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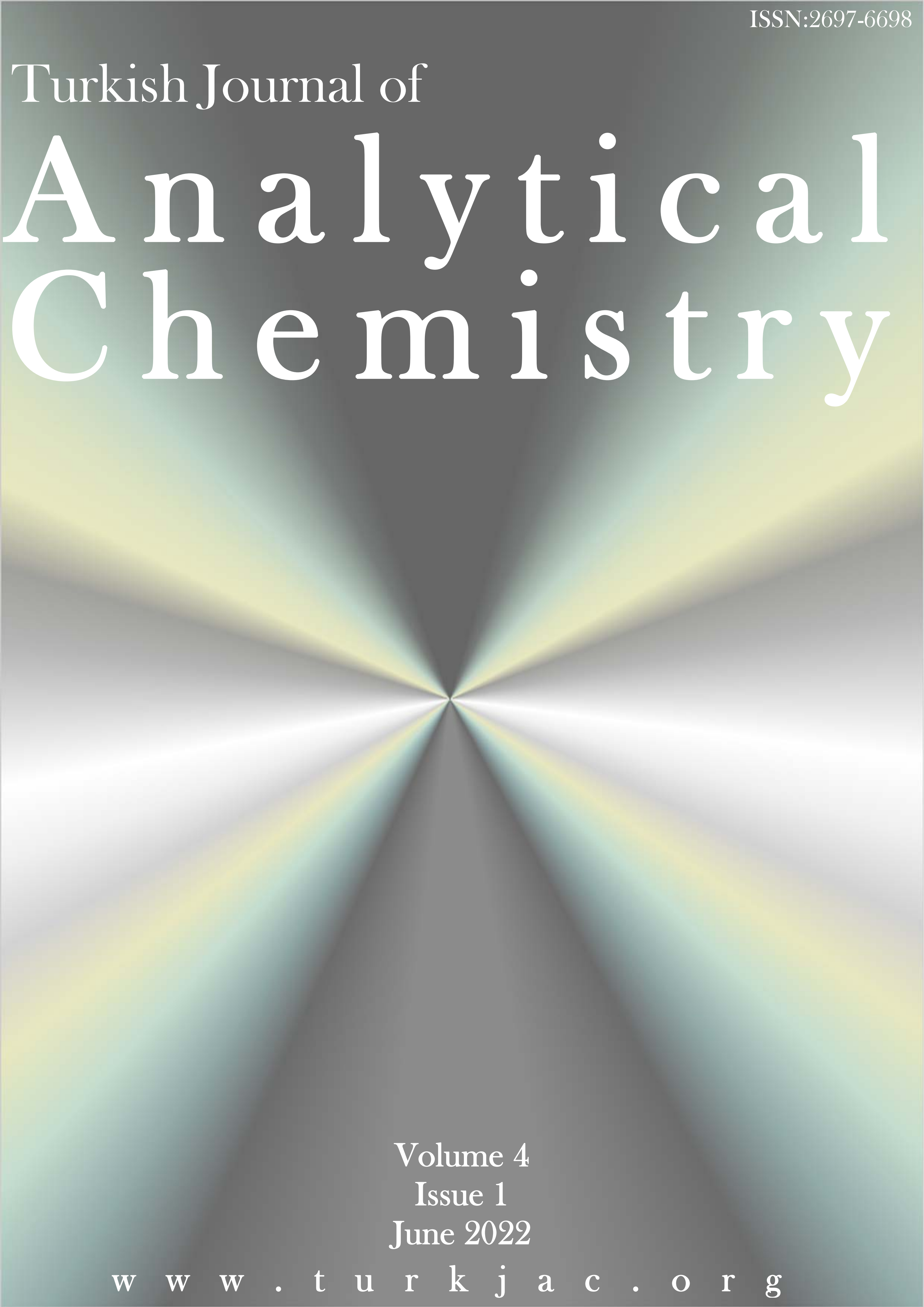

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Application of a cyclic renewable silver amalgam liquid film electrode (R-AgLAFE) to study the electroreduction processes of Bi(III) ions in the presence of cysteine

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Abstract

The cyclically refreshable liquid silver amalgam film silver-based electrode (R-AgLAFE) to study electrode processes under the “cap-pair” conditions were used. The effects of cysteine on the kinetics and the mechanism of Bi(III) electroreduction in chlorates(VII) have been studied. The methodology of the study is based on electrochemical methods: DC polarography, square wave voltammetric (SWV), and cyclic voltammetric (CV). The magnitude of the catalytic effect is related to the formation of the Bi(III)-Hg(SR)$_2$ active complexes mediating electron transfer equilibrium. This reaction controls the total rate of the process.

Keywords: Cysteine, R-AgLAFE electrode, “cap-pair” conditions, voltammetry, catalytic effect

1. Introduction

Determination of the active substance content is a routine activity in the process of pharmaceutical preparation production. Due to various structures and properties, a proper analytical method should be chosen for each analyzed substance [1]. The most commonly used analytical techniques include UV-VIS spectrophotometry, liquid chromatography, gas chromatography, mass spectrometry, and electrochemical methods. Voltammetry is an example of an electrochemical method that, in addition to chromatography, is becoming increasingly common due to its very large sensitivity at the level of pM, selectivity, or the possibility of conducting speciation analysis in real samples without their pre-treatment [2].

The fundamental factors that determine the suitability of the applied voltammetric method are the electrode and the supporting electrolyte. Supporting electrolytes can be acidic, neutral, or are many a times subsidized with organic substances which can act as catalysts or inhibitors of the electrode processes. The “cap-pair” rule [3] determines the conditions that indicate the catalytic activity of a substance [4–10] developed and used for electrode processes. It has been well known that a major role in accelerating the process is played by the ability of the organic substance to form complexes with the depolarizer and to locate the depolarizer reduction potential in the area of labile adsorption equilibrium of the organic substance.

The present study is based on the innovative electrode with the cyclic renewable liquid silver amalgam film (R-AgLAFE). It combines the advantages of a commercial mercury electrode allowing for equally accurate determination of analytical parameters and offering a significant reduction in the toxic mercury needed to produce an amalgam film and the generated waste [11,12].

The studies presented in this paper using an R-AgLAFE electrode address the effect of cysteine on the

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Bi(III) ions electroreduction process in the context of the "cap-pair" rule effect. Cysteine ((2R)-2-amino-3-mercaptopropionic acid) is an endogenous amino acid in the human body that is synthesized through two metabolic pathways. Nevertheless, it is often supplemented in the form of a drug, especially for its protective use [13,14]. The presence of a thiol group indicates that it is also involved in many reactions in the body, for example in the biosynthesis of fatty acids, many esters, and amino acids as well as in some metabolic processes [15]. Despite this, cysteine in the cells is found at very low levels [6] which is a defensive mechanism against its toxicity manifesting itself particularly dangerously against the central nervous system (CSN) [16,17]. The maintenance of physiologically low concentrations of cysteine in the cells is regulated by its incorporation into proteins and peptides, as well as by the aerobic and anaerobic metabolism. According to the literature data, it exhibits the ability to bind some metal cations in a composite unity [18,19], hence there is a need to study its action mechanism in the presence of metal ions. The essence of the bioanalytical research point of view is related to the possibilities of developing targeted drug therapies.

2. Experimental

2.1. Reagents and solutions

The following reagents were used to prepare the solutions: $NaClO_4$ (Sigma-Aldrich) (purity: 98%), $HClO_4$ (Sigma-Aldrich) (purity: 99.999%; density: 1.664 g/mL at 298 K) cysteine (Sigma-Aldrich) (purity: 96%) as well as redistilled water. 1 mol/dm^3 chlorate(VII) solution was applied as a supporting electrolyte. The choice was made by the poor complex-making properties of ClO_4^- ions, their susceptibility to water structure destruction, and the fact that they adsorb only to a small extent on the mercury surface [12].

The solutions of 1 mol/dm^3 chlorate(VII) were subsidized with the Bi(III) ions (at a concentration of 1×10^{-3} mol/dm^3. The measurements were performed with the cysteine concentration between 1×10^{-5} and 1×10^{-3} mol/dm^3. Only freshly prepared solutions of cysteine were used. The solutions were deaerated using the purge of nitrogen for cushioning. This gas was passed over the solution during the measurements. The measurements were commonly made at a temperature of 298 ± 0.1 K.

2.2. Apparatus

The experiments were performed in a thermostated vessel at 298 K using an electrochemical analyzer Autolab/GPES (version 4.9) (Eco Chemie, Ultrecht Holland) and a programmable tripod mtmanko M165D (mtmanko, Cracow, Poland).

The device is programmed for: pulse time which is 90 ms, an interval between the measurements of 800 ms, and 3 immersions of the electrode in the film of liquid silver amalgam. A three-electrode measuring cell was placed inside the tripod and the arrangement of the electrodes was as follows:

- an innovative electrode made of a cyclic renewable liquid silver amalgam film (R-AgLAFE) with an area of (0.1725 cm^2) as the working electrode,
- a silver/silver chloride electrode (Ag/AgCl) in 3 mol/dm^3 KCl as a reference electrode,
- a platinum wire as an auxiliary electrode.

The applied innovative electrode of the cyclic renewable liquid silver amalgam film R-AgLAFE [20] is characterized by: cyclic repeatability and reproducibility of the surface, excellent homogeneity of the film, and stability of the applied film thickness. This makes it an excellent alternative to the mercury drop electrode as it guarantees similar quality and performance parameters as those of HMDE. Moreover, it provides the opportunity to study electrode processes under the "cap-pair" conditions [12].

The applied electrochemical techniques that are: direct current (DC) voltammetry, cyclic voltammetry (CV), and square wave voltammetry (SWV) were used to determine the kinetic parameters of the (E_f^0), cathodic transition coefficient (α) standard rate constants (k_s) of the depolariser electroreduction and diffusion coefficient (D_{ox}) [20] of the process of Bi(III) ions electroreduction as well as in the presence of cysteine. In the voltammetric or polarographic measurements the optimal experimental operating conditions were as follows: the scan rate 5–1000 mV s^{-1} for the cyclic voltammetry (CV) and step potential 2 mV, pulse amplitude 20 mV, frequency 120 Hz for the square wave voltammetry (SWV). No fewer than three scans were performed for each measurement. The range of the potentials tested was constantly changed to study the variety of processes that can occur.

3. Results and discussion

The addition of cysteine (CE) to the basic electrolyte solution caused an increase in the current value of the SWV peaks while decreasing their width at the mid-height (Fig. 1). It was observed that the SWV curves, especially at smaller CE concentrations, were slightly distorted. This could be related to the changes in the adsorption layer of the R-AgLAFE electrode.

