

## A Sensitive Spectrophotometric Determination of Silver(I) with Blue Tetrazolium Chloride

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**Summary:** Silver(I)-assisted reduction of Blue tetrazolium chloride (3,3'-(3,3'-dimethoxy[1,1'-biphenyl]-4,4'-diyl)-bis(2,5-diphenyl-2H-tetrazolium) dichloride, BTC) by semicarbazide hydrochloride (SCH) was investigated in water-chloroform medium. The obtained colored products can be used for the visual detection and sensitive liquid-liquid extraction-spectrophotometric determination of Ag(I). Under the optimum conditions the calibration curve (which can be best approximated by a third-order polynomial;  $R^2=0.9990$ ) has two linear segments. For Ag(I) concentrations up to 0.011  $\mu\text{g/ml}$ , the linear regression equation had an intercept that was statistically indistinguishable from zero. The limit of detection, limit of quantitation, and molar absorptivity coefficient at  $\lambda_{\text{max}}=573$  nm were 0.6 ng/ml, 2 ng/ml and  $1.2 \times 10^6$  l/(mol·cm), respectively. The regression equation of the second linear segment (0.021–0.028  $\mu\text{g/ml}$ ) was  $A = 200\gamma - 3.5$  ( $R^2=0.9989$ ), where  $\gamma$  is the concentration in  $\mu\text{g/ml}$ . The relative standard deviation at the 22 ng/ml level ( $n = 5$ ) was 3.8 %. The effect of concomitant ions was studied, and the analysis of real samples tested the applicability of the developed procedure.

**Keywords:** Silver, Tetrazolium Salt, Formazan, Liquid-Liquid Extraction, Spectrophotometry, Determination.

### Introduction

Silver, a group 11 transition element, is a soft, ductile, and malleable metal, superior to all other metals in electrical conductivity, thermal conductivity, contact resistance and antimicrobial effects. It has been known for millennia and has undoubtedly played an important role in the development of human civilization. Nowadays silver finds applications in electronics, electrical engineering, optics, electroplating, photography, solar panels, disinfection installations, jewelry, coinage, catalytic processes, pharmaceuticals, and nanotechnology.

Geochemically, silver is classified as a rare metal. In terms of abundance, it ranks 67<sup>th</sup> among the elements in the earth's crust [1, 2] with a median content in its bulk of about 56 ng/g [3]. It is found in a free state (native silver), as an alloy with gold and other elements (copper, lead, cobalt), and in minerals usually containing sulfur, chlorine, copper, antimony, and arsenic [4, 5]. Most silver (*ca.* 83 %) is produced as a byproduct of lead, copper, and gold refining [5].

Various methods have been used to determine trace amounts of silver, including flame atomic absorption spectrometry, electrothermal atomic absorption spectrometry, inductively coupled plasma optical emission spectroscopy, and spectrophotometry.

The monotetrazolium salts 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) [6] and 2,5-diphenyl-3-(1-naphthyl)-2H-tetrazolium chloride (Tetrazolium violet, TV) [7] have been applied for the sensitive spectrophotometric determination of Ag(I) ions. Under certain conditions, these colorless (leuco) salts [8] are reduced by semicarbazide hydrochloride (SCH) to colored formazans. Trace amounts of silver are sufficient for the color reaction to occur, given that 1 mole of Ag(I) is responsible for converting 4 moles of the tetrazolium salt to formazan [6]. The sensitivity of the determination can be further increased by combining with liquid-liquid extraction, since the resulting formazans are sparingly soluble in water and can be easily extracted in small volumes of water-immiscible solvents [7, 9].

In the present study, we focus on the possibility of using the ditetrazolium salt Blue tetrazolium chloride (3,3'-(3,3'-dimethoxy[1,1'-biphenyl]-4,4'-diyl)-bis(2,5-diphenyl-2H-tetrazolium) dichloride, BTC) for the extractive spectrophotometric determination of Ag(I). BTC is a commercially available reagent used in many fields, such as histochemistry, biochemistry, corrosion protection, dosimetry, and analytical chemistry [9-15]. It is cheaper and more stable under the basic conditions

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[10, 16, 17] needed to "unlock" the color reaction for Ag(I) than other well-known ditetrazolium salts (e. g., Nitro blue tetrazolium and Tetranitro blue tetrazolium). As a ditetrazolium salt, BTC can form a diformazan (Fig. 1) which is more intensely colored than the formazans derived from monotetrazolium salts [10, 16]. In addition, the transformation to colored species is less dependent on the action of dissolved oxygen and light [10, 11, 18].

