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# A novel approach in dispersive liquid–liquid microextraction based on the use of an auxiliary solvent for adjustment of density UV–VIS spectrophotometric and graphite furnace atomic absorption spectrometric determination of gold based on ion pair formation

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ABSTRACT

This paper presents a novel approach to dispersive liquid–liquid microextraction (DLLME), based on the use of an auxiliary solvent for the adjustment of density. The procedure utilises a solvent system consisting of a dispersive solvent, an extraction solvent and an auxiliary solvent, which allows for the use of solvents having a density lower than that of water as an extraction solvent while preserving simple phase separation by centrifugation. The suggested approach could be an alternative to procedures described in the literature in recent months and which have been devoted to solving the same problem. The efficiency of the suggested approach is demonstrated through the determination of gold based on the formation of the ion pair $[Au(CN)_2]^-$ anion with Astra Phloxine (R) reagent and its extraction using the DLLME procedure with subsequent UV–VIS spectrophotometric and graphite furnace atomic absorption spectrometric detection. The optimum conditions were found to be: pH 3; 0.8 mmol L$^{-1}$ $K_4[Fe(CN)_6]$; 0.12 mmol L$^{-1}$ R; dispersive solvent, methanol; extraction solvent, toluene; auxiliary solvent, tetrachloromethane. The calibration plots were linear in the ranges 0.39–4.7 mg L$^{-1}$ and 0.5–39.4 μg L$^{-1}$ for UV–VIS and GFAAS detection, respectively; thus enables the application of the developed method in two ranges differing from one from another by three orders of magnitude. The presented approach can be applied to the development of DLLME procedures for the determination of other compounds extractable by organic solvents with a density lower than that of water.

## 1. Introduction

Progress in analytical chemistry focuses mainly on the improvement of parameters such as accuracy, precision and sensitivity. However, a great deal of attention has recently been given to developing new analytical methods that are environmentally friendly, or “green”. Green analytical methods are characterised by (1) reduced consumption of solvents in both the sample pre-treatment and measurement steps, which can be achieved by automation or miniaturisation, and (2) by replacing hazardous reagents [1]. Conventional liquid–liquid extraction (LLE) has a series of well-known drawbacks, such as the use of large sample volumes and hazardous organic solvents, the subsequent generation of large amounts of pollutants [2], the necessity for human handling and excessive workplace exposure to organic vapours. As a result, since the implementation of solid phase extraction (SPE), conventional LLE has been overshadowed, though it is currently undergoing further development, as is demonstrated by novel miniaturized preconcentration techniques which use only small volumes of organic solvents [2].

One of these techniques is dispersive liquid–liquid microextraction (DLLME), in which a solvent system containing a mixture of water-immiscible extraction solvent and water-miscible disperser solvent is injected rapidly by a syringe into an aqueous sample, which then allows the analyte to be extracted into fine droplets of extraction solvent during the formation of cloudy solution [2–5]. The main advantage of DLLME lies in the formation of a large surface area between the water sample and the extraction solvent, which allows for the rapid achieving of a state of equilibrium and thus significantly shortens the extraction time. After centrifugation, the settled fine droplets are removed and analysed, usually by chromatography or spectrometry. As other researchers have previ-

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ously stated in articles and reviews [2,4,5], the extraction solvent must have a density higher than that of water due to the simple separation of phases by centrifugation, and the disperser solvent has to be highly miscible in both aqueous phase and the extraction solvent. This is why the extraction solvent requirements are the main limitation of the DLLME technique [5]. In a review of the literature, we found an interesting article, written by Anthemidis et al., in which the formation of the cloudy solution takes place in a moving stream and the separation of the fine droplets of organic phase after DLLME procedure is based on retention, not centrifugation. The hydrophobic droplets of organic phase were retained into a microcolumn packed with PTFE and quantitatively eluted by isobutylmethylketone and transported directly to the FAAS nebulizer [6].

Initially, the DLLME technique was developed for the analysis of organic compounds. Recently, however, this extraction technique has been more often used for the determination of inorganics and for speciation analysis [2,5], mainly with a variety of spectrometric detection methods such as graphite furnace atomic absorption spectrometry (GFAAS), flame atomic absorption spectrometry (FAAS) or UV–VIS spectrophotometry, the latter method being the least used. In analysis of inorganics, the organic phase also frequently contains a chelating agent in addition to the extraction solvent and disperser solvent. Despite the wide use of chelating reagents, we found only one article based on the DLLME of an ion pair (ion associate): Shamsipur and Ramezani modified the long-known reaction of gold with Victoria Blue R [7] and developed the quantitative separation and preconcentration of gold by DLLME using this reagent, followed by its determination with GFAAS [8]. The limited use of the connection of DLLME with the ion pair formation as well as with UV–VIS spectrophotometric detection is probably the result of the above-mentioned requirements regarding the density of the extraction solvents. In general the most commonly applied extraction solvents in DLLME are chlorobenzene, chloroform, tetrachloromethane and tetrachloroethylene [4]. Non-polar solvents such as tetrachloromethane are not able to extract the ion pairs, while strongly polar solvents, such as chlorobenzene or chloroform, will extract not only ion pairs, but the single salt of the dye reagent as well, thus enhancing the blank test. In addition, they can extract interferents, causing the reduced selectivity of the determination.