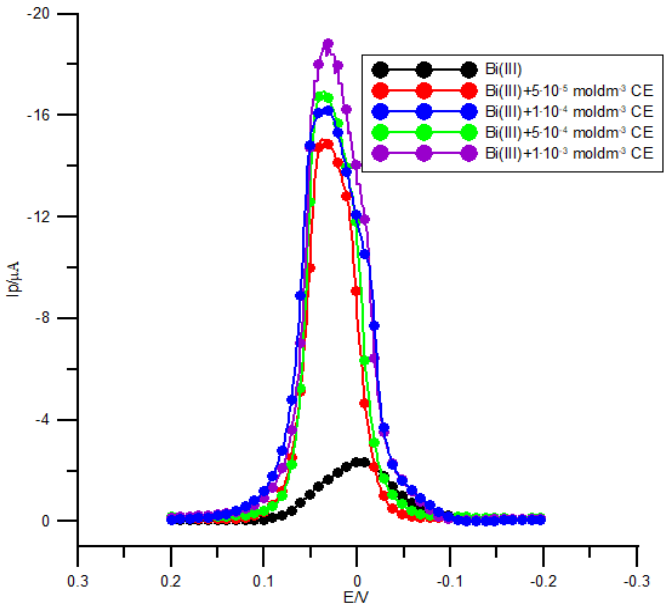

Figure 1. SWV peaks of the Bi(III) ions electroreduction in 1 mol/dm³ chlorate(VII) with the increasing cysteine concentration included in the legend

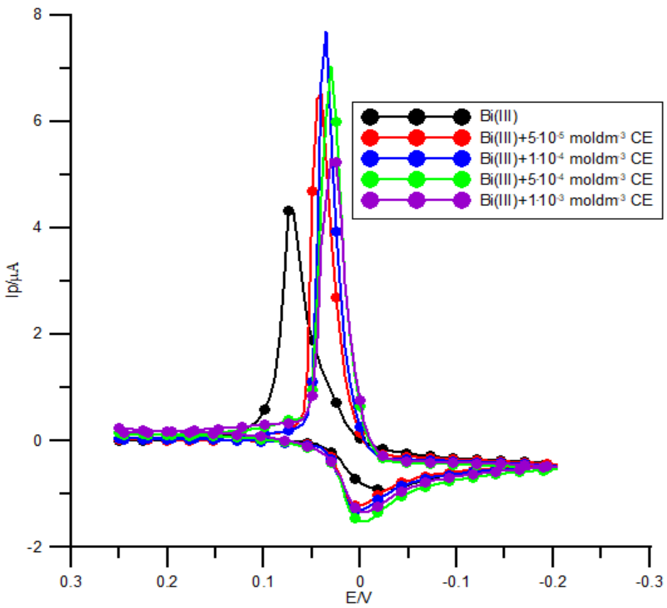

Figure 2. CV curves of the Bi(III) ions electroreduction in 1 mol/dm³ chlorate(VII) with the increasing cysteine concentration shown in the legend

It seems that the mechanism proposed by Stankovich and Bard [21], Heyrovsky and co-workers [22,23] as well as Nosal-Wiercińska [23] explains the cysteine-mercury interactions in the most effective way. The reaction of cysteine with the mercury ions is the basis of its electrolytic activity on the mercury electrodes. The reaction involves the electrooxidation of mercury in two separate steps, to cysteine mercurous thiolate $Hg_2(SR)_2$ and cysteine mercuric thiolate $Hg(SR)_2$, respectively, which are both strongly adsorbed on the electrode surface [23]. Similar behavior of a compound that contains a thiol group in its structure (–SH), on the mercury and R-AgLAFE electrodes was presented in the paper [12].

Fig. 2 shows the cyclic voltammetry curves of Bi(III) ions electroreduction in the presence of cysteine. With the addition and increase of cysteine concentration in the chlorate(VII) solution, a decrease in the potential difference between the anodic and cathodic ΔE peaks is observed which indicates an increase in the reversibility of the Bi(III) ions electroreduction process. However, no significant changes in ΔE were observed with the change of the electrode polarization rate (Table 1) as well as with the increase of CE concentration.

Table 1. Changes in *ΔE* for the Bi(III) ions electroreduction process and in the presence cysteine in 1 mol/dm³ chlorate(VII) at the polarization rate *v*

$10^3c_{Bi(III)} + 10^4c_{CE}\ /mol\ dm^3$	ΔE/ V							
	v/m Vs-1							
	5	10	20	50	100	200	500	1000
0	0.0980	0.0970	0.1001	0.1020	0.1101	0.1262	0.1482	0.1712
0.5	0.0358	0.0370	0.0374	0.0393	0.0396	0.0458	0.0604	0.0674
1.0	0.0323	0.0334	0.0340	0.0351	0.0368	0.0436	0.0596	0.0643
5.0	0.0318	0.0324	0.0329	0.0337	0.0351	0.0429	0.0578	0.0627
10.0	0.0311	0.0319	0.0326	0.0333	0.0349	0.0413	0.0521	0.0601

This confirms the changes occurring in the adsorption layer on the R-AgLAFE electrode and what is more, it indicates a chemical reaction which will be the stage controlling the rate of Bi(III) ions electroreduction process. According to the assumptions of the "cap-pair" rule and the literature data [1,23–24], it will probably be the reaction of active complexes formation on the surface of the electrode which can mediate the electron transfer [23]. On the other hand, cysteine will be a bridge in the formation of these complexes.

A lack of change in the value of formal potential E_f^0 with the increasing cysteine concentration in the chlorate(VII) solutions (Table 2) confirms that the mentioned complexes do not form in the basic electrolyte solution [25].

The literature data [24,26–27] indicate a reaction of Bi(III) with mercury cysteine thiolate(II) $Hg(SR)_2$. This is the form of anodic oxidation of mercury in the presence of cysteine that adsorbs in the reduction potential range Bi(III) (~0 V) and is bound lability to the electrode surface [24]. It is supposed that, similarly to the mercury electrode used earlier, the active Bi(III)-$Hg(SR)_2$ complexes are obviously located inside the adsorption layer (Fig. 3).

Table 2. Values of formal potentials E_f^0 of 1 × 10^{-3} mol/dm³ Bi(III) ions electroreduction in 1 mol/dm³ chlorate(VII) and in the presence of cysteine

$10^3c_{Bi(III)} + 10^4c_{CE}\ /mol\ dm^3$	E_f^0/ V
0	0.810
0.5	0.950
1.0	0.950
5.0	0.940
10.0	0.930

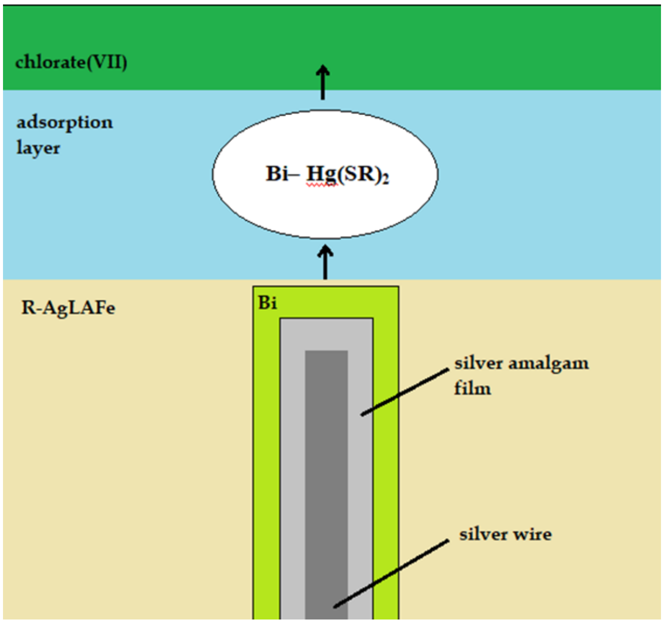

Figure 3. The reaction path obtained for the Bi(III) ions electroreduction in the presence of cysteine.

Based on the parameters of the cyclic voltammetry CV curves, the values of the transfer coefficients α and the standard rate constants k_s of the Bi(III) ions electroreduction and in the presence of cysteine (Table 3) were determined.

Table 3. Values of cathodic transition coefficients α and standard rate constants k_s of 1×10^{-3} mol/dm³ Bi(III) electroreduction in 1 mol/dm³ chlorate(VII) and in the presence of cysteine.

$10^3 c_{Bi(III)} + 10^4 c_{CE}$ / $mol\ dm^3$	α	$10^4 k_s$/ cm s^{-1}
0.00	0.25	0.360
0.50	0.53	5.370
1.00	0.57	8.110
5.00	0.59	10.05
10.0	0.61	12.78

The increase in the values of the transition coefficients α after the addition of cysteine into the basic electrolyte solution indicates an increase in the reversibility of the Bi(III) ions electroreduction process [25]. This also translates into an increase in the standard rate constants, confirming the catalytic action of the amino acid [25]. An increase in the concentration of cysteine in chlorate(VII) results in further increases in the mentioned kinetic parameters (Table 3).

4. Conclusions

The studies using an innovative electrode of cyclically renewable liquid silver amalgam film R-AgLAFE pointed out the catalytic effect of cysteine on the multistep electroreduction process of Bi(III) ions according to the "cap-pair" rule. The magnitude of the catalytic effect is related to the formation of active complexes mediating electron transfer equilibrium. The complex formation on the electrode surface is due to the adsorption of $Hg(SR)_2$, which increases the activity of the electrode without limiting its surface area. The composition of these complexes varies, which can be inferred from Marcus' theory [25], predicting the change of complexes in the ionic layer after the partial charge loss.

The "cap-pair" studies on the R-AgLAFE electrode proved to be valid and provide satisfactory results.

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Seasonal variations in tap water quality parameters in Çanakkale, Türkiye

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Abstract

Water pollutants are a major problem for the world. Especially, heavy metals are significant environmental pollutants due to their tendency to accumulate in organisms causing toxic effects on humans, animals, and aquatic organisms. Therefore, qualitative and quantitative analyses of pollutants are important. Especially the analysis of contaminants in tap water is important. In this study, unlike previous studies, physicochemical water quality parameters were also studied for the first time along with heavy metal analysis. Water-quality parameters (pH, temperature, conductivity, and dissolved oxygen) were determined in tap water by YSI 556 MPS, and other parameters such as biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were determined by electrometric and titrimetric methods. Various anions were analyzed with the UV-VIS spectroscopy technique. Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn were analyzed as pollutants by ICP-OES. Amounts of Cr, Cd, Ni, and Pb were found within detectable limits, but Cu, Fe, Mn, and Zn were found at 0.003 ppm, 0.173 ppm, 0.009 ppm, and 2.343 ppm, respectively. The pH of the tap water was in the interval between 6.64 and 7.12 (mean: 6.85 ± 0.12). Nitrite varied between 0.20 and 0.60 mg/L (average: 0.36 ± 0.001 mg/L) revealing that the tap waters exceeded the TSI standards for first quality drinking waters (0.20 mg/L). The data were evaluated using the relevant statistical analyses.

Keywords: Tap water, water quality, heavy metal, physicochemical, biochemical

1. Introduction

The development of industry and technology has provided better living conditions for humans, but previously unknown problems have emerged with the arrival of industry. Sometimes, wastewaters may contain high concentrations of heavy metals. After discharging these wastewaters, organisms in the receiving environment may be harmed. Also, wastewaters that have high concentrations of heavy metals may contaminate drinking waters, lake waters, or groundwater. It is estimated that approximately 80.0% of all diseases are caused by poor hygiene conditions and a lack of safe water. Millions of people die due to water pollution [1–4].