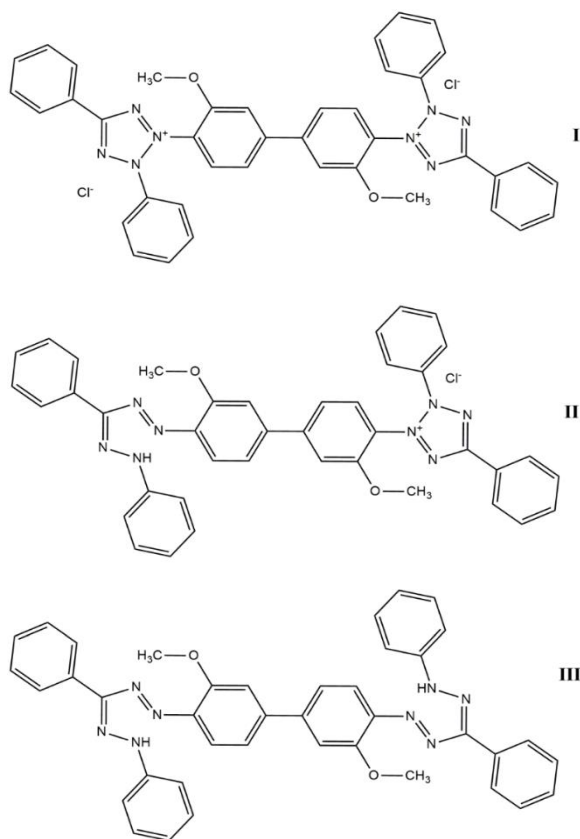


Fig. 1: Formulae of Blue tetrazolium chloride (BTC) (I), Blue tetrazolium half-formazan (II) and Blue tetrazole diformazan (III).

## Experimental

### Reagents and Instruments

The stock solution of Ag(I) was prepared from analytically pure AgNO<sub>3</sub> (Raj-Khim Product EOOD, Bulgaria) following Nagaraja et al. [6]. Working  $5 \times 10^{-6}$  mol/l Ag(I) solutions were obtained by diluting the stock solution. The other chemicals were supplied from Merck: BTC (for microbiology), SCH ( $\geq 99\%$ ) and NaOH (for analysis). Aqueous solutions of these chemicals were made at the following concentrations:  $2 \times 10^{-3}$  mol/l of BTC,  $6 \times 10^{-2}$  mol/l of SCH and 5 mol/l of NaOH. Deionized water

(18.2 M $\Omega$  cm, ELGA-Veolia LabWater, UK) was used throughout the work. Chloroform (Honeywell Riedel-de Haen) was redistilled and used repeatedly. Absorbance was recorded on an Ultrospec 3300 pro UV-Vis spectrophotometer (UK), supplied with 1 cm quartz cells. The pH was evaluated with a WTW Inolab 7110 pH meter.

### Procedure for Establishing the Optimum Conditions

Solutions of Ag(I), BTC, SCH and NaOH were introduced into a glass separatory funnel with a PTFE stopcock. The contents were diluted with water to a total volume of 25 ml and 4 ml of chloroform was added. The funnel was then shaken for a fixed period (1–5 min). A portion of the chloroform extract was filtered through a paper filter into the cell and the absorbance was measured against a blank. To achieve good repeatability, the separatory funnels were cleaned with 2 mol/l NaOH by shaking for ca. 1 min.

### Recommended Procedure for Silver(I) Determination

In a separatory funnel were successively added an aliquot of the analyzed solution, 0.5 ml of  $2 \times 10^{-3}$  mol/l BTC solution, and 1.5 ml of  $6 \times 10^{-2}$  mol/l SCH solution. Water and NaOH solution were added to increase the pH to  $12.2 \pm 0.2$  and the volume to 25 ml. Then 4 ml of chloroform was added, and the funnel was shaken for 2 min. A portion of the chloroform layer was poured into the cell and absorbance was recorded against a blank at  $\lambda = 573$  nm. The concentration of Ag(I) was determined using a calibration curve.