Besides the mentioned method with Victoria Blue R [8], only one another method has been described for Au determination using the DLLME procedure. This method is based on the reaction of gold(III) with dicyclohexylamine followed by electrothermal atomic absorption spectrometry detection [9]. No article was found in literature in which DLLME was combined with a UV–VIS detection technique for the determination of gold.

This paper presents a method for determination of gold based on the dispersive liquid–liquid microextraction of the ion pair of $[Au(CN)_2]^-$ complex anion with Astra Phloxine (R) reagent (Fig. 1) with subsequent UV–VIS spectrophotometric and graphite furnace atomic absorption spectrometric detection. To the best of our knowledge, for the first time an auxiliary solvent (tetrachloromethane) has been used for adjustment of the density of the mixture of solvents, and the same reaction has been used in DLLME for both UV–VIS and GFAAS detection techniques, which thus enables the application of the developed method in two ranges differing from one another by three orders of magnitude.

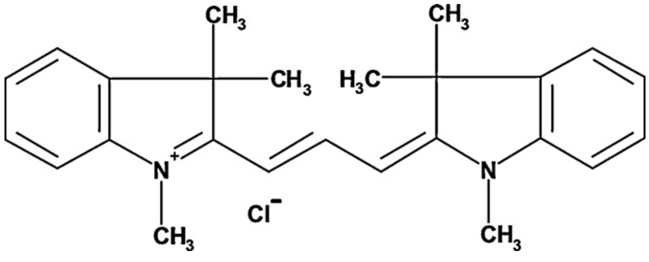

**Fig. 1.** The structure of Astra Phloxine.

**Table 1**
Graphite furnace-heating program.

| Step | Temperature (°C) | Ramp time (s) | Hold time (s) | Internal flow (mL min$^{-1}$) |
|---|---|---|---|---|
| Drying | 120 | 1 | 20 | 250 |
| Drying | 140 | 5 | 20 | 250 |
| Pyrolysis | 800 | 10 | 10 | 250 |
| Atomisation | 1800 | 0 | 3 | 0 |
| Cleaning | 2450 | 1 | 3 | 250 |

## 2. Experimental

### 2.1. Reagents

All chemicals and solvents used were of analytical grade purity (unless stated otherwise). Ultra pure water from Millipore Milli-Q RG (Millipore, USA) was used throughout the work. Gold(III) chloride trihydrate ($HAuCl_4 \times 3H_2O$) was purchased from Sigma–Aldrich. The Astra Phloxine dye reagent was obtained from Jiacheng-Chem Enterprises Ltd. (China); the organic solvents amylacetate, ethylacetate and benzene from Fluka; toluene (for UV spectroscopy) from Slavus (Slovakia); methanol from Merck; $CCl_4$ from Sigma–Aldrich; acetone (puriss) and ethanol (96% for UV spectroscopy) from Spektrum 3D (Hungary). A stock solution containing 0.01 mol L$^{-1}$ of Au(III) was prepared by dissolving $HAuCl_4 \times 3H_2O$ in 1 mol L$^{-1}$ HCl and standardised gravimetrically [10]. The working solutions ($4 \times 10^{-5}$–$1 \times 10^{-3}$ mol L$^{-1}$ Au) were prepared by appropriate dilution of the stock solution with water prior to use. A 1 mmol L$^{-1}$ Astra Phloxine (R) solution was prepared by dissolving 0.0393 g of reagent in 0.5–1 ml methanol and diluting with water up to 100 mL.

### 2.2. Apparatus

A Lambda 35 UV–VIS scanning spectrophotometer (Perkin Elmer, USA) and a Spekol 11 spectrophotometer (Carl Zeiss, Germany) with matched quartz cells of appropriate path length or ultra-micro cells of 10 mm path length were used for recording the absorbance spectra and daily routine absorbance measurements.

Atomic spectrometric measurements were carried out using a Perkin Elmer AAnalyst 600 atomic absorption spectrometer equipped with an AS 800 autosampler device and a transversely heated graphite atomizer (THGA). A Cathodeon type 3QNY/Au hollow cathode lamp operated at 10 mA was used. Argon (99.99% purity) obtained from Messer was used as a carrier gas. The wavelength at 242.8 nm was used with a slit 0.7 nm in width. For correcting non-specific absorbance, a Zeeman background correction was used throughout. The graphite furnace-heating program used in this study is given in Table 1. In order to avoid contamination in AAS measurements, all vessels used were washed with 0.1 mol L$^{-1}$ $HNO_3$.

Laser desorption ionisation mass spectrometry (LDI-MS) measurements were performed with a Bruker Biflex III (Daltonics, Germany) mass spectrometer equipped with a time of flight (TOF) analyser. A total acceleration voltage of 19 kV was used with 3 kV pulse (extraction) voltage and with a delay time of 200 ns. A nitrogen laser (337 nm, 3 ns pulse width, $10^6$–$10^7$ W/cm$^2$) operating at 4 Hz was used to produce laser desorption, and 500 shots were summed. The sample solution (0.5–1.0 μL) was dropped onto the stainless steel sample table and allowed to dry. Mass spectra were recorded in linear negative ion mode as well as in reflectron mode.