Metals that are denser than 5.00 g/mL are called heavy metals. More than 60 metals are included in this group, including Zn, Pb, Cd, Fe, Cu, Ni, and Co [5–7]. Heavy metals which are consumed by organisms via the food chain may cause bioaccumulation, and in some situations, there are toxic impacts when they exceed certain amounts [8–10]. Toxic heavy metals such as Ag, As, Ba, Cd, Cr, Pb, Mn, Hg, Ni, Se, V, and Zn are harmful to life [11]. Heavy metals with toxic properties enter the environment from various sources and constitute one of the most important causes of environmental pollution today [12]. Heavy metal accumulates in the body [8,9,13]. As, Cd, Cr, Ni, Pb, and Se are highly toxic, and have carcinogenic, mutagenic, and teratogenic effects [14]. Heavy metals create important problems in aquatic environments and for creatures living in these environments via the usage of natural resources and human activities [6–9,13,15].

The conductivity of substances that ionize in water depends on the temperature, salinity, and other water properties. Temperature influences the displacement speed of ions [16].

Determining the amount of dissolved oxygen in water is important both for living organisms and for determining whether industrial wastewaters have mixed with drinking waters or not. Dissolved oxygen (DO) is also a parameter used in the determination of the

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assimilation capacity of the receiving environment and pollution potential [17]. Chemical oxygen demand (COD) is one of the most used parameters in environmental pollution. The chemical oxygen demand of organic matter in water is mainly an indicator of the total amount of all organic materials [18]. Biochemical oxygen demand (BOD) in water is defined as the amount of oxygen required for the decomposition of organic matter used by microorganisms under aerobic conditions.

Sulfate is another anion widely known anion in water coming after bicarbonate and chloride. Sulfate in the soil passes into water systems over time. Among the water-insoluble sulfate salts, barium sulfate and calcium sulfate are the most resistant sulfate salts, and they are in the mid and low soluble groups. Sodium sulfate and magnesium sulfate have laxative effects in humans at 250 mg/L. Whereas animals are affected above the limit of 1000 mg/L. Sulfates also give water a bitter taste and form calcium sulfate and magnesium sulfate that precipitate in boiling water thereby their dissolved concentrations are very low in this type of water [19].

Nitrate and nitrite are commonly available in nature. Domestic and industrial wastes, especially nitrogen fertilizers commonly used in agriculture, contaminate soil and water systems in terms of nitrogen and so indirectly increase algal bloom levels in lakes, dams, and other freshwater resources which provide drinking water for humans' populations. In Türkiye, although there are many freshwater resources, most of these freshwater resources have been polluted with nitrogen from domestic, agricultural, and industrial wastewaters [20]. Freshwater resources such as the Atikhisar Dam in Çanakkale city are also reported to be exposed to pollution, especially in the mining industry.

Cyanide is an extremely volatile and toxic substance known as hydrocyanic acid. Hydrogen cyanide in the gas phase is colorless and has a bitter almond smell. Cyanide in the liquid phase is colorless, as in the gas phase. Cyanide is a toxic substance in the environment and used in rubber and fertilizer industries, the manufacture of plastics, gold, silver, certain chemicals, and pesticides. Apart from the non-tobacco smoke resulting from the combustion of - polymeric materials and structures containing nitrogen, cyanide is also found in some plants [21]. The upper limit value for cyanide is 0.200 mg/L in drinking water [22].

Heavy metals and bacteriological parameters were studied in tap water from Çanakkale (Türkiye) city [23]. Çanakkale tap water samples from 40 different points were collected between 20 April and 5 May 2007, and the water samples were analyzed for As, Pb, Cu, Zn Cd, and some microbiological parameters [23]. According to the study, Cd, Cu, Pb, and Zn were found to be under the limit values (Cd: 0.95–11.9, Cu 0.004–0.580, Pb <0.08, and Zn 0.00–5.30 mg/L) [23]. In addition, according to microbiological analyses of the water samples which were completed using the membrane filter method, bacteria were found in 28 of 40 water samples, 18 of these contained coliform bacteria and one of them was *Escherichia coli* TS 266. Microbiological values for the water samples were higher than the Limit Values according to WHO, EPA, and EC [23]. In addition, there are a few studies about heavy metals combined with physicochemical parameters in the Strait of Çanakkale [24] and Biga Kocabaş River [25,26].

Although there are many studies on the analysis of heavy metals to determine the quality of drinking water in the Marmara region [27–29], there are a few studies on other quality parameters such as pH, dissolved oxygen (DO), biochemical oxygen demand (BOD) and chemical oxygen demand (COD), combined with heavy metals analysis in wastewater of Kocabaş Stream of Marmara basin [25,26].

Therefore, in this study, unlike previous studies, physicochemical (pH, temperature, conductivity, and DO, BOD, and COD water quality parameters in tap waters of the Çanakkale center were also studied for the first time along with heavy metal (Pb, Cd, Fe, Zn, Cu, Mn, Cr) analysis. Besides, nitrite (NO_2^-) and nitrate (NO_3^-) levels were studied in this study due to the toxic effect of nitrite.

2. Materials and Methods

2.1. Study area and period

The study area, Çanakkale, is located in the northwest of the Republic of Türkiye at 25°40'–27°30' east longitude and 39°27'–40°45' north latitude. In this study, a total of 80 tap water samples were analyzed. The tap water samples were collected seasonally (Spring: May 2012; Summer: July 2012; Autumn: October 2012; Winter: February 2013) from five different neighborhoods known as Esenler (St.ER), Cevatpaşa (St.CR), Ismetpaşa (St.IR) Kemalpaşa (St.KR) and Barbaros (St.BR) in Çanakkale province (Fig. 1).

2.2. Sampling and preservation

The tap water samples were taken 3 minutes after the tap was turned on. The water samples collected from taps were placed in clean polythene sample bottles (1.00 L) and stored in a deep freezer at -21.0 °C for measurements of sulfate, nitrite, and nitrate. For the analyses of cyanide anions, the water samples were stored in 250 mL PE flasks and preserved with sodium hydroxide solution (pH = 12.0) for ten days. The water samples for heavy metal measurements were preserved with nitric acid (HNO_3) and stored in clean polythene sample bottles

(250 mL) at 4.00 °C. In this study, a total of 80 samples collected from five sampling points were analyzed for each parameter. For samples at each station, three separate measurements were performed and the obtained values were averaged. Relative Standard Deviations (% RSD) of measurements were under 5.00%.

Figure 1. Sampling locations for drinking water quality parameters in Çanakkale, Türkiye

2.3. Reagents and apparatus

Analytical reagents used in all analyses were analytical grade (Merck, Germany). Double distilled deionized water (<0.100 µS/cm conductivity) was used throughout the experiments. In order to minimize contamination and interference, all the materials and glassware were carefully cleaned. The stock standard solution was prepared daily (AccuTrace, the reference Standard Merck).

2.4. Metal analysis using the ICP-OES method

Concentrations of heavy metals in tap waters were measured by a Perkin Elmer Optima 8000 Model (ICP-OES). Analyses of all samples were repeated three times. The ICP-OES device was calibrated before each measurement via Merck multi-element standard solution.

2.5. Temperature, conductivity, and pH

Water quality parameters such as temperature, pH, conductivity, and dissolved oxygen were measured with a water probe "YSI 556 MPS" and Hach Lange apparatus HQ40D *in situ*.

2.6. Sulfate, nitrite, and nitrate

Sulfate, nitrite, and nitrate anions were analysed according to the colorimetric method. A Hach-Lange DR2800 VIS spectrophotometer was used for the analyses.

2.7. Cyanide

Cyanide was analyzed according to the spectrophotometric (colorimetric) method. For this, a Hach-Lange DR2800 VIS spectrophotometer was used. In the method, cyanide was converted to cyanogen chloride (CNCl) by the reaction of cyanide with chloramine-T at a pH of less than 8. After the reaction was completed color formation by the addition of pyridine-barbituric acid reagent was determined by the absorbance formed at 578 nm for the complex formed with pyridine-barbituric acid reagent and CNCl. To obtain colors of comparable intensity, it is essential to have the same salt content in both the sample and the standard [30].

2.8. Biochemical oxygen demand (BOD$_5$)

For the BOD$_5$ measurement, 250 mL tap water samples were placed in Winkler glass bottles (Wheaton-USA) and incubated in a dark location for 5 days.

Table 1. Devices and operation parameters for analyses

Parameters	Device Used	Method	Reference*
Sulfate (mg/L)	Hach-Lange DR2800 Spectrophotometer	Turbidimetric Method at 420 nm wavelength	SM 4500-SO$_4$ E.
Cyanide (mg/L)	Hach-Lange DR2800 Spectrophotometer	Colorimetric Method at 578 nm wavelength	SM 4500-CN- E.
Nitrite (mg/L)	Hach-Lange DR2800 Spectrophotometer	Colorimetric Method at 543 nm wavelength	SM 4500-NO$_2$- B.
Nitrate (mg/L)	Hach-Lange DR2800 Spectrophotometer	UV Spectrophotometric Method at 220–275 nm wavelength	SM 4500-NO$_3$- B.
BOD$_5$ (mg/L)	Hach-Lange Limunescence Dissolved Oxygen Electrode	5-Day BOD Test	SM 5210 B.
COD (mg/L)	Merck Pharo 100 Spectrophotometer	Closed Reflux, Colorimetric Method at 420 and/or 600 nm wavelength	SM 5520 D.
Temperature (°C)	TFA 30.1040 Thermemeter	Laboratory and Field Methods	SM 2550 B
pH	Hach-Lange pH-Meter	Electrometric Method	SM 4500 H+ B.
Conductivity (µS/cm)	Hach-Lange Conductivity Meter	Electrical Conductivity Method	SM 2510 B.
Dissolved Oxygen (mg/L)	Hach-Lange Limunescence Dissolved Oxygen Electrode	Limunescence Method	ASTM D888-12
Heavy metals (mg/L)	ICP-OES Perkin Elmer Optima 8000"	ICP-OES Method	EPA 200.7

*SM: Standard Methods for the Examination of Water and Wastewater. APHA, AWWA, WEF. 22nd Edition; ASTM: American Society for Testing and Materials

