep eutectic solvents. *Separation and Purification Technology*, *174*, 554-560.

Kazi, T. G., Shah, F., Afridi, H. I., Khan, S., Arian, S. S., & Brahman, K. D. (2012). A green preconcentration method for determination of cobalt and lead in fresh surface and waste water samples prior to flame atomic absorption spectrometry. *Journal of analytical methods in chemistry*, *2012*.

Khezeli, T., Daneshfar, A., & Sahraei, R. (2015). Emulsification liquid–liquid microextraction based on deep eutectic solvent: an extraction method for the determination of benzene, toluene, ethylbenzene and seven polycyclic aromatic hydrocarbons from water samples. *Journal of Chromatography A*, *1425*, 25-33.

Klaassen, C. D., Casarett, L. J., & Amdur, M. O. (1986). *Toxicology: the basic science of poisons*. Macmillan.

Komjarova, I., & Blust, R. (2006). Comparison of liquid–liquid extraction, solid-phase extraction and co-precipitation preconcentration methods for the determination of cadmium, copper, nickel, lead and zinc in seawater. *Analytica chimica acta*, *576*(2), 221-228.

Kovacs, C. S., & Ward, L. M. (2020). Physiology of calcium, phosphorus, and bone metabolism during fetal and neonatal development. In *Maternal-Fetal and Neonatal Endocrinology* (pp. 573-586). Elsevier.

Kubilay, Ş., Gürkan, R., Savran, A., & Şahan, T. (2007). Removal of Cu (II), Zn (II) and Co (II) ions from aqueous solutions by adsorption onto natural bentonite. *Adsorption*, *13*(1), 41-51.

Liao, J. J., Capen, R. C., & Schofield, T. L. (2006). Assessing the reproducibility of an analytical method. *Journal of chromatographic science*, *44*(3), 119-122.

Mahmoud, M. E., Osman, M. M., Hafez, O. F., Hegazi, A. H., & Elmelegy, E. (2010). Removal and preconcentration of lead (II) and other heavy metals from water by alumina adsorbents developed by surface-adsorbed-dithizone. *Desalination*, *251*(1-3), 123-130.

Makoś, P., Fernandes, A., Przyjazny, A., & Boczkaj, G. (2018). Sample preparation procedure using extraction and derivatization of carboxylic acids from aqueous samples by means of deep eutectic solvents for gas chromatographic-mass spectrometric analysis. *Journal of Chromatography A*, *1555*, 10-19.

Makoś, P., Przyjazny, A., & Boczkaj, G. (2018). Hydrophobic deep eutectic solvents as “green” extraction media for polycyclic aromatic hydrocarbons in aqueous samples. *Journal of Chromatography A*, *1570*, 28-37.

Mesli, M., & Belkhouche, N.-E. (2018). Extraction and pre-concentration of lead from copper by emulsion liquid membrane technique using an ionic liquid. *Euro-Mediterranean Journal for Environmental Integration*, *3*(1), 14.

Molleman, B., & Hiemstra, T. (2017). Time, pH, and size dependency of silver nanoparticle dissolution: the road to equilibrium. *Environmental Science: Nano*, *4*(6), 1314-1327.

Musa, M., Sulaiman, A., Bello, I., Itumoh, J., Bello, K., Bello, A., & Arzika, A. (2012). Physicochemical properties of some commercial groundnut oil products sold in Sokoto metropolis. Northwest Nigeria. *Journal of Biological Science and Bioconservation*, *4*, 17-24.

Ng, K. L. (2010). *Determination of lead, cadmium, zinc and nickel in edible oil products by stripping voltammetry/Ng Khan Loon* University of Malaya].

Ooi, E., & Ng, Y. S. (2018). Removal of Lead from Water by Liquid-Liquid Extraction using trihexyltetradecylphosphonium Chloride in Different Diluents. E3S Web of Conferences,

Parthasarathi, R., Subramanian, V., & Sathyamurthy, N. (2005). Hydrogen bonding in phenol, water, and phenol− water clusters. *The Journal of Physical Chemistry A*, *109*(5), 843-850.