Centrifugation was performed using a Z 323 K centrifuge (Hermle Labortechnik, Germany), a Heraeus Biofuge Stratos microlitre centrifuge (Kendro Laboratory Products, Germany) or a KA 1455-2 centrifuge (Zuglói Gépgyár, Hungary) depending on the volumes of the samples.

## 3. General procedure

### *3.1. Conventional liquid–liquid extraction procedure*

Appropriate volumes of the $HAuCl_4$ working solution with a proper concentration of gold were pipetted into glass test tubes. Then 0.4 mL of 10 mmol $L^{-1}$ $K_4[Fe(CN)_6]$ and 0.6 mL of 1 mmol $L^{-1}$ Astra Phloxine were added; the desired pH of 3 was reached, and the volumes filled up to 5 mL with water. After each reagent was added, the mixture was shaken well. Finally, 5 mL toluene was added, and extraction by manual shaking for 1 min was carried out. After the two phases separated, the absorbance of the organic layer was measured against that of a blank test.

### *3.2. Dispersive liquid–liquid microextraction procedure*

A 5-mL sample solution containing Au(III) as well as all the necessary reagents in concentrations described above in Section 3.1 were prepared in conical micro centrifugal tubes. Then 0.5 mL of a mixture of solvents containing methanol as disperser solvent as well as 145 μL of toluene as extraction solvent and 145 μL of $CCl_4$ as auxiliary solvent was vigorously injected using a 0.5 mL glass syringe. Afterwards, the mixture was gently shaken 3 times and centrifuged at 3000 rpm for 2 min. When finished, a layer of sediment (ca. 250 μL) containing the mixture of toluene and $CCl_4$ was found in the bottom of each tube. This was removed by a proper Hamilton syringe and inserted into matched quartz cell or ultramicro cell of appropriate path length (for UV–VIS measurement) or transferred into graphite atomizer (for AAS measurement).

## 4. Results and discussion

### *4.1. Theoretical aspects and reaction mechanism*

It has been previously confirmed [11] by voltamperometric and polarographic studies that the reaction between $[AuCl_4]^-$ and $[Fe(CN)_6]^{4-}$ gives rise to the $[Au(CN)_2]^-$ complex, as can be seen from the reactions:

$$3\,[Au^{III}Cl_4]^- + 6\,[Fe(CN)_6]^{4-} = 3\,[Au^{I}Cl_2]^- + 6\,[Fe(CN)_6]^{3-} + 6\,Cl^-$$

$$3\,[Au^{I}Cl_2]^- + [Fe(CN)_6]^{3-} = 3\,[Au^{I}(CN)_2]^- + Fe^{3+} + 6\,Cl^-$$

and the final reaction:

$$3\,[Au^{III}Cl_4]^- + 6\,[Fe(CN)_6]^{4-} = 3\,[Au^{I}(CN)_2]^- + 5\,[Fe(CN)_6]^{3-} + Fe^{3+} + 12\,Cl^-$$

Consequently, besides the reduction of Au(III) to Au(I), a complex-forming reaction (the forming of $[Au(CN)_2]^-$ anion) has taken place. Formation of the $[Au(CN)_2]^-$ complex can also be confirmed by calculations based on standard potentials of the $AuCl_4^-/Au$, $AuCl_2^-/Au$, $Au(CN)_2^-/Au$, $AuCl_4^-/AuCl_2^-$, $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ redox systems, as well as on the stability constants of the $[AuCl_2]^-$, $[Au(CN)_2]^-$, and $[Fe(CN)_6]^{3-}$ complex ions [11]. Of all the known cyanide complexes, the $[Au(CN)_2]^-$ complex has the highest stability constant ($\beta = 38.3$), which repeatedly confirms the formation of dicyanoaurate complex anion.

After these redox and complex-forming reactions have run in aqueous phase, the procedure is followed by ion pair formation and extraction, which can be expressed by the following scheme:

$$[Au(CN)_2]^-{}_{(aq)} + R^+{}_{(aq)} = [Au(CN)_2]^-R^+{}_{(aq)}$$

$$[Au(CN)_2]^-R^+{}_{(aq)} + nS_{(org)} = [Au(CN)_2]^-R^+ \times nS_{(org)}$$

where aq is the aqueous phase, org means the organic phase and S is the organic solvent.

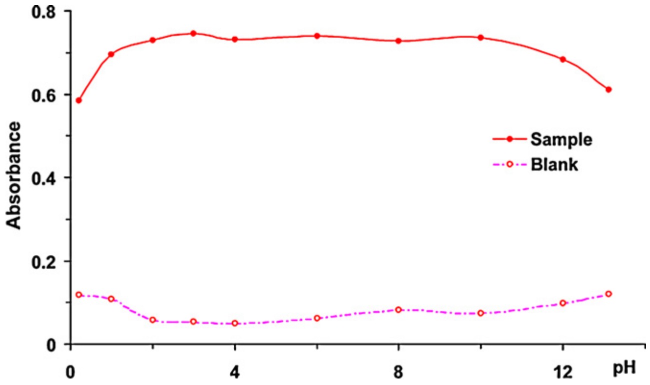

**Fig. 2.** The effect of the pH of the aqueous phase on the extraction of $[Au(CN)_2]^-\,R^+$ ion pair by toluene $2 \times 10^{-5}$ mol $L^{-1}$ Au(III), 0.8 mmol $L^{-1}$ $K_4[Fe(CN)_6]$, 0.12 mmol $L^{-1}$ R; $V_{(aq)} = V_{(org)} = 5$ mL; $l = 3$ mm; $\lambda = 556$ nm.