Table 2. Physicochemical parameters in the tap waters from Çanakkale, Türkiye

Seasons	Sampling station	Temperature (°C)	pH	Conductivity (µS/cm)	Dissolved oxygen (mg/L)
Winter	Esenler Region	10.1	6.98	360.0	7.60
	Cevatpaşa Region	9.4	7.01	327.0	7.30
	Ismetpaşa Region	7.3	6.95	362.0	8.40
	Kemalpaşa Region	6.6	6.84	364.0	7.20
	Barbaros Region	7.9	6.72	364.0	6.90
	Winter average	8.3 ± 0.1	6.90 ± 0.12	355.0 ± 19.3	7.48 ± 0.17
Spring	Esenler Region	12.7	6.76	412.0	7.10
	Cevatpaşa Region	11.8	6.87	414.0	6.80
	İsmetpaşa Region	10.9	6.84	421.0	7.40
	Kemalpaşa Region	13.6	6.65	417.0	6.90
	Barbaros Region	11.8	6.89	431.0	6.80
	Spring average	12.2 ± 0.1	6.80 ± 0.12	419.0 ± 22.8	7.00 ± 0.16
Summer	Esenler Region	20.1	6.91	530.0	6.90
	Cevatpaşa Region	18.7	6.99	526.0	6.40
	İsmetpaşa Region	18.0	7.01	532.0	6.80
	Kemalpaşa Region	21.2	7.12	483.0	6.10
	Barbaros Region	18.9	6.96	564.0	6.10
	Summer average	19.4 ± 0.2	7.00 ± 0.12	527.0 ± 28.2	6.46 ± 0.14
Autumn	Esenler Region	16.3	6.64	471.0	7.30
	Cevatpaşa Region	14.3	6.71	481.0	7.10
	İsmetpaşa Region	16.9	6.65	478.0	8.00
	Kemalpaşa Region	15.1	6.73	399.0	7.40
	Barbaros Region	14.5	6.79	452.0	7.10
	Autumn average	15.4 ± 0.2	6.70 ± 0.12	456.0 ± 24.8	7.38 ± 0.17
	Annual average	13.8 ± 0.2	6.85 ± 0.12	439.0 ± 23.8	7.08 ± 0.16

The BOD levels were analyzed by comparing the DO levels of a water sample taken immediately with the DO levels of tap water samples incubated in a dark location for 5 days.The difference between the two DO levels represents the amount of oxygen required for the decomposition of any organic material in the tap water samples. DO concentrations were measured using a dissolved oxygen test kit.

2.9. Chemical oxygen demand (COD)

For the chemical oxygen demand (COD), Winkler glass bottles (250 mL) acidified with hydrochloric acid were used. The tap water samples were preserved with sulphuric acid to a pH < 2 and maintained at 4 °C until analysis. Analyses of the samples began within 24 hours. In this application, quantitative analyses of COD were performed using the Hach-Lange DR2800 VIS spectrophotometer reading absorbance values between 200–400 nm and Merck Spectroquant COD mercury-free cell test.

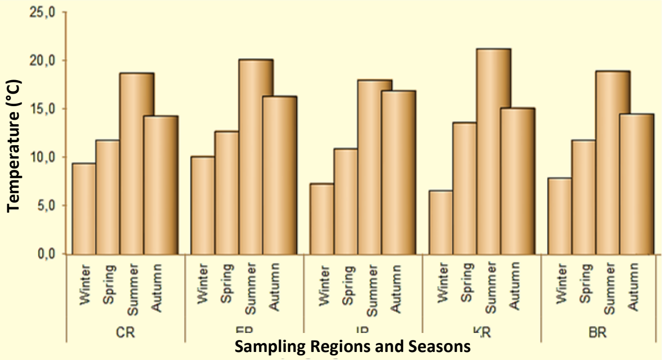

Figure 2. Seasonal and regional variations in temperatures in the tap waters of Çanakkale

2.10. Statistical analysis

Two-way analysis of variance (ANOVA) was used for statistical analysis.

2.11. Operation parameters for the methods

Devices and operation parameters for all methods are reported in Table 1.

3. Results and Discussion

3.1. Physicochemical parameters in the tap-waters

Results of the physicochemical parameter results such as temperature, conductivity, pH, and dissolved oxygen (DO) of the tap water samples which were collected from 5 different locations in Çanakkale are presented in Table 2. Moreover, drinking water quality limit values according to Turkish standard TS 266 and other international standards (limits) are presented in Table 3.

3.2. Temperature variations

The temperature variations in the tap water, which were taken from 5 various locations in Çanakkale, were given in Table 2 and Fig. 2. As expected, the temperature of the tap water samples from all sampling sources reached the highest levels in the summer.

Table 3. Turkish standard TS 266 and other international drinking water standards and limit values

Parameters	**Turkish Standards Istitute (TS 266; 2005)**	**Word Health Organization (WHO, 2011)**	**USA Environmental Protection Agency (EPA, 2008)**	**European Union (EC, 1998)**
Temperature (°C)	25.00	-	-	-
pH	6.50–9.50	6.50–8.00	6.50–8.50	6.50–9.50
Dissolved Oxygen (DO) (mg/L)	8.000	-	-	-
Sulfate (mg/L)	250.0	500.0	250.0	250.0
Cyanide (mg/L)	0.050	0.070	0.200	0.050
Nitrite-N (mg/L)	0.200	-	-	-
Nitrate-N (mg/L)	5.000	-	-	-
Chemical Oxygen Demand (COD) (mg/L)	25.00	-	-	-
Biological Oxygen Demand (BOD) (mg/L)	4.000	-	-	-
Ni (mg/L)	0.020	0.020	-	-
Cd (mg/L)	0.005	0.003	0.005	0.005
Cr (mg/L)	0.050	0.050	0.100	0.050
Pb (mg/L)	0.010	0.010	0.015	0.010
NO_3^- (mg/L)	50.00	50.00	45.00	50.00
Cu (mg/L)	2.000	2.000	1.000	2.000
Mn (mg/L)	0.050	0.100	0.050	0.050
Fe (mg/L)	0.200	0.300	0.300	0.200
TSS (mg/L)	-	1000	500	-
Zn (mg/L)	2.000	3.000	5.000	-
NH_4^+ (mg/L)	0.500	1.500	-	0.500
Hardness (as $CaCO_3$)	-	500.0	-	-
Colour (PC Unit)	20.00	15.00	15.00	-
Turbidity (NTU)	1.000	5.000	1.000	1.000

Both the highest (in summer: 21.20 °C) and the lowest temperature value (in winter 6.60 °C) were observed in the KR. However, while the seasonal temperature variations were high, the regional temperature variations were low (Table 2 and Fig. 2).When regional variations of average temperature in the tap waters during the year are examined, there were no important variations between sampling regions (Table 1). During the year, the annual average temperature in the tap waters was 13.80 ± 0.2 °C (Table 2). Both the average value and other values for temperature were acceptable according to Turkish standard TS 266 limit values (Table 3).

Although the drinking water temperature was not at a level that directly causes health problems, it is well known that as water temperature increases, the corrosive effect level increases on equipment within the water network system, and the solubility level of heavy metals increases. Therefore, temperature increases in drinking waters indirectly create a negative effect on human health, so monitoring of temperature levels of drinking waters is important for public health [31].

3.3. pH variations

pH variation in the tap water which was taken from 5 various locations in Çanakkale is presented in Table 2 and Fig. 3.

Seasonal variations of pH values of the Tap-Water in all regions showed that there was no linear correlation between samples. The highest pH value was detected in KR in winter (7.12) and the lowest value was detected in ER in autumn (6.64). The pH values measured in the tap water samples in Çanakkale were in the interval between minimum and maximum values of both national and international standards (Table 3 and Fig. 3). When both regional and seasonal variations in average pH in the tap waters during the year are examined, the values were under neutral pH value (pH: 7.00), except for summer values in the KR and IR (Table 3). During the year, all pH levels were under the neutral value for pH (7.00) (Fig. 3), and thereby the average pH value in samples was 6.85 ± 0.12 (Table 2). So, it can be concluded that the tap-waters of Çanakkale city were partly unhealthy in terms of pH or acidity.

Although the direct effect of low and high pH levels in drinking water on human health is generally unknown lower pH values are thought to treat many diseases, even cancer while acidic waters can cause cancer and various diseases directly.

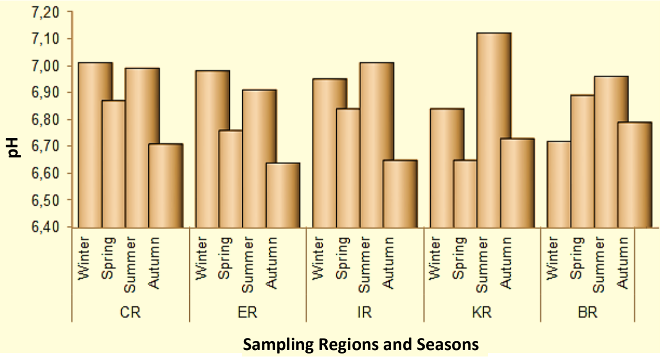

Figure 3. Seasonal changes in pH value in the tap waters of Çanakkale

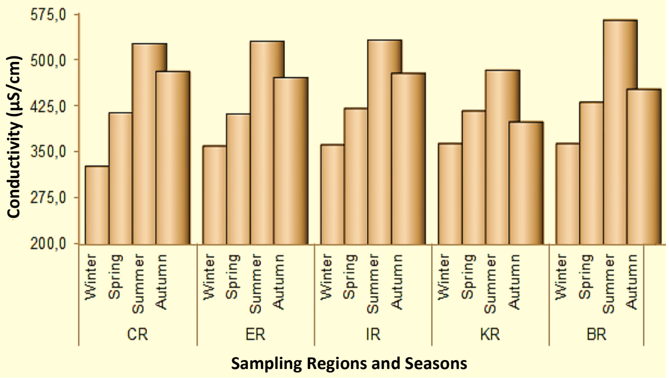

Figure 4. Seasonal changes of conductivity in the tap waters of Çanakkale

However, there are some claims that while alkaline waters treat many diseases and even cancer, acidic waters cause various diseases, even cancer. But it is well known that acidity increases in drinking water both cause corrosion of equipment used in the water distribution system and increase the solubility of heavy metals conductivity and temperature. Considering this situation, although not directly, high acidity indirectly creates a negative effect on human health. Therefore, monitoring pH levels in drinking waters is important for public health [31].

3.4. Conductivity variations

The conductivity variations in the tap-water samples, which were collected from 5 different locations of Çanakkale, are presented in Table 2 and Fig. 4.

Conductivity levels reached their highest values in summer. The highest conductivity value was detected in BR in summer (564.0 µS/cm) and the lowest value was detected in CR in winter (327.0 µS/cm). The conductivity values measured in the tap water samples from Çanakkale, were in the interval between minimum and maximum values of both national and international standards (Table 2 and Fig. 4). When the regional variations of average conductivity in the tap waters during the year are examined, the values varied between 416.0 ± 22.1 in the KR and 453.0 ± 24.6 in the BR (Table 2). During the year, the annual average conductivity value in the tap waters was 439.0 ± 23.8 µS/cm (Table 2).

An increase in conductivity causes corrosion and oxidation of equipment in the water distribution systems. Considering that the major source of heavy metals in drinking water is the corrosion and oxidation of water distribution equipment such as household plumbing, taps, and pipes, conductivity increases in drinking waters indirectly create a negative effect on human health. Therefore, monitoring conductivity values, in addition to temperature and pH measurements, in drinking waters is important for public health [31].