Peng, S., Li, L., Tan, H., Wu, Y., Cai, R., Yu, H., . . . Srinivasan, M. (2013). Monodispersed Ag nanoparticles loaded on the PVP-assisted synthetic Bi 2 O 2 CO 3 microspheres with enhanced photocatalytic and supercapacitive performances. *Journal of Materials Chemistry A*, *1*(26), 7630-7638.

Pourrut, B., Shahid, M., Dumat, C., Winterton, P., & Pinelli, E. (2011). Lead uptake, toxicity, and detoxification in plants. *Reviews of environmental contamination and toxicology volume 213*, 113-136.

Rad, A. S., Rahnama, R., Zakeri, M., & Jamali, M. R. (2019). Dispersive liquid–liquid microextraction based on green type solvents—" deep eutectic solvents"—for highly selective separation and efficient preconcentration of nickel in water samples. *Journal of the Iranian Chemical Society*, *16*(8), 1715-1722.

Rahnama, R., & Ghadiri, R. (2015). Separation and preconcentration of trace amounts of lead from water samples using solvent-assisted dispersive solid phase extraction. *Journal of the Brazilian Chemical Society*, *26*(8), 1642-1647.

Razi Asrami, M., Tran, N. N., Saien, J., & Hessel, V. (2020). Mass Transfer Characterization of Ionic Liquid Solvents for Extracting Phenol from Aqueous Phase in a Microscale Coiled Flow Inverter. *Industrial & Engineering Chemistry Research*, *59*(37), 16427-16436.

Salonia, J., Wuilloud, R., Gáquez, J., Olsina, R., & Martinez, L. (1999). Determination of lead in tap water by ICP-AES with flow-injection on-line adsorption preconcentration using a knotted reactor and ultrasonic nebulization. *Journal of Analytical Atomic Spectrometry*, *14*(8), 1239-1243.

Sharrett, A. R., Carter, A. P., Orheimt, R. M., & Feinleib, M. (1982). Daily intake of lead, cadmium, copper, and zinc from drinking water: the Seattle study of trace metal exposure. *Environmental research*, *28*(2), 456-475.

Sumesh, E., Bootharaju, M., & Pradeep, T. (2011). A practical silver nanoparticle-based adsorbent for the removal of Hg2+ from water. *Journal of hazardous materials*, *189*(1-2), 450-457.

Szyczewski, P., Frankowski, M., Zioła-Frankowska, A., Siepak, J., Szyczewski, T., & Piotrowski, P. (2016). A comparative study of the content of heavy metals in oils: linseed oil, rapeseed oil and soybean oil in technological production processes. *Archives of Environmental Protection*, *42*(3), 37-40.