### *4.2. Investigation of appropriate experimental conditions*

Various factors influencing the ion pair formation of gold with R (pH, the concentration of ferrocyanide ions and dye reagent), as well as factors influencing the dispersive liquid–liquid microextraction procedure (the nature of the extraction solvent, auxiliary solvent, disperser solvent and extraction time), were studied. In addition, the main spectrophotometric characteristics of the extracted ion pairs were established.

#### *4.2.1. Effect of pH*

The concentration of $H^+$ ions can markedly influence some of the reaction equilibrium (see Section 4.1). To gain knowledge about the effect of the pH on the absorbance of the extracted ion pairs, a pH in the range from 0.2 to 13 was examined. An experiment was carried out as described in Section 3. The required concentration of $H^+$ ions for various pH levels was reached by the addition of HCl (in the range of pH 0.2–3) or NaOH (in the range of pH 10–13) having the appropriate concentrations, and a 1 mol $L^{-1}$ $HOAc–NH_4OH$ buffer solution (in the range of pH 4–9). As seen in Fig. 2, the absorbance of the extracted ion pairs is the highest (and at the same time the absorbance of the blank is the lowest) in the range from pH 2 to 10. A decrease in absorbance at pH < 2 can be explained by protonation of the complex anion of analyte and formation of poorly dissociated $HAu(CN)_2$ [12], which is not able to form an ion pair with R that is extractable into organic solvents. The decrease in absorbance at pH > 12 is probably due to the hydrolysis of dye. For further study, a pH of 3 was chosen.

#### *4.2.2. Effect of ferrocyanide ions*

Besides the acidity of the medium, the concentration of ferrocyanide ions also plays an important role in the formation of $[Au(CN)_2]^-$ complex anion and consequently in the extraction of Au(I) ion pairs with R. The effect of $K_4[Fe(CN)_6]$ concentration up to 1.2 mmol $L^{-1}$ was studied (Fig. 3). The extraction of gold in the absence of ferrocyanide is probably caused by the extraction of Au(III) in the form of an ion pair of $[AuCl_4]^-$ with R. The addition of ferrocyanide in the first step caused a decrease in absorbance, and in further steps an increase. This phenomenon can be explained by the

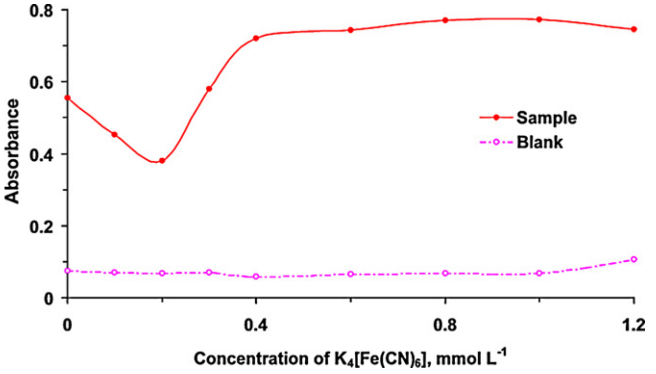

**Fig. 3.** The effect of the concentration of ferrocyanide ions on the extraction of $[Au(CN)_2]^-$ $R^+$ ion pair by toluene $2 \times 10^{-5}$ mol $L^{-1}$ Au(III), pH 3, 0.12 mmol $L^{-1}$ R; $V_{(aq)} = V_{(org)} = 5$ mL; $l = 3$ mm; $\lambda = 556$ nm.

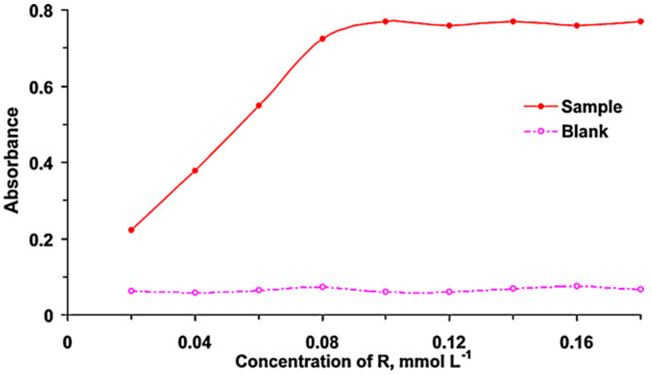

**Fig. 5.** The effect of the concentration of Astra Phloxine on the extraction of $[Au(CN)_2]^-$ $R^+$ ion pair by toluene $2 \times 10^{-5}$ mol $L^{-1}$ Au(III), pH 3, 0.8 mmol $L^{-1}$ $K_4[Fe(CN)_6]$; $V_{(aq)} = V_{(org)} = 5$ mL; $l = 3$ mm; $\lambda = 556$ nm.