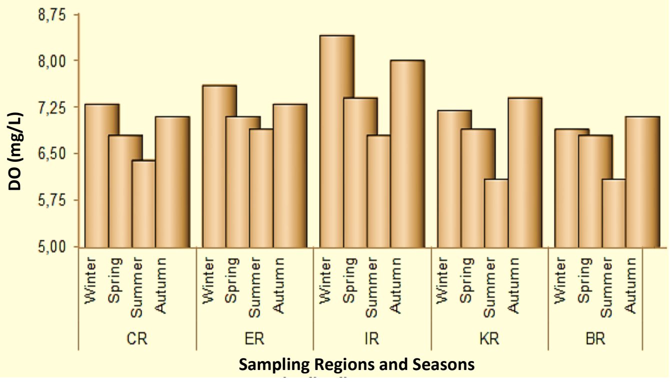

Figure 5. Seasonal changes in DO values in the tap waters of Çanakkale

3.5. DO variations

DO variation in the tap-water samples, which were seasonally taken from 5 various points in Çanakkale, are presented in Table 2 and Fig. 5.

Seasonal variations in the DO values showed that there was an inverse (negative) correlation between both temperature and conductivity with DO values. When the samples had the lowest temperature and conductivity, DO concentrations were measured at the highest levels. The highest DO value was measured in the IR in winter (8.40 mg/L) and the lowest DO value was determined in BR and KR in summer (6.10 mg/L). The DO values measured in the tap water samples in Çanakkale, were in the interval between minimum and maximum values of both national and international standards (Table 2 and Fig. 5). When regional variations of average DO in the tap waters during the year are examined, the values were between 6.70 ± 0.15 in the BR and 7.65 ± 0.17 IR (Table 2). During the year, the annual average DO value in the tap waters was 7.08 ± 0.16 (Table 2).

High DO value has vital importance for life in the aquatic environment, and moreover, it has vital importance for public health in drinking water. High DO levels in drinking water are a good situation since it makes drinking water taste better. However, high DO speeds up corrosion of the water network systems, with high temperature, conductivity, and acidity levels. The level of oxygen in water is in close relation to temperature, pressure, and salinity. Many sources of drinking water such as lakes and ponds have anoxic conditions in the summer period, due to oxygen deficiency linked to water pollution and biological processes. Therefore, considering that there are positive and negative effects of low and high DO levels, it is desirable for public health that DO levels of drinking water are neither too low nor too high [32].

3.6. Seasonal changes of biochemical parameters in the tap waters

The biochemical parameter results such as sulfate, cyanide, nitrite, nitrate, BOD, and COD in the tap water

Table 4. Seasonal variations of sulfate, cyanide, nitrite, nitrate, BOD, and COD in tap waters from Çanakkale, (nd: not detected)

Seasons	Sampling	Sulfate (mg/L)	Cyanide (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)	BOD_5 (mg/L)	COD (mg/L)
Winter	Esenler Region	73.0	nd	0.30	2.90	1.22	4.05
	Cevatpaşa Region	87.0	nd	0.40	2.10	3.08	12.80
	İsmetpaşa Region	76.0	nd	0.30	3.00	2.41	8.26
	Kemalpaşa Region	77.0	nd	0.40	2.60	2.64	8.50
	Barbaros Region	75.0	nd	0.30	2.40	3.02	9.28
	Winter average	77.6 ± 1.5	nd	0.34 ± 0.01	2.60 ± 0.05	2.47 ± 0.37	8.58 ± 0.81
Spring	Esenler Region	96.0	nd	0.40	3.40	1.61	6.12
	Cevatpaşa Region	91.0	nd	0.30	3.40	2.84	10.1
	İsmetpaşa Region	82.0	nd	0.40	3.10	1.76	5.12
	Kemalpaşa Region	92.0	nd	0.20	2.40	1.43	5.54
	Barbaros Region	90.0	nd	0.20	2.60	3.33	10.1
	Spring average	90.2 ± 1.7	nd	0.30 ± 0.01	2.98 ± 0.05	2.19 ± 0.32	7.40 ± 0.69
Summer	Esenler Region	116.0	nd	0.30	4.10	1.88	5.12
	Cevatpaşa Region	103.0	nd	0.30	3.70	2.71	11.2
	İsmetpaşa Region	96.0	nd	0.30	3.80	1.93	7.12
	Kemalpaşa Region	108.0	nd	0.20	3.60	3.01	6.24
	Barbaros Region	103.0	nd	0.40	3.70	2.36	8.16
	Summer average	105.2 ± 2.0	nd	0.30 ± 0.01	3.78 ± 0.07	2.38 ± 0.35	7.57 ± 0.71
Autumn	Esenler Region	101.0	nd	0.50	3.20	0.32	2.06
	Cevatpaşa Region	96.0	nd	0.60	3.20	1.74	8.04
	İsmetpaşa Region	86.0	nd	0.40	2.90	2.69	9.14
	Kemalpaşa Region	101.0	nd	0.50	3.40	2.06	4.68
	Barbaros Region	84.0	nd	0.40	3.10	1.99	7.13
	Autumn average	93.6 ± 1.8	nd	0.48 ± 0.02	3.16 ± 0.06	1.76 ± 0.26	6.21 ± 0.64
	Annual average	91.70 ± 1.74	nd	0.36 ± 0.01	3.13 ± 0.06	2.20 ± 0.33	7.44 ± 0.71

Table 5. Average values for sulfate, cyanide, nitrite, nitrate, BOD and COD in tap waters from Çanakkale, during the year (nd: not detected)

Stations	Sulfate (mg/L)	Cyanide (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)	BOD5 (mg/L)	COD (mg/L)
ER	96.5 ± 1.8	nd	0.40 ± 0.01	3.40 ± 0.06	1.26 ± 0.19	4.34 ± 0.41
CR	94.3 ± 1.8	nd	0.40 ± 0.01	3.10 ± 0.06	2.59 ± 0.39	10.5 ± 0.99
IR	85.0 ± 1.6	nd	0.30 ± 0.01	3.20 ± 0.06	2.20 ± 0.33	7.41 ± 0.70
KR	94.5 ± 1.8	nd	0.30 ± 0.01	3.00 ± 0.06	1.27 ± 0.34	6.22 ± 0.59
BR	88.0 ± 1.7	nd	0.30 ± 0.01	3.00 ± 0.06	2.68 ± 0.40	8.67 ± 0.88
Annual Average	91.7 ± 1.7	nd	0.360 ± 0.001	3.13 ± 0.06	2.20 ± 0.33	7.44 ± 0.71

samples, which were collected from 5 different locations in Çanakkale, are presented in Table 4 and Table 5.

3.7. Sulfate variations

The sulfate variations in the tap water, which were seasonally measured from 5 various locations in, were given in Table 4 and Fig. 6.

Sulfate is one of the most abundant anions in water. Sodium sulfate and magnesium sulfate have laxative effects in humans. Therefore, the sulfate concentration has an upper limit of 250 mg/L [19]. The highest sulfate concentration was detected in ER in the summer (116.0 mg/L) and the lowest sulfate concentration was determined in ER in winter (73.0 mg/L) among the Çanakkale city tap waters. Compared to TS 266 and other international drinking water standards, the sulfate concentration findings were below the upper limit values (Table 3).

3.8. Nitrite and nitrate variations

The nitrite and nitrate variations in the tap-water samples, which were collected seasonally from 5 different locations in Çanakkale, are presented in Table 4, Fig. 7, and Fig. 8.

While the lowest nitrite concentration (0.20 mg/L) in this study was measured in KP in spring and summer and in BR in spring, concentration (0.60 mg/L) was found in CR in autumn. As is known, according to the drinking water quality classification of the TSI, nitrite values above 0.50 mg/L and over indicate 4th quality drinking waters.

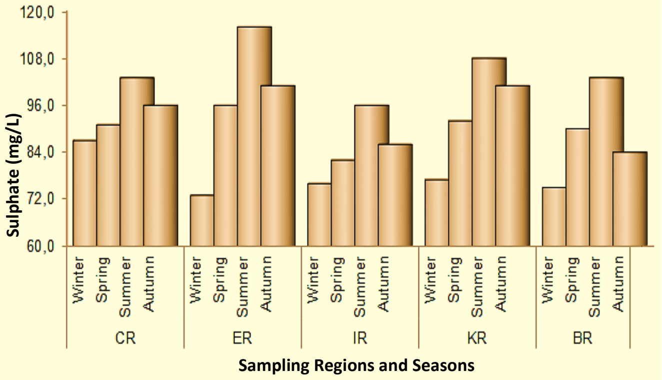

Figure 6. Seasonal changes of sulfate concentrations in the tap waters of Çanakkale

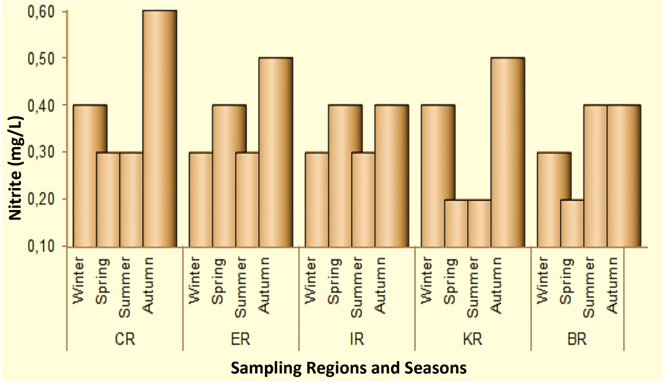

Figure 7. Seasonal changes in nitrite concentrations in the tap waters of Çanakkale

Average nitrite values in Table 4 (0.360 ± 0.001 mg/L) revealed that the tap waters from Çanakkale exceeded the TSI standards for first quality drinking waters (nitrite upper limit value: 0.200 mg/L) (Table 3). In other words, the nitrite findings were high above the upper limit values according to TS 266 drinking water standards (Table 3). It is known that nitrite interacts with hemoglobin and the formation of methemoglobin occurs in this way. As a result, the oxygen-carrying capacity of the blood decreases with the increase of nitrite in the blood [33]. It is known that nitrite has more toxicity than nitrate. For instance, it was reported that LD50 values of sodium nitrite are between 85 and 220 mg per kilogram of body weight in mice and rats [34,35].

The highest nitrate concentration was determined in ER in summer (4.10 mg/L) and the lowest nitrate concentration was determined in CR in winter (2.10 mg/L). The average nitrate (NO_3^-) value in Table 4 (3.13 ± 0.06 mg/L) revealed that the tap waters were lower than the upper Limit Values according to TS 266 Standard (Table 3).

As it is known, nitrate and nitrite are part of the nitrogen cycle and nitrate is the final product of the biochemical oxidation of nitrogen. Nitrate (NO_3^-) is found in stable forms of combined nitrogen for systems rich in oxygen. Therefore, NO_3^- does not have a direct toxic effect. NO_3^- can be reduced by microbial action.