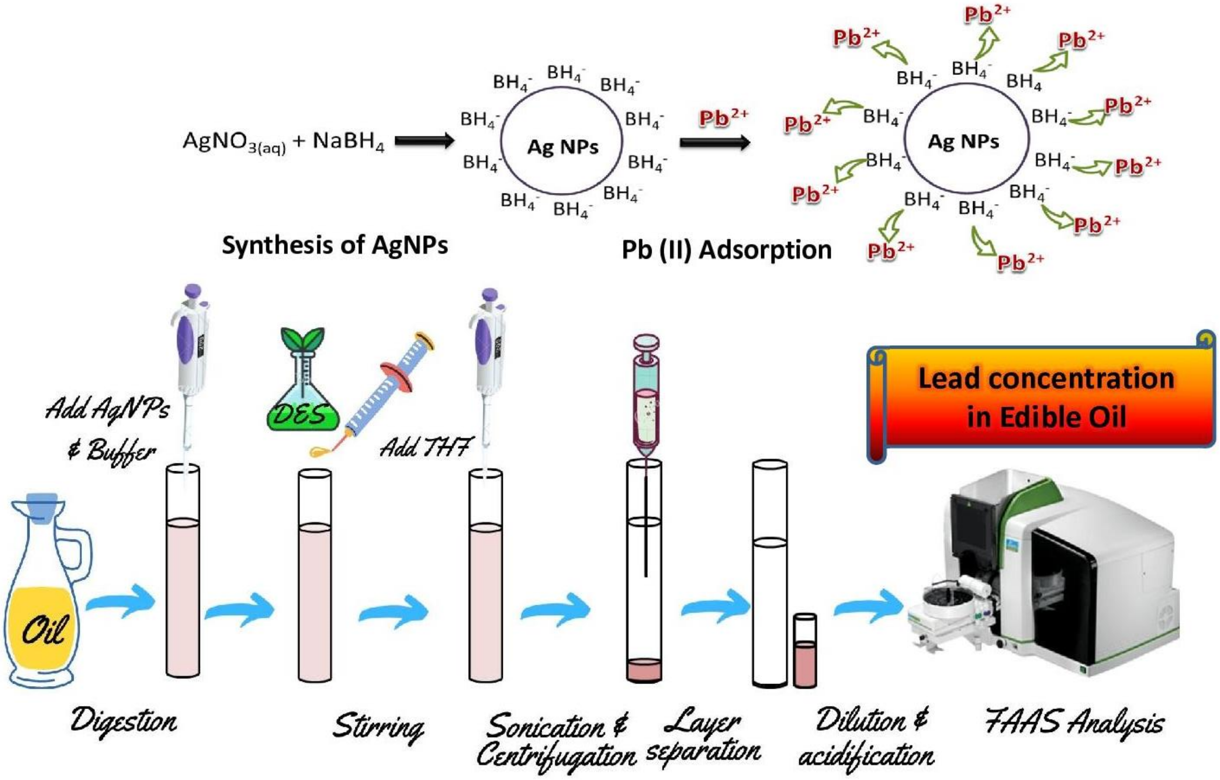
Wenzl, T., Haedrich, J., Schaechtele, A., Piotr, R., Stroka, J., Eppe, G., & Scholl, G. (2016). *Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Food and Feed*.

Yusof, N. A., & Ahmad, M. (2002). A flow cell optosensor for lead based on immobilized gallocynin in chitosan membrane. *Talanta*, *58*(3), 459-466.

Zeiner, M., Steffan, I., & Cindric, I. J. (2005). Determination of trace elements in olive oil by ICP-AES and ETA-AAS: A pilot study on the geographical characterization. *Microchemical Journal*, *81*(2), 171-176.

Zhuravlev, A., Zacharia, A., Gucer, S., Chebotarev, A., Arabadji, M., & Dobrynin, A. (2015). Direct atomic absorption spectrometry determination of arsenic, cadmium, copper, manganese, lead and zinc in vegetable oil and fat samples with graphite filter furnace atomizer. *Journal of Food Composition and Analysis*, *38*, 62-68.