reduction of Au(III) to Au(I) (see first reaction in Section 4.1) and the formation of $[AuCl_2]^-$, which is extracted poorly with R into organic solvents in comparison with $[AuCl_4]^-$, probably due to the different hydrations of these two anions. The hydration energy of $[AuCl_2]^-$ is significantly higher than that of $[AuCl_4]^-$ [13], thus influencing the extraction of complex anion. It is important to emphasise that further addition of ferrocyanide ions leads to an increase in extraction efficiency of approximately 40%, probably due to the formation of $[Au(CN)_2]^-$, which is extracted better with R into organic solvents. The $[Au(CN)_2]^-$ can be much more easily extracted than $[AuCl_2]^-$ and $[AuCl_4]^-$ complex ions. The existence of both $[AuCl_2]^-$ and $[Au(CN)_2]^-$ complexes in aqueous phase was verified by the LDI-TOF-MS method, which during the past decade has been successfully used for speciation analysis in solution [14]. It is important to note that only $[Au(CN)_2]^-$ was found in the organic phase (Fig. 4), thus proving that the proposed reaction mechanism is correct. As a result (Fig. 3), a concentration 0.8 mmol $L^{-1}$ of $K_4[Fe(CN)_6]$ was chosen for further experiments.

### 4.2.3. Effect of the concentration of Astra Phloxine

The effect of the concentration of Astra Phloxine on the extraction of the Au(I) ion pairs in the range 0.02–0.18 mmol $L^{-1}$ of R was studied (Fig. 5). The absorbance of the extracts was the highest at concentration of R more than 0.08 mmol $L^{-1}$. A concentration 0.12 mmol $L^{-1}$ of R was thus chosen for further studies.

### 4.2.4. Effect of an organic solvent

The absorbance of the extracted ion pairs greatly depends on the nature of the organic solvent. Aliphatic and aromatic hydrocarbons, higher alcohols, ketones, acetic esters and halogen derivatives of aliphatic and aromatic hydrocarbons were tested as extractants. For efficient extraction, the organic solvent should thoroughly dissolve the ion pair formed and at the same time dissolve the simple salt of the dye to the least extent possible. Therefore, the choice of organic solvent was based on the following characteristics: molar absorptivity, recovery percentage and signal-to-noise ratio. The absorption spectra of the Au(I) ion pairs during the extraction by various organic solvents under optimum experimental conditions were recorded (Fig. 6), and the main spectrophotometric characteristics were calculated (Table 2). The best extractants appeared to be the aromatic hydrocarbons and acetic esters, both of which showed very good extractability of the ion pairs. The recovery percentages varied from 72.1 (*o*-xylene) and 77.2 (ethyl acetate) to 86.7 (benzene) and 86.8 (butyl acetate). The molar absorptivity of Au(I) ion pairs with R decreases in the following order: benzene > toluene > xylene and ethyl acetate > butyl acetate > amyl acetate. The signal-to-noise ratios were better for the aromatic hydrocarbons. The main drawback of acetic esters in comparison with aromatic hydrocarbons is their higher solubility in water. Another limitation of their use may arise from the higher value of the blank test, which can be explained by their higher solvation energy in comparison with aromatic hydrocarbons [15]. A comparison of the results obtained (molar absorptivity, recovery percentage

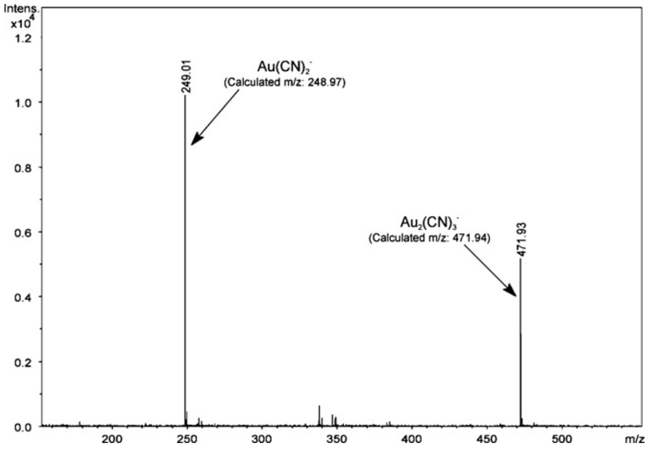

**Fig. 4.** The negative reflectron LDI-TOF MS spectrum of the organic phase. The compositions and the calculated *m*/*z* values are indicated.

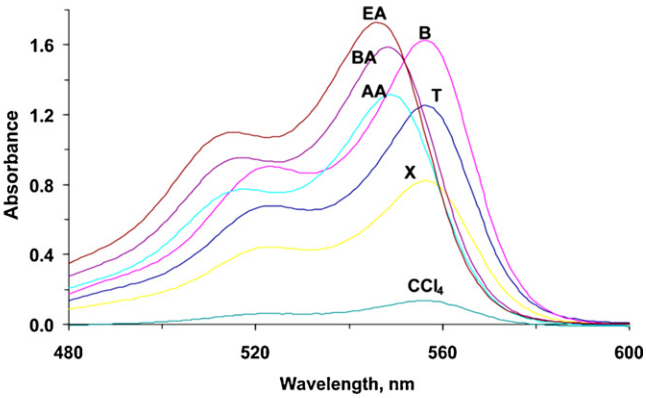

**Fig. 6.** The spectra of Au(I) ion pairs with Astra Phloxine during extraction with benzene (B), toluene (T), xylene (X), amyl acetate (AA), butyl acetate (BA), ethyl acetate (EA) and tetrachloromethane ($CCl_4$) measured against that of a blank test $2 \times 10^{-5}$ mol $L^{-1}$ Au(III), pH 3, 0.8 mmol $L^{-1}$ $K_4[Fe(CN)_6]$, 0.12 mmol $L^{-1}$ R; $V_{(aq)} = V_{(org)} = 5$ mL; $l = 5$ mm.