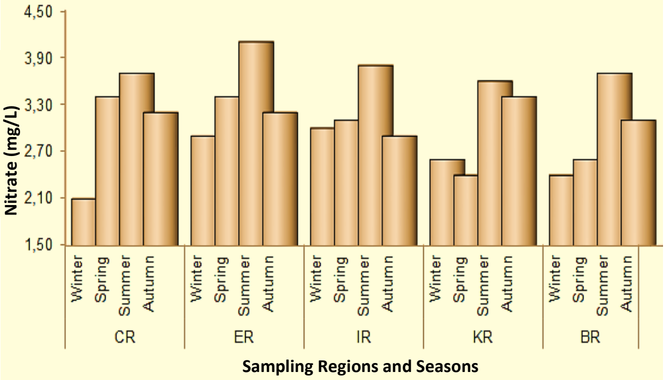

Figure 8. Seasonal changes in nitrate concentrations in the tap waters of Çanakkale

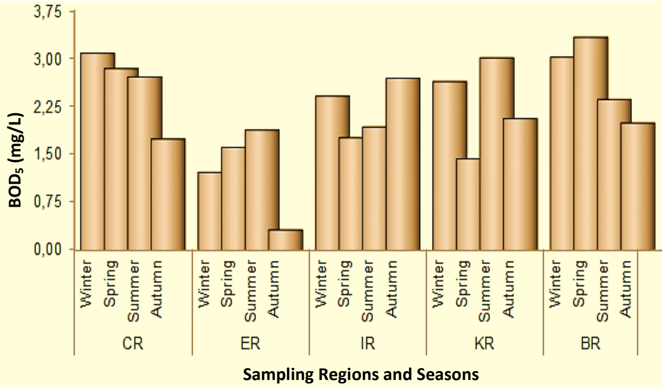

Figure 9. Seasonal changes in BOD in the tap waters of Çanakkale

For instance, NO_3^- is transformed into harmful nitrite ions by the activity of bacterial nitrate reductase [33]. Sometimes, the biochemical reaction can reduce nitrite ions (NO_2^-) to different compounds or oxidize them to NO_3^- [36].

3.9. BOD variations

The biological (biochemical) BOD and COD variations of the tap-waters, which were seasonally taken from 5 various locations of Çanakkale, are presented in Table 4, Fig. 9, and Fig. 10.

BOD is also called biological oxygen demand. BOD refers to the amount of organic matter which can be decomposed biologically under aerobic conditions at a certain temperature over a specific period [37,38]. The BOD value is most stated in terms of mg of oxygen consumed per liter of water sample during 5 days of incubation in the dark at 20.0 °C and is often used as a parameter for the degree of organic pollution in the water [37]. In this study, the highest BOD value was determined in BR in spring (3.33 mg/L) and the lowest value of BOD was determined in ER in autumn (0.32 mg/L) (Table 4 and Fig. 9).

The values for BOD vary proportionally with the values for COD. BOD is similar in function to COD, since both measures the level of organic compounds in water.

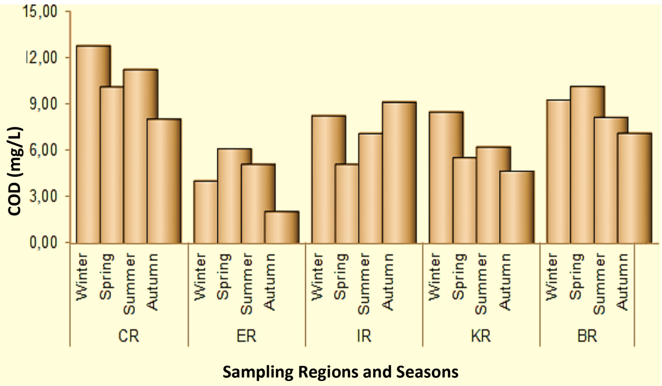

Figure 10. Seasonal changes in COD in the tap waters of Çanakkale

However, because it measures everything that can be chemically oxidized, COD is less specific than BOD which refers only to levels of biodegradable organic matter. The highest value of COD was determined in CR in winter (12.80 mg/L) and the lowest value was determined in ER in autumn (2.06 mg/L) (Table 4 and Fig. 10). Most pristine rivers which have drinking water quality generally have BOD under 1.00 mg/L. Moderately polluted rivers may have BOD values in the range of 2–8 mg/L. Rivers are generally considered severely polluted when BOD values exceed 8.00 mg/L [39]. Both in this framework and according to national and international water standards (Table 3), the tap (drinking) waters of Çanakkale are moderately polluted in view of the average values for BOD (2.20 ± 0.33 mg/L) and COD (7.44 ± 0.76 mg/L) (Table 4, Fig. 9, and Fig. 10).

3.10. Seasonal variations of heavy metals in the tap waters

Seasonal and regional variations of heavy metals (Pb, Cd, Cr, Ni, Fe, Mn, Cu, Zn) in the tap water samples, which were collected from 5 different locations in Çanakkale, are presented in Table 6 and Table 7. Their seasonal variations are also illustrated in Fig. 11–Fig. 14.

3.11. Iron (Fe) variations

Fe variations in the tap waters which were seasonally taken from 5 different locations of Çanakkale, were given in Table 6 and Fig. 11.

During the study, minimum and maximum Fe concentration values were found between nd and 0.173 mg/L (Table 6). The highest Fe concentration value was found in IR and BR compared to other sampling regions (Fig. 11). The highest Fe value (0.173 mg/L) detected in the BR in spring was below the limit value in the TS 266 and other international drinking water standards such as WHO, EPA, and European Union (EC) (Table 3). In conclusion, according to the average Fe value in the drinking waters of Çanakkale, Türkiye (Fe: 0.040 ± 0.055), there was no risk in terms of human health in the study area.

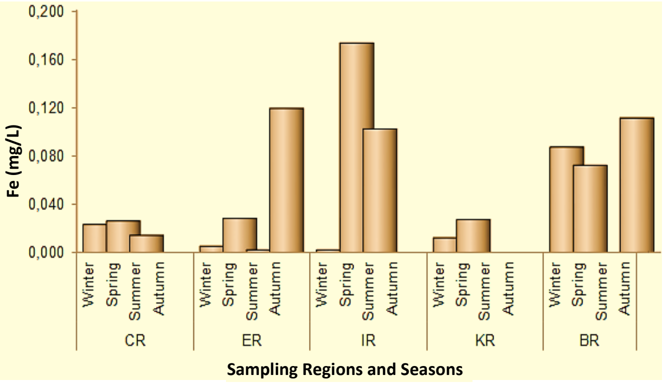

Figure 11. Seasonal changes in Fe concentrations in the tap waters of Çanakkale

The average Fe amount in rivers was reported to be 0.70 mg/L. The concentrations in anaerobic groundwater are usually between 0.50 and 10.0 mg/L, but these concentrations can sometimes reach over 50.0 mg/L [39]. In the study area, even the highest Fe concentration (0.170 mg/L) did not reach this upper limit value (0.30 mg/L) for drinking waters. Fe is known to be an essential element for the human body. The minimum daily requirement for the element varies between 10 and 50 mg/day, depending on age, sex, physiological activity, and bioavailability [40,41].

3.12. Manganese (Mn) variations

Mn variations in the waters, which were collected from various locations in Çanakkale, were given in Table 6 and Fig. 12.

Mn is abundant in the earth's crust [42]. Mn is an essential element for both humans and animals because it is required for the functioning of many cellular enzymes (e.g., manganese superoxide dismutase, pyruvate carboxylase) and can activate many others such as kinases, decarboxylases, hydrolases, and transferases [43]. Manganese ion species are Mn^{2+}, Mn^{4+} or Mn^{7+} [44].

During the study, minimum and maximum Mn concentrations varied from nd to 0.009 mg/L (Table 6). Mn concentrations were higher in IR and BR than in any other sampling region (Fig. 12). The highest Mn value (0.009 mg/L) detected in the IR region in spring (0.009 mg/L) was under the limit value of the WHO, USA, EPA, and EC (Table 3) and is suitable for human health.

It is known that Mn occurs naturally in many surface and ground water sources and soils which may enter these waters. Ambient manganese concentrations in fresh waters typically vary between 0.01 and 2.00 mg/L [45].

3.13. Copper (Cu) variations

Cu variations in water, which were seasonally taken from 5 various places in, were given in Table 6 and Fig. 13.

Table 6. Variations of metal concentrations in the tap-waters of Çanakkale, (nd: not detected)

Seasons	Sampling	Pb	Cd	Cr	Ni	Fe	Mn	Cu	Zn
Winter	Esenler	nd	nd	nd	nd	0.005	0.003	0.002	0.262
	Cevatpaşa	nd	nd	nd	nd	0.023	0.003	0.001	0.087
	İsmetpaşa	nd	nd	nd	nd	0.002	0.003	0.001	nd
	Kemalpaşa	nd	nd	nd	nd	0.012	0.003	0.003	nd
	Barbaros	nd	nd	nd	nd	0.087	0.006	0.001	0.229
	Winter	nd	nd	nd	nd	0.026 ± 0.035	0.004 ± 0.001	0.002 ± 0.001	0.116 ± 0.124
Spring	Esenler	nd	nd	nd	nd	0.028	0.003	nd	0.489
	Cevatpaşa	nd	nd	nd	nd	0.026	0.002	nd	0.058
	İsmetpaşa	nd	nd	nd	nd	0.173	0.009	nd	1.599
	Kemalpaşa	nd	nd	nd	nd	0.027	0.002	nd	nd
	Barbaros	nd	nd	nd	nd	0.072	0.004	nd	0.052
	Spring	nd	nd	nd	nd	0.065 ± 0.063	0.004 ± 0.003	nd	0.440 ± 0.677
Summer	Esenler	nd	nd	nd	nd	0.002	0.001	nd	0.084
	Cevatpaşa	nd	nd	nd	nd	0.014	0.001	nd	0.076
	İsmetpaşa	nd	nd	nd	nd	0.102	0.008	nd	2.343
	Kemalpaşa	nd	nd	nd	nd	nd	nd	nd	0.016
	Barbaros	nd	nd	nd	nd	nd	nd	nd	0.167
	Summer	nd	nd	nd	nd	0.024 ± 0.044	0.002 ± 0.003	nd	0.537 ± 1.011
Autumn	Esenler	nd	nd	nd	nd	0.119	0.002	nd	1.06
	Cevatpaşa	nd	nd	nd	nd	nd	nd	nd	0.084
	İsmetpaşa	nd	nd	nd	nd	nd	nd	nd	1.246
	Kemalpaşa	nd	nd	nd	nd	nd	nd	nd	0.031
	Barbaros	nd	nd	nd	nd	0.111	0.001	nd	0.401
	Autumn	nd	nd	nd	nd	0.046 ± 0.063	0.001 ± 0.001	nd	0.564 ± 0.560
	Averages	nd	nd	nd	nd	0.040 ± 0.052	0.003 ± 0.003	0.001 ± 0.001	0.414 ± 0.644

Table 7. Average values for metal amounts in the tap-waters of Çanakkale, Türkiye during the year (nd: not detected)

Stations	Pb (mg/L)	Cd (mg/L)	Cr (mg/L)	Ni (mg/L)	Fe (mg/L)	Mn (mg/L)	Cu (mg/L)	Zn (mg/L)
ER	nd	nd	nd	nd	0.039	0.002	0.001	0.474
CR	nd	nd	nd	nd	0.016	0.002	nd	0.076
IR	nd	nd	nd	nd	0.069	0.005	nd	1.297
KR	nd	nd	nd	nd	0.010	0.001	0.001	0.012
BR	nd	nd	nd	nd	0.068	0.003	0.001	0.212
Annual Average	nd	nd	nd	nd	0.040 ± 0.055	0.003 ± 0.001	0.0004 ± 0.001	0.4142 ± 0.524

In this study, Cu concentrations of the tap water in Çanakkale were found between nd and 0.003 mg/L (average: 0.001 ± 0.001 mg/L). The highest Cu concentration was determined in KR in winter with a level of 0.003 mg/L (Table 6 and Fig. 13). Fig. 13 revealed that Cu concentrations in KR (0.003 mg/L) and partly in ER (0.002 mg/L) were much higher than the values in any other region. Even these highest Cu values during the study were much lower than the limit value (2.00 mg/L) in the TS 266, WHO, and EPA.