Graphic abstract



Figures

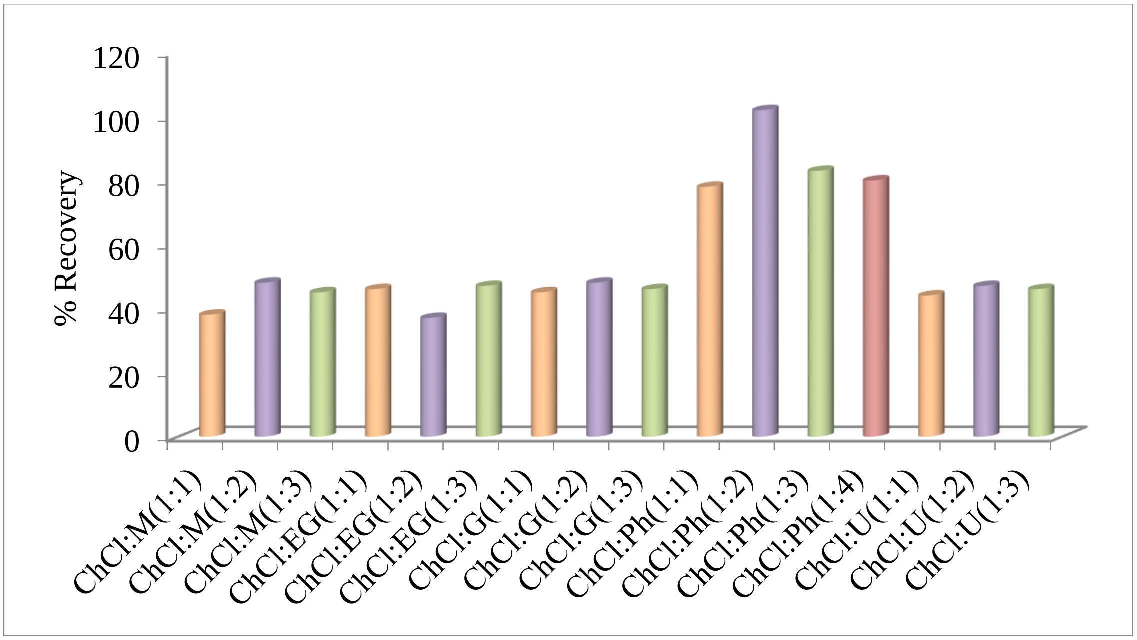


Figure 1. Evaluation and selection of DES for maximum recovery of lead (II) ions. ChCl: Chlorine chloride, M: Malonic acid, EG: Ethylene glycol, G: Glycerol, Ph: Phenol, U: Urea.

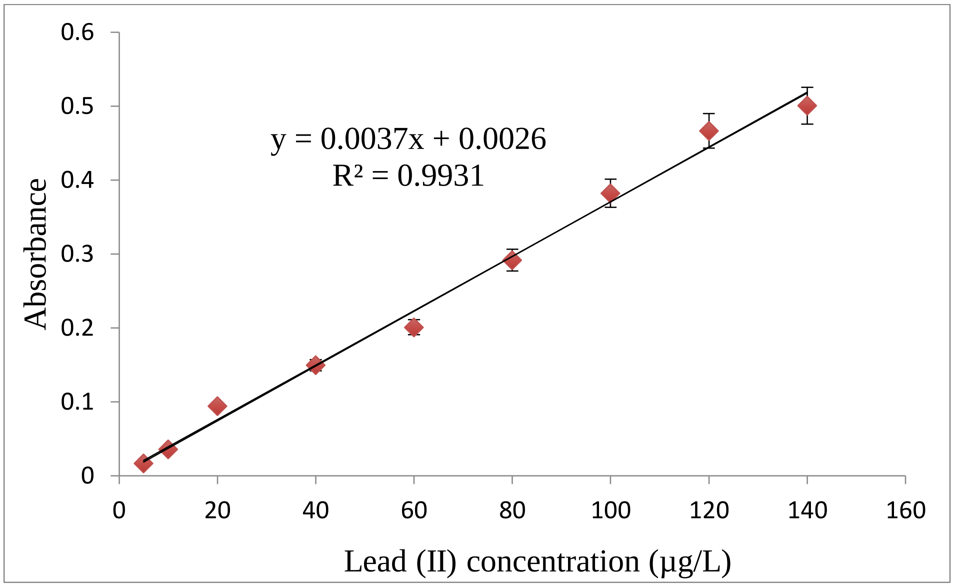


Figure 2. Calibration curve of lead (II) at different concentrations.

Tables

Table 1. Comparative study of the new method with reported methods in the literature.