**Table 2**
Spectrophotometric characteristics of the extracted ion pairs of Au(I) with Astra Phloxine.

| Extractant | λ (nm) | $\varepsilon \times 10^{-4}$ ($\mathrm{L\,mol^{-1}\,cm^{-1}}$) | $R$[a] (%) | $A_x/A_0$[b] |
|---|---|---|---|---|
| Benzene | 556 | 16.3 | 86.7 | 12.5 |
| Toluene | 556 | 12.5 | 81.9 | 24.0 |
| Xylene | 557 | 8.19 | 72.1 | 40.1 |
| Amyl acetate | 549 | 13.1 | 84.1 | 11.3 |
| Butyl acetate | 548 | 15.8 | 86.8 | 8.2 |
| Ethyl acetate | 546 | 17.3 | 77.2 | 5.6 |

[a] $R$ is the recovery of the extraction procedure calculated according to following equation $R\% = (A_1/(A_1+A_2)) \times 100$ where $A_1$ is the absorbance of the organic phase after the first extraction, $A_2$ is the absorbance of the organic phase after the second extraction.

[b] $A_x/A_0$ is the ratio of the absorbance of the extracted ion pair and absorbance of the blank test.

**Table 3**
Effect of foreign ions (1.97 mg $\mathrm{L^{-1}}$ Au).

| Foreign ion | Au found ($\mathrm{mg\,L^{-1}}$) | Au:ion | Error (%) |
|---|---|---|---|
| Ag(I) | 2.03 | 1:50 | 3.0 |
| | 2.09 | 1:60 | 6.1 |
| Cd(II) | 1.92 | 1:65 | −2.5 |
| | 1.86 | 1:80 | −5.6 |
| Co(II) | 2.05 | 1:55 | 4.1 |
| | 2.12 | 1:70 | 7.6 |
| Cu(II) | 2.04 | 1:50 | 3.6 |
| | 2.15 | 1:60 | 9.1 |
| Ni(II) | 1.90 | 1:50 | −3.5 |
| | 1.82 | 1:70 | −7.6 |
| Tl(III) | 2.06 | 1:50 | 4.6 |
| | 2.11 | 1:60 | 7.1 |
| Zn(II) | 1.93 | 1:50 | −2.0 |
| | 1.87 | 1:60 | −5.1 |
| Pd(II) | 2.05 | 1:50 | 4.1 |
| | 2.14 | 1:65 | 8.6 |
| Pt(IV) | 2.03 | 1:55 | 3.0 |
| | 2.11 | 1:65 | 7.1 |
| Hg(II) | 1.92 | 1:50 | −2.5 |
| | 1.87 | 1:70 | −5.1 |
| Fe(II) | 1.94 | 1:50 | −1.5 |
| | 1.87 | 1:100 | −5.1 |
| Fe(III) | 1.92 | 1:50 | −2.5 |
| | 1.87 | 1:70 | −5.1 |
| Ga(III) | 2.02 | 1:100 | 2.5 |
| In(III) | 2.03 | 1:100 | 3.0 |
| Cr(III) | 2.01 | 1:100 | 2.0 |
| Pb(II) | 1.93 | 1:50 | −2.0 |
| | 1.81 | 1:100 | −8.1 |
| Al(III) | 2.06 | 1:1000 | 4.6 |
| Mg(II) | 2.08 | 1:1000 | 5.6 |
| Ca(II) | 2.05 | 1:1000 | 4.1 |
| Na(I), K(I) | 2.04 | 1:2000 | 3.6 |
| $\mathrm{Cl^-}$ | 2.01 | 1:1000 | 2.0 |
| $\mathrm{Br^-}$ | 2.04 | 1:500 | 3.6 |
| $\mathrm{CH_3COO^-}$ | 1.92 | 1:200 | −2.5 |
| $\mathrm{SO_4{}^{2-}}$ | 1.90 | 1:1000 | −3.5 |
| $\mathrm{NO_3{}^-}$ | 2.06 | 1:100 | 4.6 |
| $\mathrm{H_2PO_4{}^-}$ | 1.88 | 1:40 | −4.6 |

and signal-to-noise ratio) demonstrated that benzene is the best organic solvent for the extraction of the Au(I) ion pair. However, due to its lower toxicity, toluene was used as an extractant for further experiments.

### 4.2.5. Interferences

Various ions were tested as possible interferents. The stability constant of $[\mathrm{Fe(CN)_6}]^{4-}$ is substantially higher ($\beta = 36.9$) than those of cyanide complexes of silver ($\beta = 19.42$), cadmium ($\beta = 17.11$), cobalt ($\beta = 19.09$), copper(I) ($\beta = 30.03$), nickel ($\beta = 30.03$), thallium ($\beta = 35$) and zinc ($\beta = 19.62$). For this reason, these metal ions are not able to form cyanide complexes in the presence of $[\mathrm{Fe(CN)_6}]^{4-}$. This therefore allows for the selective determination of gold without interference from a 50–100-fold excess of the above-mentioned elements. In addition, the oxidation states of palladium(II), platinum(IV), thallium(III), mercury(II) and cobalt(II) ions tend to change in the presence of $\mathrm{K_4[Fe(CN)_6]}$, and consequently, these elements also do not interfere with the determination. The results of the study of interferences are summarized in Table 3. The tolerable amount of each ion was taken as an Au:interference ion ratio that resulted in an error not exceeding ±5%.