Cu is present in surface water and groundwater in fixed concentrations [46]. Cu concentration in surface waters is 0.0005 to 1.000 mg/L in many studies in the USA [46]. In UK, the average Cu concentration in the river-stour was between 0.003 and 0.019 mg/L (average: 0.006 mg/L). Background concentration based on an upper control station was 0.001 mg/L. This six-fold increase in copper levels was due to the effect of a sewage treatment plant found downstream [47]. Cu levels varied between 0.0008 and 0.010 mg/L in an unpolluted location on the River Periyar in India [47]. Finally, Cu concentrations in drinking water vary widely due to different water physicochemical characteristics [47–50].

On the one hand, the concentration of Cu is to be low [46, 51,52]. On the other hand, the Cu amount in drinking water is increasing [19]. Although Cu concentrations in our tap waters are much lower and suitable in terms of human health, the tap water in our region is slightly acidic (pH: 6.85 ± 0.12).

3.14. Zinc (Zn) variations

Zn variations in the tap-waters, which were seasonally taken from 5 various locations in Çanakkale were given in Table 6 and Fig. 14.

Zn is found in small amounts in almost all volcanic rocks. The fundamental zinc minerals are in the form of sulfides such as sphalerite and wurtzite [53]. The natural zinc level of the soil is generally between 1 and 300 mg/kg [54]. The concentration of Zn is usually under 10.0 µg/L in surface waters and between 10–40 µg/L in groundwaters [53].

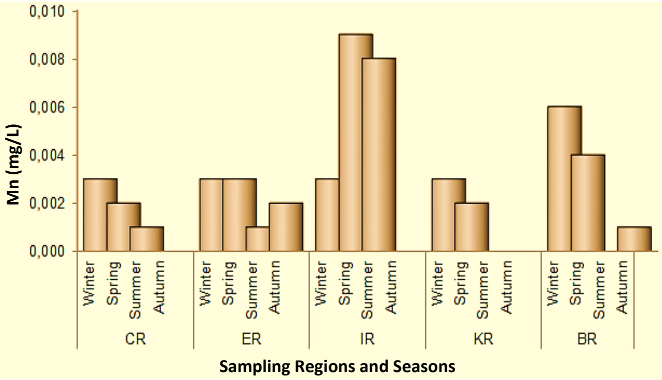

Figure 12. Seasonal changes in Mn concentrations in the tap waters of Çanakkale

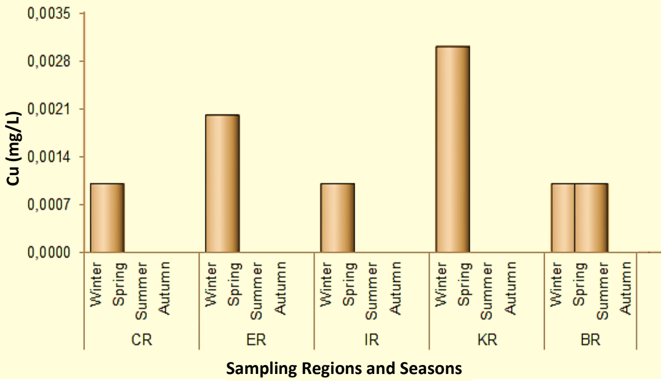

Figure 13. Seasonal changes of copper concentrations in the tap waters of Çanakkale

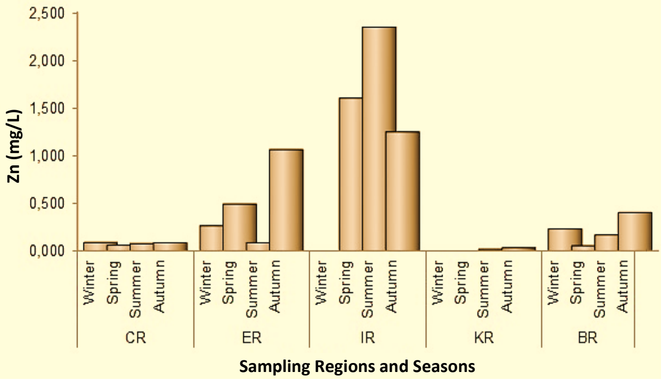

Figure 14. Seasonal changes in zinc concentrations in the tap waters of Çanakkale

In tap water, the Zn concentration can be much higher than usual as a result of the leaching of Zn from piping and relevant equipment [54]. The most corrosive waters in terms of Zn are ones that have low pH, high CO_2 content, and low mineral salts. In a study including 67% of public water supplies in Finland, the average Zn concentration in water samples taken from upstream and downstream parts of the water networks was below 20.0 µg/L. There were much higher concentrations reaching 1.10 mg/L in tap water [55]. Even Zn levels in groundwater were much higher than in surface waters. For instance, there were higher concentrations of Zn reaching 24 mg/L in waters pumped from about 6000 wells in Finland [56].

In our study, the highest Zn concentration was determined in IR in summer with a level of 2.343 mg/L (Table 6 and Fig. 14). Fig. 14 revealed that Zn concentrations in IR and partly in ER were much higher than the values in any other region. The highest Zn value during the study was above the limit value (2.00 mg/L) for TS 266. However, the average value of Zn (0.414 ± 0.644 mg/L) was below the limit values for one national (TS 266) and two international standards of the WHO and EPA. Therefore, Zn levels in the tap water in Çanakkale, Türkiye were within the range of acceptable values. Zn concentrations in the tap water samples in Çanakkale (0.414 ± 0.644 mg/L) were much lower than the concentrations in tap waters from Finland (1.10 mg/L) [55].

3.15. Other toxic heavy metals

During the study, concentrations of Pb, Cd, Cr, and Ni, which are toxic heavy metals for human health, were not found at measurable values (not detection: nd). Considering that toxic heavy metal concentrations were found not detected (nd), there are no health risks for tap waters in Çanakkale for some metal amounts.

4. Conclusion

In this study, unlike previous studies, physicochemical water quality parameters were also studied for the first time along with heavy metal analysis. Results of this study about tap water quality parameters in Çanakkale, Türkiye, unfavorable situations were not detected in terms of physicochemical parameters, except for pH and nitrite. Although pH values measured in the tap water samples from Çanakkale were in the interval between minimum and maximum values of both national and international standards, the average pH value of the drinking waters (6.85 ± 0.12) was under the neutral pH value (pH: 7.00) except for summer values in the KR and IR (Table 3 and Fig. 3). According to the drinking water quality classification of TS 266, nitrite values higher than 0.50 mg/L represent 4th quality drinking waters. The average nitrite value in this study (0.36 ± 0.01 mg/L) revealed that the tap waters in Çanakkale exceeded the TSI standards for first quality drinking waters (Table 3). Therefore, the study showed that Çanakkale tap waters were unhealthy in terms of acidity and nitrite ions. As it is known, the presence of even a low nitrite level in drinking water reduces the quality of drinking water [57].

Considering toxic heavy metals such as Pb, Cd, Cr, and Ni and non-toxic metals such as Fe, Mn, and Zn, it is clear that tap water in Çanakkale does not pose any health risks to human health. To summarize, heavy metal contamination, exceeding national or international standards, was not found in the main water sources in Çanakkale province. However, the findings revealed that although heavy metal concentrations in our tap waters were very low and there was no risk in view of human health, it is necessary to consider that there may be an increase in heavy metal concentrations in the water network system, due to the fact that the tap-waters in our region are slightly acidic (pH: 6.85 ± 0.12). An increase in acidity and temperature is known to increase heavy metal solubility. As it is known that the major source of heavy metals such as lead, iron, copper, and zinc in drinking water is corrosion of household plumbing, taps, and water distribution pipe equipment. Water absorbs a lot of heavy metals such as copper and iron by leaching from plumbing materials such as pipes, fittings, and brass faucets. The number of heavy metals in the water depends on the types and amounts of minerals in the water, how long water stays in the pipes, and the water temperature, and acidity.

Finally, we suggest the public use cold water coming from the taps after running for one minute for drinking and cooking in order to decrease the level of some heavy metals that accumulate when household plumbing is not in use as heavy metals from plumbing corrosion can accumulate overnight. Moreover, because the dissolution of heavy metals is much easier in hot water than in cold water, hot water consumption should be limited, and it should not be used to prepare food for babies.

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Author Contributions

Hasan Kaçar: Collected the tap water samples and performed the analysis.

Selehattin Yılmaz: Conceived the analysis and wrote the paper.

Muhammet Türkoğlu: Performed statistical analysis and wrote the paper.

Murat Sadıkoğlu: Provided scientific contribution.

Conflicts of Interest

The authors declare no conflict of interest.

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Effects of spray and freeze-drying methods on aroma compounds, sensory characteristics, physicochemical composition, antioxidant and antimicrobial properties of instant sage (*Salvia rosifolia* Sm.) tea

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Abstract

Sage is used as a flavoring and spice in foods around the world. Besides its strong and bitter taste, sage has been traditionally used as an effective solution to many health problems for centuries. It is a rich plant with many bioactive compounds. In this study, instant tea production was performed from the dried samples of the sage plant (*Salvia rosifolia* Sm.) using two drying methods: freeze-drying and spray-drying. The sensorial, physicochemical, antioxidant, and antimicrobial properties of the products obtained by both methods were analyzed. Color values, solubility in water, moisture analysis (21.3% spray-dried; 4.0% freeze-dried), free radical scavenging activity (DPPH), Trolox Equivalent Antioxidant Capacity (TEAC), Ferric reducing/antioxidant power assay (FRAP), aroma analysis, protein analysis (5.5% spray-dried; 5.7% freeze-dried), ash, mineral, antimicrobial, and sensory analyses were performed. Significant differences were found between the two soluble teas obtained by the spray-drying and the freeze-drying methods. In general, the antioxidant capacities are higher in freeze-dried samples. All the 61 aroma components were detected in freeze-dried samples while only 18 of them were detected in spray-dried samples. In addition to their differences, plenty of bioactive components, easy to use, ready-to-drink herbal tea have been produced with both techniques.