Method	Detection techniques	LOD (µg/L)	LOQ (µg/L)	Linearity (µg/L)	RSD (%)	Estimated time of sample preparation* (minutes)	References
Cloud point extraction	TS-FF-AAS	0.43	1.44	5-50	8.7	>35	(Coelho et al., 2008)
Cloud point extraction	ICP-OES	0.8	2.60	---	13	>67	(Blanchet-Chouinard & Larivière, 2018)
Solid phase extraction	FAAS	0.2	1	1-60	3.2	>180	(Gouda & Zordok, 2018)
Cloud point extraction	FAAS	1.33	6.65	20-320	3.06	>35	(Citak & Tuzen, 2012)
Cloud point extraction	FAAS	0.26	0.86	20-100	1.88	>60	(Kazi et al., 2012)
SADSPE	FAAS	1.3	4.30	4-100	5	>21	(Rahnama & Ghadiri, 2015)
Cloud point extraction	FAAS	3.42	11.31	5-10	4.8	>45	(Citak & Tuzen, 2010)
DES-based extraction	FAAS	2.4	7.9	5-60	0.9-4.3	37	(Soylak & Koksal, 2019)
DES-based extraction with Fe_3O_4 sorbent	FAAS	0.4	2	2-250	1.8	>75	(Karmini et al. 2016)
DES-based extraction with Ag-NPs	FAAS	0.28	0.92	5-140	4.5	6.5	*Present work*

*after digestion

SADSPE; Solvent-assisted dispersive solid-phase extraction, FAAS; Flame atomic absorption spectroscopy, ICP-OES; Inductively coupled plasma optical emission spectroscopy, TS-FF-AAS; Thermo spray flame furnace atomic absorption, DES; Deep eutectic solvent

Table 2. Determination of lead (II) in edible oil samples.

Samples	Lead spiked (µg/mL)	Lead found (µg/mL)	% Recovery
Olive oil	--	1.21± 0.01	-------
	2.5	3.90± 0.02	105.12
	5.0	6.64± 0.02	106.92
Sesame oil	--	1.26± 0.03	-------
	2.5	3.65± 0.017	97.07
	5.0	6.11± 0.04	97.60
Canola oil	--	1.19± 0.01	-------
	2.5	3.62± 0.01	98.10
	5.0	6.19± 0.04	100.00

Supplementary data

Deep eutectic solvent (DES) with silver nanoparticles (Ag-NPs) based assay for analysis of lead (II) in edible oils

Hameed Ul Haq[1], Rafiat Bibi[2], Muhammad Balal Arain[3], Faisal Safi[2], Sana Ullah[2], Roberto Castro-Muñoz[1,4], Grzegorz Boczkaj[1,5,*]

[1]*Gdansk University of Technology, Faculty of Chemistry, Department of Process Engineering and Chemical Technology, 80 – 233 Gdansk, G. Narutowicza St. 11/12, Poland. E-mail: grzegorz.boczkaj@pg.edu.pl / hameed.haq@pg.edu.pl

[2]Department of Chemistry, Abdul Wali Khan University, Mardan, 23200, Pakistan. E-mail: hulhaqchem@gmail.com / rafiatmohmand@hotmail.com / faisalsafi1993@gmail.com

[3]Department of Chemistry, University of Karachi, Karachi, Sindh, 75270 Pakistan. E-mail: bilal_ku2004@yahoo.com

[4]Tecnologico de Monterrey, Campus Toluca, Avenida Eduardo Monroy Cárdenas 2000 San Antonio Buenavista, 50110, Toluca de Lerdo, Mexico. Email: food.biotechnology88@gmail.com

[5]EkoTechCenter, Gdansk University of Technology, G.Narutowicza St. 11/12, 80-233 Gdansk, Poland

Corresponding author: Dr. Grzegorz Boczkaj, Assoc. Prof., PhD. Sc. Eng. Gdansk University of Technology, Faculty of Chemistry, Department of Process Engineering and Chemical Technology, 80 – 233 Gdansk, G. Narutowicza St. 11/12, Poland. Fax: (+48 58) 347-26-94; Tel: (+48) 697970303; e-mail: grzegorz.boczkaj@gmail.com or grzegorz.boczkaj@pg.edu.pl