### Samples and Sample Preparation

Studying silver films for medical applications is a multi-step task involving the dissolution of the incorporated silver [19, 20]. For this purpose, silver films deposited under different experimental conditions on anodized aluminum alloy (EN AW 1050A) were dissolved at room temperature in a mixture (40 ml) of concentrated nitric acid and water (1:1). Before analysis, the obtained solutions were diluted with water in 100-ml volumetric flasks.

## Results and Discussion

The reduction of tetrazolium salts to formazans can be affected by many factors, including dissolved gases [21, 22], irradiation [21–24], organic solvents [22, 24, 25], ligands [26], colloids [23], suspended particles [24], and heavy metal ions [6, 7, 17, 23, 26, 27]. Moreover, the spectral characteristics of the obtained colored species are dependent on the

nature of the solvent [10], parameters of irradiation [18, 21, 22], pH [10], temperature [10], complex formation processes [28–30], aggregation [31], and ability to break down into simpler structures [10]. Knowledge of the effect of these factors is important for developing a reliable method for spectrophotometric determination and/or visual detection of various species [7, 14, 32, 33].

BTC can be converted into two different colored species: half-formazan (containing one intact tetrazolium ring) and diformazan (containing no tetrazolium rings) (Fig. 1). Literature data [10] for their absorption maxima and molar absorption coefficients are shown in Table 1. Spectra obtained under different conditions are given in Fig. 2. When the concentration of NaOH is low (spectrum 2), the absorbance at 520–530 nm (expected for half-formazan or the so-called “red component” [10]) is close to that at 570–580 nm (expected for diformazan or the so-called “blue component”). In spectrum 1, however, the “blue component” predominates, and the absorption is higher. This indicates that a greater amount of diformazan is produced with increasing basicity.

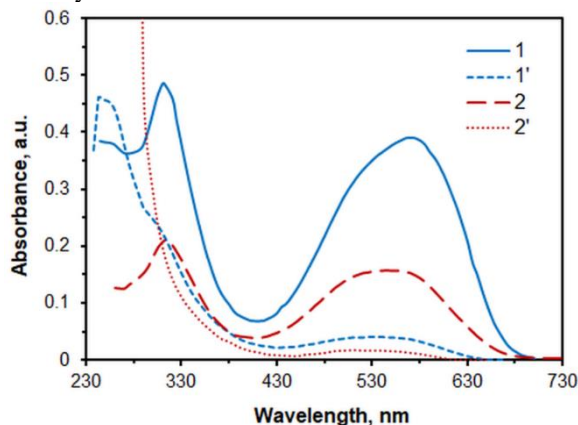


Fig. 2: UV-Vis spectra of the chloroform extracts in the presence (1 and 2) and absence (1' and 2') of Ag(I) at different NaOH concentrations.  $c_{Ag} = 1.6 \times 10^{-7}$  mol/l,  $c_{BTC} = 4.0 \times 10^{-5}$  mol/l,  $c_{SCH} = 3.6 \times 10^{-3}$  mol/l,  $V_{chloroform} = 4$  ml,  $V_{aq. phase} = 25$  ml,  $t_{ex} = 2$  min. (1, 1')  $c_{NaOH} = 1.4 \times 10^{-1}$  mol/l; (2, 2')  $c_{NaOH} = 2.0 \times 10^{-2}$  mol/l.

Table-1: Absorption maxima ( $\lambda_{max}$ ) and molar absorptivity coefficients ( $\epsilon_{max}$ ) of BTC and the resulting half-formazan and diformazan [10].