## 4.3. Investigation of the dispersive liquid–liquid microextraction procedure

The vigorous addition of a mixture of three solvents – methanol, tetrachloromethane, and toluene – into the aqueous phase leads to the formation of a cloudy solution and thereby to the extraction of Au(I) ion pair with R into the fine droplets of toluene dispersed into the aqueous sample. In general, the effect of the nature of the extraction solvent and disperser solvent used as well as extraction time were studied in this step.

### 4.3.1. Choice of extraction solvent

The requirements for the extraction solvent in DLLME are the same as in conventional LLE, namely low solubility in water and high efficiency for extraction of the target analyte [16]. In the case of UV–VIS detection there is one more requirement, namely minimum extraction of the blank. Therefore, the influence of the extraction solvent was achieved using conventional LLE, and the results obtained are described in Section 4.2.4 above. From these facts it is clear that the aromatic hydrocarbons (benzene and toluene) are the most suitable extraction solvents.

### 4.3.2. Choice of auxiliary solvent

Toluene ($0.8669\,\mathrm{g\,cm^{-3}}$), which seems to be the best extraction solvent, has a density lower than that of water, thus making its use in conventional DLLME (without the use of special vessels or additional steps) impossible. For this reason we used not one solvent, but a mixture of two solvents, for the extraction procedure. One of these (toluene) ensures the efficient extraction of the target analyte; the second (tetrachloromethane) serves as an auxiliary solvent and ensures that the mixture's density is higher than that of water in order to allow for a simple phase separation by centrifugation. When choosing an auxiliary solvent, the following parameters were taken into account: (a) density, (b) solubility in water and (c) ability to extract the reagent. (a) Solvents having a density markedly higher than that of water, such as chlorobenzene ($1.107\,\mathrm{g\,cm^{-3}}$), chloroform ($1.483\,\mathrm{g\,cm^{-3}}$), 1,2-dichloroethane ($1.253\,\mathrm{g\,cm^{-3}}$), and tetrachloromethane ($1.595\,\mathrm{g\,cm^{-3}}$), were considered as auxiliary solvents in order to make it possible to prepare a mixture of solvents with a density higher than that of the aqueous phase while using minimal volumes of auxiliary solvent. (It is important to note that since our aim was to develop a versatile method, it is suitable to prepare a mixture of solvents with a density markedly higher than that of water, because in some cases the density of the aqueous phase may itself be higher than that of water, if, for example the aqueous phase contains a great deal of dissolved salts, etc.) (b) The solubility of the auxiliary solvent in water should be as low as possible in order to ensure better separation of aqueous and organic phase after extraction. (c) In addition, the auxiliary solvent should not extract the Astra Phloxine used, or should do so only to the least possible extent, in order to achieve the best possible signal-to-noise ratio. When extracting the reagent from the aqueous phase with solvents considered as auxiliary solvents, the highest absorbance observed was for 1,2-dichloroethane; the absorbance were lower for other solvents (given as percentage) of the absorbance obtained for 1,2-dichloroethane: chlorobenzene (68.8%), chloroform (31.3%),

**Table 4**
Precision and accuracy data for the determination of Au using GFAAS detection.

| Added | Determined[a] ($\mu$g L$^{-1}$) | RSD (%) | *R* (%) |
|---|---|---|---|
| 1.00 | 1.03 ± 0.05 | 3.9 | 103.0 |
| 12.70 | 12.87 ± 0.30 | 1.9 | 101.3 |
| 25.30 | 26.03 ± 0.80 | 2.5 | 102.9 |
| 38.07 | 39.18 ± 1.20 | 2.5 | 102.9 |

[a] Confidence limit $\Delta x = st/\sqrt{n}$ where *s* means standard deviation, *t* is Student coefficient for $n-1$ degrees of freedom; RSD%, relative standard deviation percentage; *R*%, recovery percentage.

and tetrachloromethane (6.3%). Given these results, $CCl_4$, which is characterised by high density and low solubility in water and, more importantly, extracts the Astra Phloxine reagent to the least extent, was chosen as the auxiliary solvent for further experiments.

### 4.3.3. *The effect of disperser solvent*

The disperser solvent has to be highly miscible with both water and the extraction solvent. Therefore, acetone, ethanol and methanol were tested as disperser solvents. Sample solutions containing Au(III) as well as all necessary reagents under optimum experimental conditions were treated with 0.5 mL of solvent mixture containing the disperser solvent being investigated as well as 145 $\mu$L of toluene and 145 $\mu$L of $CCl_4$, and were analysed as described in Section 3.2. The best disperser solvent seems to be methanol due to the lowest value of the blank test among those solvents investigated.