Supplementary figures

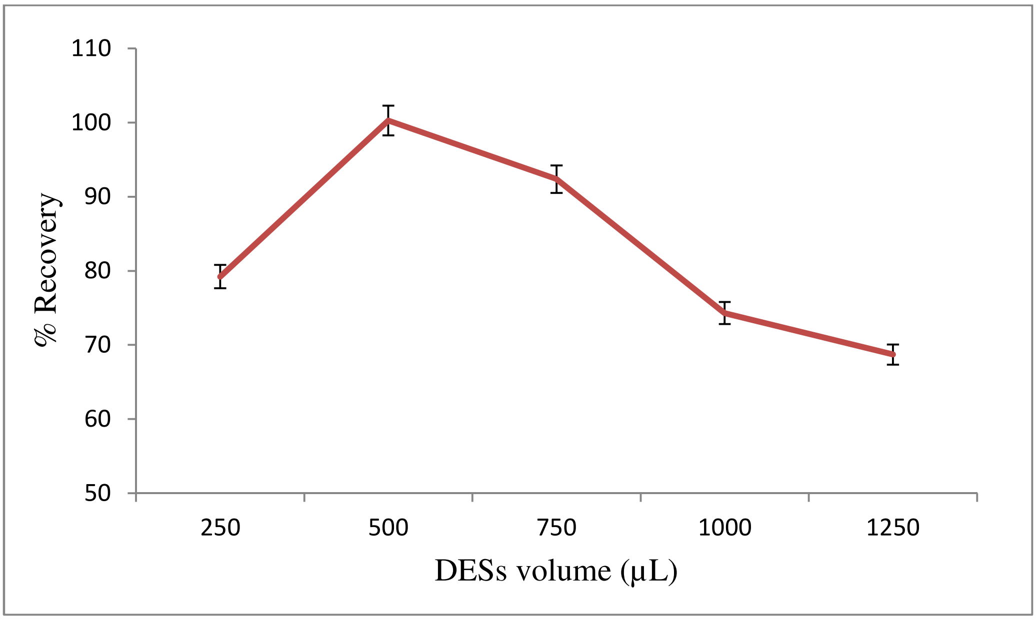


Figure S1: Volume optimization of DESs

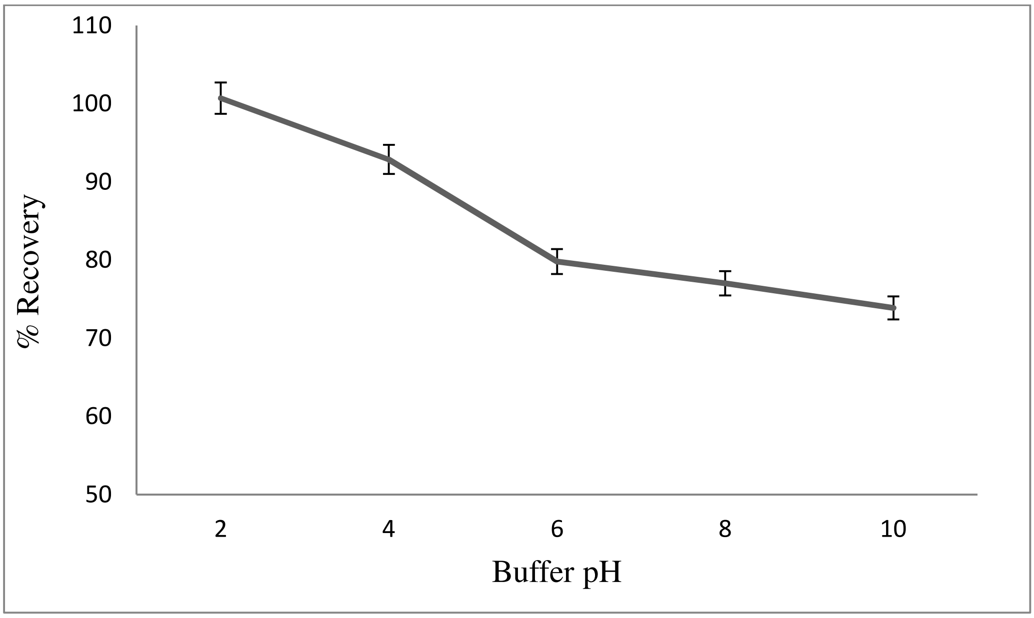


Figure S2: Optimization of pH

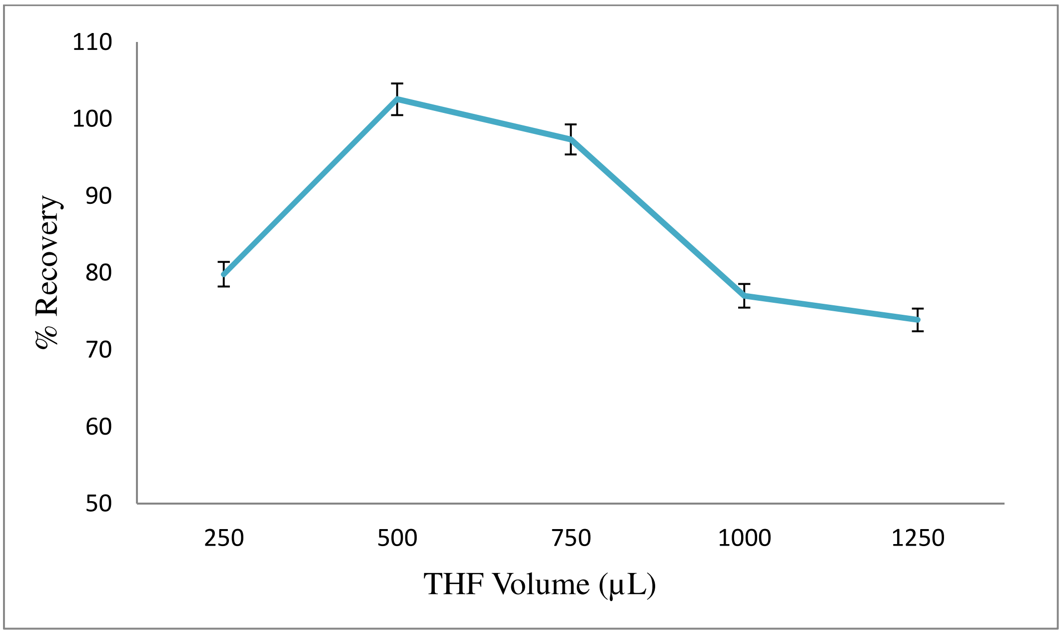


Figure S3: Optimization of THF

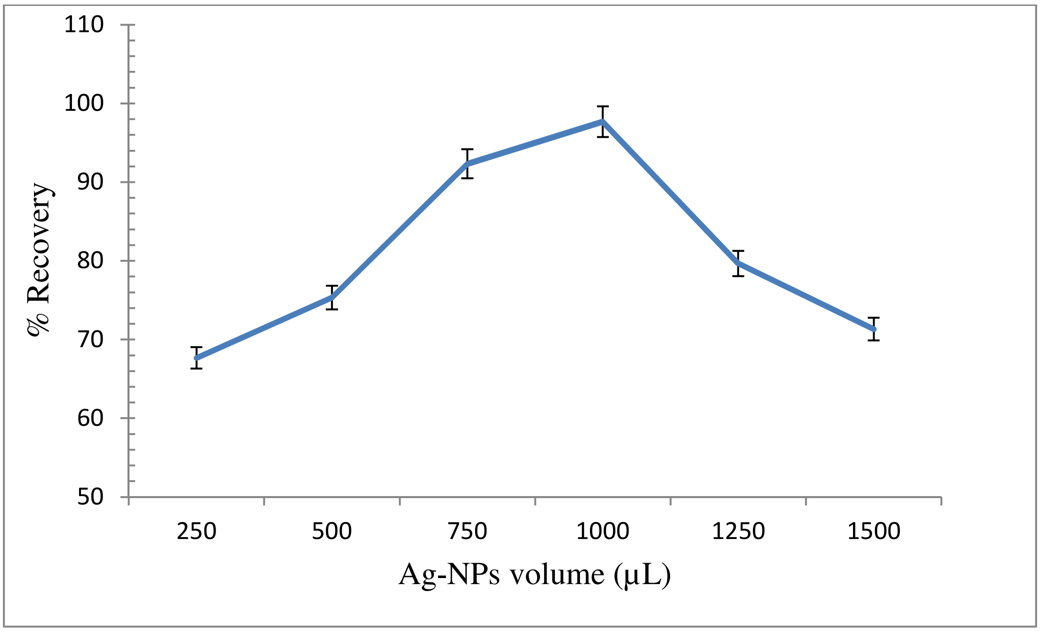