Compound	$\lambda_{max}$ , nm	$\epsilon_{max}$ , l/(mol·cm)	Solvent(s)
BTC	250–252	42,200–50,200	H <sub>2</sub> O or H <sub>2</sub> O/methanol (9:1)
Half-formazan	520	29,400	Ethanol/H <sub>2</sub> O
	530–540	–	Tetrahydrofuran
Diformazan	600	48,100	Dimethylformamide
	580	37,136	Ethanol/chloroform, Dimethylformamide
	590	44,900	Dimethylformamide
	590	49,400	Tetrahydrofuran

We were unable to obtain pure diformazan in our experiments. In all cases, the recorded spectra contain a spectral band at about 310–313 nm that can be attributed to the half-formazan [10].

Spectral peculiarities can also be observed at high Ag(I) concentrations (Fig. 3, spectrum 3). As shown in Fig. 3 a characteristic “tooth” and a minimum at about 560 nm are observed when  $c_{Ag} = 2.7 \times 10^{-7}$  mol/l. These spectral features can probably be attributed to aggregation of the obtained colored species [31].

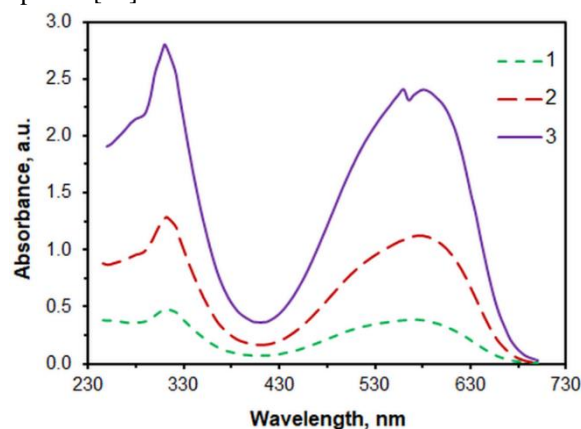


Fig. 3: UV-Vis spectra of the chloroform extracts at different Ag(I) concentrations.  $c_{BTC} = 4.0 \times 10^{-5}$  mol/l,  $c_{SCH} = 3.6 \times 10^{-3}$  mol/l, pH = 12.2,  $V_{chloroform} = 4$  ml,  $V_{aq. phase} = 25$  ml,  $t_{ex} = 2$  min. (1)  $c_{Ag} = 1.6 \times 10^{-7}$  mol/l; (2)  $c_{Ag} = 2.2 \times 10^{-7}$  mol/l; (3)  $c_{Ag} = 2.7 \times 10^{-7}$  mol/l.

#### Effect of Light, Dissolved Oxygen, and Waiting Time

BTC belongs to the group of so-called “modern tetrazolium salts”, which are little affected by the presence of daylight [10, 18]. Our experiments conducted under light and dark conditions were consistent with this statement. Unlike the redox system involving TV [7], the presence of dissolved oxygen does not “quench” the color reaction. Therefore, it is not necessary to purge the working solutions with N<sub>2</sub> prior to use [7]. This simplifies the procedure and increases its reliability.

Fig. 4 shows the effect of waiting time on absorbance at three representative wavelengths: 573 nm (absorption maximum), 520 nm ("red component" or half-formazan), and 312 nm (half-formazan). The obtained parallel straight lines show no redistribution of the colored species during the studied period.

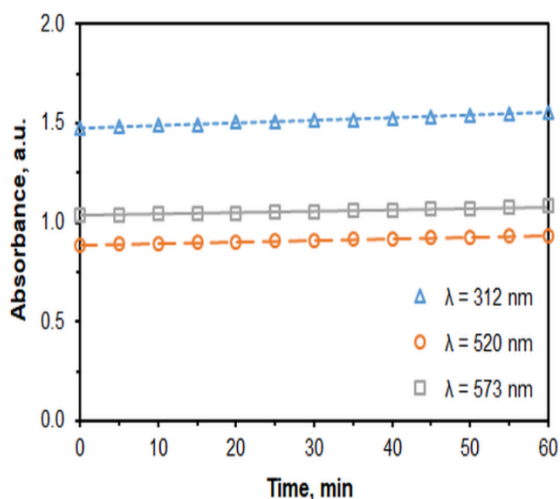


Fig. 4: Effect of waiting time on absorbance at 312 nm (1), 520 nm, and 573 nm (3).  $c_{\text{Ag}} = 2.1 \times 10^{-7}$  mol/l,  $c_{\text{BTC}} = 4.0 \times 10^{-5}$  mol/l,  $c_{\text{SCH}} = 3.6 \times 10^{-3}$  mol/l, pH = 12.2,  $V_{\text{chloroform}} = 4$  ml,  $V_{\text{aq. phase}} = 25$  ml,  $t_{\text{ex}} = 2$  min.