### 4.3.4. *Extraction time*

The effect of extraction time (the time interval between injection of the extraction mixture and the beginning of centrifugation) in DLLME of gold using Astra Phloxine was examined step-wise in 0.5 min increments up to 5 min. A sample solution containing Au(III) and all the required reagents at optimum concentration levels (pH 3; 0.8 mmol L$^{-1}$ $K_4[Fe(CN)_6]$, 0.12 mmol L$^{-1}$ R) were added into different test tubes. The mixture of organic solvents was then injected into each test tube and analytical signals were measured by means of the UV–VIS or GFAAS method. Results clearly revealed that the extraction time does not significantly affect the efficiency of DLLME of gold. This is in good accordance with the observations of other researchers published in the literature that DLLME is a very fast process, a fact which can be considered as an important advantage of this technique. In later experiments, the phase separation by centrifugation was performed immediately after mixing the reagents and gentle manual shaking. Despite the fact that the DLLME process is rapid and begins immediately after adding the mixture of solvents, centrifugation facilitates a better extraction, which is evident in the video and photo (Supplementary material).

### 4.3.5. *Validation*

The suggested method was evaluated under the selected experimental conditions by checking the calibration range, accuracy and precision. The calibration plots were linear in the ranges 0.39–4.7 mg L$^{-1}$ and 0.5–39.4 $\mu$g L$^{-1}$ for UV–VIS and GFAAS detection, respectively. The standard addition principle (the model samples spiked with various known amounts of Au) was used to evaluate the precision (characterised by relative standard deviation percentage, RSD%) and accuracy (characterised by the recovery percentage, *R*%) of the suggested method; the results obtained are given in Table 4.

### 4.3.6. *Analytical application*

Recently, the application of gold and nano-gold to many fields of science and technology has been intensively studied and has been the subject of numerous published articles [17,18]. Gold and its compounds have been applied as a form of medication since ancient times for the treatment of several diseases [18]. As a real sample (obtained from Dr. József Jekö), an intermediate product from the production of Nano Gold was used, which results from dissolving 99.9% pure Au$^0$ in aqua regia, evaporating the solution to dryness and then subsequently dissolving it in ultrapure water. Gold prepared in this way as Au(III) was subsequently subjected to analysis. The results obtained are given in Table 5. The suggested method was also applied to the analysis of a real pharmaceutical sample containing sodium aurothiomalate as the active component. The content of one ampoule was diluted with 100 mL water. A 1 mL of this volume was then drawn off and 2 mL 1 mol L$^{-1}$ HCl and 3 mL 30% $H_2O_2$ were added to it. The mixture was thoroughly shaken and heated for 30 min in a water bath. Afterwards, the mixture was cooled and diluted. Various aliquots were then analysed using the suggested DLLME procedure. The results obtained are given in Table 6.

**Table 5**
Application of the suggested method to the determination of Au in an intermediate product from the production of Nano Gold.

| Labeled | Determined | RSD (%) | *R* (%) |
|---|---|---|---|
| UV–VIS (mg L$^{-1}$) | | | |
| 0.90 | 0.94 ± 0.05 | 4.3 | 104.4 |
| 3.15 | 3.02 ± 0.14 | 3.7 | 95.9 |
| 4.14 | 4.04 ± 0.12 | 2.4 | 97.6 |
| GFAAS ($\mu$g L$^{-1}$) | | | |
| 1.97 | 1.87 ± 0.20 | 5.2 | 94.9 |
| 5.90 | 6.10 ± 0.20 | 2.6 | 103.4 |
| 15.76 | 15.24 ± 0.60 | 3.2 | 96.7 |

**Table 6**
Determination of Au in a real pharmaceutical sample.

| Calculated (mg L$^{-1}$) | Determined (mg L$^{-1}$) | RSD (%) | *R* (%) |
|---|---|---|---|
| 0.92 | 0.96 ± 0.04 | 3.4 | 104.3 |
| 1.84 | 1.90 ± 0.06 | 2.5 | 103.3 |
| 2.76 | 2.69 ± 0.07 | 2.1 | 97.5 |

## 5. Conclusion

Recently, articles have been discovered which describe other approaches, for example a dispersive liquid–liquid microextraction method based on the solidification of a floating organic drop (DLLME-SFO) [19,20]. After preparing the final version of this manuscript, we found a few of articles devoted to using a mixture of toluene and carbon tetrachloride as extraction solvent [21] and to using solvents lighter than water as extraction solvent [22–24]. While it is true that in [21] “using the mixture of toluene and carbon tetrachloride as extraction solvent” was described “to reduce the use of chlorinated solvent ($CCl_4$) and study of possibility of using less toxic solvents”, this is actually done for very different purposes than those indicated in our work. In [22] the use of a solvent lighter than water is made possible by designing a new special extraction vessel and by an additional step “elevating the sample surface by injecting 1 mL distilled water through the septum in the bottom of glass test tube by the use of a syringe”. It is important to note that centrifugation is also needed in this process. In [23] a capillary tube was used to collect the extraction solvent lighter than water after DLLME, and in [24] the addition of terminating solvent to break up the emulsion is needed.

In our opinion, the above-mentioned approaches complicate the entire procedure, and are probably not suitable for the extraction of ion pairs with subsequent spectrophotometric detection. Our procedure could thus be an alternative to the procedures described in the literature in recent months and surely offers another view on solving the problem of using solvents less dense than water in DLLME. It is important to note that in the procedure we are suggesting, one of the main advantages of DLLME is preserved, namely the possibility of simply separating the phases by centrifugation. The presented approach can thus be applied to the development of DLLME procedures for determination of other compounds.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2010.08.028.

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