Figure S4: Optimization of Ag-NPs concentration

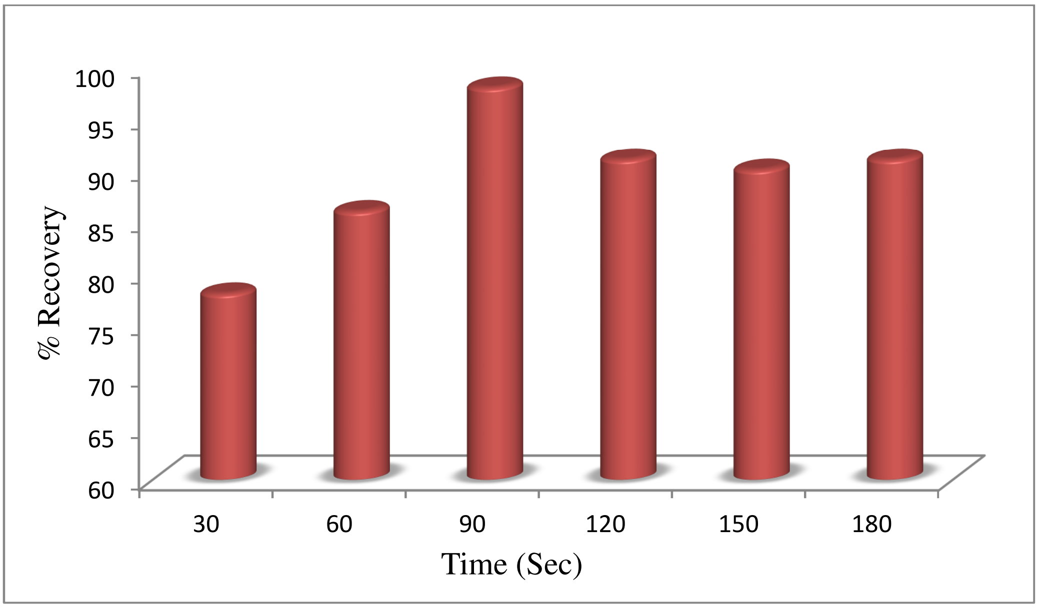


Figure S5: Optimization of sonication time for maximum recovery

Supplementary table

Table S1: Effects of interfering ions on the recovery of lead (II)

Ions	Added as	Concentration (mg/L)	Foreign ion	Recovery
Na^+	$NaNO_3$	2000	500	99.75
K^+	KCl	1000	250	100.62
Cd^{2+}	$CdCl_2$	10	2.5	94.86
Pb^{2+}	$Pb(NO_3)_2$	10	5	92.04
Mg^{2+}	$Mg(NO_3)_2.6H_2O$	500	1250	96.73
Co^{2+}	$CoCl_2.6H_2O$	1000	250	91.21
Ni^{2+}	$NiCl_2.6H_2O$	20	5	105.73
SO_4^{2-}	K_2SO_4	2000	500	90.61