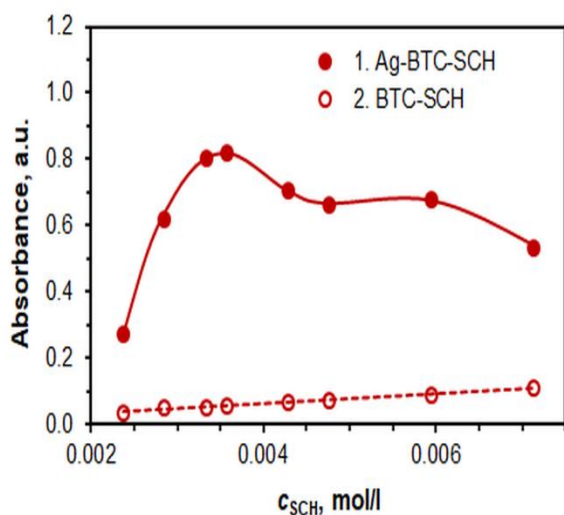


Fig. 5: Effect of SCH concentration in presence (1) or absence (2) of Ag(I).  $c_{\text{Ag}} = 1 \times 10^{-7}$  mol/l,  $c_{\text{BTC}} = 4.0 \times 10^{-5}$  mol/l, pH = 12.2,  $V_{\text{chloroform}} = 4$  ml,  $V_{\text{aq. phase}} = 25$  ml,  $t_{\text{ex}} = 2$  min,  $\lambda = 573$  nm.

### Effect of SCH Concentration

The effect of SCH concentration is shown in Fig. 5. Maximum absorbance in the presence of Ag(I) was obtained at about  $c_{\text{SCH}} = 3.6 \times 10^{-3}$  mol/l. All subsequent experiments were performed at this SCH concentration.

### Effect of pH

A basic medium is required for color development. Repeatable absorbance values were obtained at pH  $12.2 \pm 0.1$ , adjusted by adding appropriate amounts of 5 mol/l NaOH solution.

### Choice of Volumes of The Two Phases and Shaking Time

As in the previous work [7], the selected volumes of the aqueous and organic phases were 25 ml and 4 ml, respectively. Shaking for 2 min was enough for the formation of colored species and discoloration of the aqueous phase. Therefore, the procedure is faster compared to those using TTC (30 min waiting time) [6] or TV (5 min shaking) [7].

### Choice of BTC Concentration. Calibration Curve and Analytical Characteristics

The choice of tetrazolium salt concentration is important to achieve satisfactory calibration graph parameters. Experiments at three different BTC concentrations were performed:  $8.0 \times 10^{-5}$ ,  $6.0 \times 10^{-5}$ , and  $4.0 \times 10^{-5}$  mol/l. The most convenient calibration curve was obtained at  $c = 4.0 \times 10^{-5}$  mol/l (Fig. 6; the optimal conditions are presented in Table 2). Two linear segments can be seen in the calibration curve. At low Ag(I) concentrations (up to  $0.011 \mu\text{g/ml}$ ) the linear regression equation was  $A = 11.1\gamma - 0.0027$  ( $R^2 = 0.9959$ ), where  $\gamma$  is the concentration in  $\mu\text{g/ml}$ . The intercept was statistically identical to zero ( $-0.0027 \pm 0.003$ ), and the molar absorptivity at  $\lambda = 573$  nm was  $\epsilon = 1.2 \times 10^6$  l/(mol·cm). At higher Ag(I) concentrations ( $0.021$ – $0.028 \mu\text{g/ml}$ ) the linear regression equation was  $A = 200\gamma - 3.5$  ( $R^2 = 0.9989$ ). Throughout the whole concentration range studied ( $0.002$ – $0.028 \mu\text{g/ml}$ ), a third-order polynomial equation can best approximate the experimental results:  $A = 180263\gamma^3 - 3256.4\gamma^2 + 25.867\gamma - 0.0183$  ( $R^2 = 0.9990$ ). The limits of detection and quantitation, calculated as 3 and 10 times the standard deviation of the blank ( $n = 10$ ) divided by the slope of the first linear segment (11.1), were  $\text{LOD} = 6 \times 10^{-4} \mu\text{g/ml}$  and  $\text{LOQ} = 2 \times 10^{-3} \mu\text{g/ml}$ . The relative standard deviation at the  $0.022 \mu\text{g/ml}$  level ( $n = 5$ ) was 3.8 %.

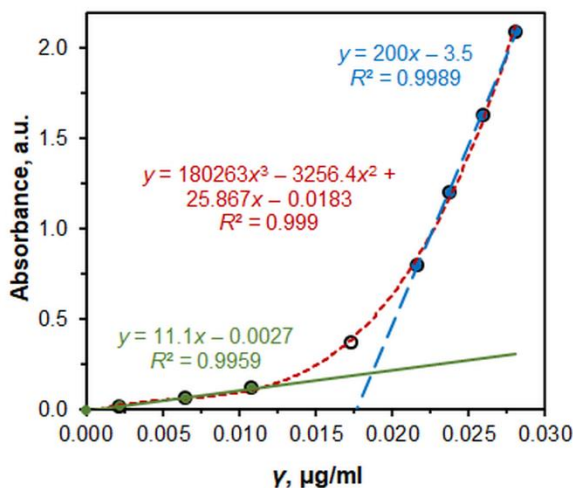


Fig. 6: Calibration graph for determination of Ag(I). The experimental conditions are given in Table-2.

Table-2: Liquid-liquid extraction-spectrophotometric optimization\*.

Parameter	Optimization range	Optimal value
Wavelength, nm	190–800	573
Concentration of SCH, mol/l	$(2.4-7.1) \times 10^{-3}$	$3.6 \times 10^{-3}$
Concentration of BTC, mol/l	$(4.0-8.0) \times 10^{-5}$	$4.0 \times 10^{-5}$
pH	12.0–12.7	12.2**
Volume of chloroform, ml	3–10	4
Extraction time, min	1–5	2

\* Optimization experiments performed at room temperature (22 °C) and volume of the aqueous phase of 25 ml; \*\* an aliquot of 0.5 ml NaOH solution (5 mol/l) was added for 1 ml of the Ag(I) solution ( $5 \times 10^{-6}$  mol/l).

The course of the resulting calibration curve can be explained based on the following considerations:

1. The linear segment at low Ag(I) concentrations is mainly due to the formation of half-formazan.
2. As the concentration of Ag(I) increases, the amount of diformazan produced increases, leading to positive deviations from Beer's law.
3. At high Ag(I) concentrations, the colored species begin to aggregate, and the salt amount decreases. This leads to negative deviations from Beer's law, compensating for the positive deviations described above. Therefore, the second linear segment (0.021–0.028 µg/ml), is due to the balance of two opposing trends.

#### Effect of Concomitant Ions and Colloidal Silver

The effect of concomitant ions on the determination of Ag(I) is shown in Table 3. Most of the tested ions (in amounts greater than those listed in Table 3) produce a slight decrease in absorbance.

However, in the case of Al(III), a slight increase is observed, accompanied by a hypsochromic shift of the absorption maximum. It is important to note that none of the investigated ions, including Al(III), can initiate a color reaction in the absence of Ag(I).

Table-3: Tolerance limits\* of foreign ions in the determination of 0.54 µg of Ag(I).

Foreign ion (FI)	FI to Ag <sup>I</sup> ratio
Li <sup>+</sup> , Na <sup>+</sup> , F <sup>-</sup> , tartrate, citrate	5000 **
K <sup>+</sup> , Zn(II), ReO <sub>4</sub> <sup>-</sup>	1000
Pb(II), Co(II), borate, sulphate	500
Al(III)	300
Mg(II), Ca(II), Ba(II), Cd(II)	200
Cl <sup>-</sup>	100
Fe(III)	50
Hg(II)	25
Mo(VI)	10
V(V), Cu(II)	5
Cr(III)	0.5

\* Defined as the FI-to-Ag(I) mass ratio that cause a relative error less than 5%.

\*\* Above which was not considered.

Silver is present in both dissolved (ionic) and colloidal forms in various samples [34, 35]. To assess the influence of colloidal silver, we used the pharmaceutical product Collargol (Colloidal silver, Chemax Pharma AD, Bulgaria). It was found that the presence of colloidal silver resulted in higher absorbance values than expected. This is consistent with the observations of other authors that colloids facilitate the conversion of tetrazolium salts to formazans [23, 36].

#### Determination of Silver(I)

The recommended procedure was successfully applied to analyze samples containing ionic silver. The obtained results (Table 4) agree with those obtained by ICP-OES.

Table-4: Determination of Ag(I) in dissolved silver films ( $n = 4$ ).

Sample	Ag(I) found, µg/ml	
	Present method (± SD)	ICP-OES
#1	0.95 ± 0.04	0.94
#2	2.1 ± 0.1	2.18
#3	2.52 ± 0.09	2.47

#### Comparison with Existing Procedures

A comparison with other spectrophotometric and liquid-liquid extraction (LLE) procedures for the Ag(I) determination is made in Table 5. The proposed LLE-spectrophotometric procedure can be described as very sensitive, simple, and cost-effective. The apparent molar absorptivity coefficients of the UV/Vis procedures usually vary between  $5.04 \times 10^3$  l/(mol·cm) [37] and  $4.51 \times 10^5$  l/(mol·cm) [6]. Procedures involving preconcentration techniques, such as flotation [38] and dispersive liquid-liquid microextraction [39], also fall within these limits.

Table-5: Comparison with existing procedures.

Reagent(s)	Technique	Sample	$\lambda$ , nm	Linear range(s), ng/ml	$\epsilon$ , l/(mol-cm)	LOD, ng/ml	Ref.
BDIC+KI	DLLME–UV/Vis	Nano Silver, semiconductor thin films	566	70–2,100	–	30	[39]
DC18C6	LLE–FAAS	Chemical reagents	–	Up to 10,000	–	13	[40]
DTPAB	UV/Vis	Synthetic mixtures	540	100–30,000	$4.3 \times 10^4$	1,000	[41]
MBO+TX-100	UV/Vis	Water samples, photographic solutions	294	100–9,000	–	1.6	[42]
Meloxicam+TX-100	UV/Vis	Water samples	412	1,000–15,000	$1.124 \times 10^4$	296	[43]
MPMP	Flotation–UV/Vis	Water samples, lead-concentrate reference material	330	10.8–540	$2.02 \times 10^5$	–	[38]
TTC+SCH	UV/Vis	Photographic film waste waters	510	20–340	$4.51 \times 10^5$	8	[6]
TV+SCH	LLE–UV/Vis	–	512	13–28	–	13	[7]
BTC+SCH	LLE–UV/Vis	Silver films for medical applications	573	2–11; 21–28	$1.2 \times 10^6$	0.6	This work

Abbreviations: BDIC, bisindocarbocyanine chloride; DC18C6, dicyclohexano-18-crown-6; DLLME, dispersive liquid-liquid microextraction; DTPAB, 2,2'-di(2,3,4-trihydroxyphenylazo)biphenyl; FAAS, flame atomic absorption spectrometry; LLE, liquid-liquid extraction; MBO, 2-mercaptobenzoxazole; MPMP, 2-[(2-mercaptophenylimino)methyl]phenol; SCH, semicarbazide hydrochloride; TTC, 2,3,5-triphenyl-2H-tetrazolium chloride; TV, Tetrazolium violet; TX-100, Triton X-100, UV/Vis, UV-visible spectrophotometry

## Conclusion

A new extraction-chromogenic system for Ag(I) was studied. It allows the spectrophotometric determination or visual detection of traces of Ag(I) in an easy and economical way. The developed procedure is characterized by high sensitivity. It outperforms some other procedures in the same category in terms of speed, simplicity, and reliability.

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