Keywords: Freeze-drying, instant tea, PCA, sage, spray-drying

1. Introduction

The sage plant (*Salvia* spp) is a member of the Lamiaceae family also known by the names ‘Leadwort’ and ‘Common sage’. It is rich in bioactive compounds such as thujone, cineol, borneol, pinene, saponin, tannin, fumaric acid, and flavone. Scientific studies reveal that it has antioxidant, antibacterial, anti-diabetic and anti-tumor properties and it has a positive effect on memory and Alzheimer’s disease [1,2]. Sage is one of the most important medicinal and aromatic plants used for a long time in the world. In Türkiye *Salvia* species are usually known as Adaçayı and consumed as tea [3]. Sage has been used for the treatment of some diseases such as epilepsy, colds, hemorrhoids, pains, tuberculosis, and menstruous disorderliness since ancient times [4]. There are many studies that report the antibacterial, anti-viral, anti-inflammatory, and anticarcinogenic effects of *Salvia* species [5–7].

Spray-drying is used for producing high-quality products in terms of particle size distribution, residual moisture content, bulk density, and particle morphology. Spray drying is generally used in the food industry to ensure microbiological stability by reducing the water content and water activity of the products, avoiding the risk of chemical and/or biological degradation, reducing storage and transport costs, and final yield of products with specific properties such as immediate solubility [8].

Freeze-drying is a drying technique of a frozen food product under a vacuum at a low temperature This process provides the vaporization of frozen water without becoming liquid under a vacuum. Freeze-drying has advantages such as uniformity, high quality, long shelf life, lightweight, and ease of transport for the products. Nowadays, in addition to the protection of food products (coffee, tea, crispy fruits and vegetables, ready-to-eat foods, and some aromatic herbs), biotechnological products and pharmaceutical products are successfully produced by freeze-drying [9].

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Spray and freeze-drying methods have been extensively studied in many food areas, but no comparative study has been reported on instant sage (*S.rosifolia* Sm.) tea that can provide better insight into the potential of these two methods. Hence, this study aimed to compare the effect of both drying methods in terms of sensory, physical, and chemical properties; antioxidant and antimicrobial properties, and explore the potential of both methods. It is also important for producing first-time instant sage tea with a freeze drier and this study can be a preliminary resource for the researchers who work in this area. The produced soluble herbal tea will provide an ability to consume natural herbal tea rich in bioactive components and easy to use.

2. Materials and methods

2.1. Materials

Samples of the sage plant (*S. rosifolia* Sm.) used in the study were collected in Gümüşhane region during the flowering season (40° 16' 19.23" N 39° 28' 53.96" E, above sea level: 1933 m, Kelkit, Gümüşhane). *S. rosifolia* Sm. species were identified by M. Gültepe (Gültepe 722 (KTUB). The samples were dried in the shadow and natural environment until they reached an 8% moisture level. Samples were stored in locked polyethylene bags during storage.

2.2. Extraction process of the sage leaves

The same extraction process was used for both techniques. For this, 1250 g of plant samples were weighed and steeped for 10 minutes in 10 L of boiled water. During the steeping stage, sage was mixed with a spoon at intervals of several minutes. At the end of 10 minutes, the herbal tea was filtered with filter paper (grade 1: 11 µm; medium flow filter paper) and cooled to room temperature. The obtained herbal tea was stored at +4 °C in capped glass bottles.

2.3. Manufacturing of instant sage tea with freeze-drying

All of the aqueous extracts were divided into splay glass containers and lyophilized for 72 hours after being frozen at -65 °C for 0.1 mbar in the freeze dryer (Scientz-12N Laboratory Lyophilizer, Ningbo Scientz Biotechnology, China). At the end of this period, freeze-dried samples were transferred to dark-capped glass bottles and stored at +4 ° C. Production was done in three replicates. 297.0 ± 12.2 g product was obtained.

2.4. Manufacturing of instant sage tea with spray drying

All of the aqueous extract obtained was dried in a laboratory-type spray dryer (The SD-06 spray, Labplant UK, England). It was conducted for 4 hours at a liquid flow rate of 5 mL/s and the internal temperature was set at 200 °C. Powdered samples were taken from the machine's flask and stored at +4 °C in dark-capped bottles. Production was carried out in 3 replications. 246.0 ± 9.8 g product was obtained.

2.5. Analysis of the spray and freeze-dried sage tea samples

2.5.1. *Moisture analysis*

Moisture contents of the freeze-dried and spray-dried samples were weighed at 1 g and kept at 70 °C until the fixed weighing of the sample was reached. The measurement was made in triplicate. Moisture content was expressed as gram loss of water/100 g sample [10].

2.5.2. *Crude protein and ash analyses*

For the protein analysis, a 1.0 g sample was weighed into a Kjeldahl tube and 1 tablet catalyst and 20 mL H_2SO_4 were added and burned for 4 hours. Then 4% H_3BO_3 was added to samples on the distillation unit. Distillate titrated with 0.1 N HCl [11].

For the ash analysis, samples were weighed 2.5 g into the porcelain crucible, and the following pre-burning samples were kept at 550 °C in the ash oven. % Ash was calculated by weighing [12].

2.5.3. *Yield % analysis*

The yield of products obtained by two different methods using the classical brewing method was calculated according to the following formula.

$$Yield\ (\%) = \frac{P}{R} \times 100 \qquad (1)$$

P: the amount of the obtained powder (g), R: the amount of the used plant (g).

2.5.4. *Color analysis*

Color analysis was performed using a Minolta Chromameter (CR-200) (Konica Minolta Sensing, Inc. Japan). For the hunter scale; L (dark/whiteness), a (greens/redness), b (blue/ yellow) are color parameters [13]. About 5 g of instant sage was placed in the sample vessel in front of the light source. The color values of the freeze-dried and spray-dried products were determined by studying on average six replicates. For the calibration of the device, L* 97.96, a* 0.08, and b* 1.78 white tiles were used.

2.5.5. *Analysis of solubility in water*

Determination of the water solubility of the products; 1.0 g of powder product was weighed and 100 mL of deionized water at room temperature was added. Thereafter, the mixture was stirred in a magnetic stirrer at 600 rpm for 5 minutes. 20 mL of the solution was transferred to a petri dish and dried at 70 °C for 24 hours, after the drying period petri dish was weighed and the difference was expressed as 100 mL resolution [10].

2.6. Antioxidant activity analysis

Sample instant tea at a concentration of 200 µg/mL in distilled water was used as samples in the analysis. UV-1800 (Shimadzu, Japan) spectrophotometer was used to measure absorbances in all of the antioxidant analyzes.

2.6.1. *Free radical scavenging activity (DPPH)*

The free radical scavenging activity was determined by the DPPH method [14]. Diluted samples (100 µL each) were mixed with 3000 µL of freshly prepared 80 µg/mL DPPH methanol solution and allowed to stand for 30 min in the dark at room temperature for any reaction to take place. The ultraviolet (UV) absorbance of these solutions was recorded on a spectrometer at 517 nm using a blank containing the same concentration of extracts without DPPH.

2.6.2. *Trolox equivalent antioxidant capacity (TEAC)*

The ABTS solution was prepared by storing 7 mM aqueous ABTS solution and 2.45 mM potassium persulfate solution (1/1, v/v) for 6 hours in a dark place at room temperature. At the beginning of the analysis, the ABTS stock solution was diluted with methanol and the absorbance at 734 nm was set to 0.700 ± 0.020. The mixture of methanol (900 µL), stock solutions of isolated molecules (100 µL), and ABTS solution (3mL) were transferred to the spectrophotometer cuvettes. The absorbance of 734 nm was measured after incubation at room temperature for 120 min [15]. Trolox was used as a standard substance.

2.6.3. *Ferric reducing/antioxidant power (FRAP) assay*

For the FRAP activity assay of water-soluble tea, 300 mM sodium acetate buffer solution (pH 3.6), 20 mM aqueous $FeCl_3$ solution, and 10 mM aqueous TPTZ solution were mixed in a ratio of 10/1/1. The FRAP solution (3 mL) was mixed with the herbal tea sample (100 µL) and methanol (900 µL) into the spectrophotometer cuvettes, and the measuring of absorbance was done at 593 nm after storing for 30 minutes at room temperature [16].

2.7. Phenolic substance analysis

HPLC-UV-DAD analyzes were performed on the Agilent 1200 series (DAD 1200) instrument for total phenolic content. The device was checked with the Agilent Chemstation program. C18 column (250 mm × 4.6 mm i.d., 5 µm particle) was used for all analyzes. The mobile phase gradient was A; ultra-pure water, B; ACN (acetonitrile HPLC purity), C; 3% acetic acid + ultra-pure water, D; 3/25% acetic acid + ACN + ultra-pure water. The flow rate was 0.800 ml/min. Gradient flow; C90%-D10% for 0 min, C80%-D20% for 10 min, C50%-D50% for 22 min, C20%-D80% for 32 min, B80%-D20% for 45 min, C90%-D10% for 55 min (5 min).

The injection volume of the samples was used as 20 µL. The flow rate was set to 0.800 mL/min. 232, 246, 260, 272, 280, 290, 308 and 328 nm were recorded with the DAD detector [17]. 5 g of sample was extracted with acetone (10 mL) in an ultrasonic bath for 10 min. It was centrifuged at 1500 g for 10 min at +4 °C. The procedure was repeated using 70% acetone (10 mL). The acetone was evaporated under nitrogen gas at 37 °C and dissolved in 15 mL of purified water and filtered through a 0.45 µm filter (Millipore Corp.) was injected 20 µL into the device [18].

2.8. Mineral analysis of water-soluble tea

For the mineral analysis, the sample was weighed about 0.5 g and acidified with 5 mL of concentrated nitric acid. Burned in a microwave. At the end of the burning, the sample was transferred to a 25 ml balloon and the volume was completed with ultrapure water. Minerals were determined by using a coupled plasma mass spectrometer. The calibration curves were drawn with 5 different concentrations for all the analytes. The calibration standards were analyzed at regular intervals to control instrument drift. Also, ultrapure deionized water blanks were analyzed after each standard to control cross-contamination [19].

2.9. Aroma analysis of the water-soluble tea

Two grams of weighed sage samples for both products were left in 100 mL of hot water for 10 minutes. 40 mL of these solutions were taken into separation funnels, then 10 mL of saturated sodium chloride solution was added. The mixture was extracted four times with 40 mL of binary solvent (diethyl ether and pentane, volume ratio 2: 1). The extracts were then combined and washed two times with 50 mL of saturated sodium chloride solution and 50 mL of deionized water, respectively. The extracts were stirred with 10 g of anhydrous sodium sulfate and filtered. The filtrate was concentrated to the final volume of 0.5 mL at 39 °C with a rotary vacuum evaporator. A 1.0 µL sample was injected into the GC-MS / FID device [20]. Instrument: Agilent 5975 GC-MS Detector. Column: 30 m × 0.25 mm ID, 0.2 µm HP-5MS. Oven: 50 °C, 4 °C/min. to 260 °C, 15 min. Carrier Gas: Helium, constant flow 1.2 mL/min. Injection: 250 °C Detector: MS, 230 °C FID Detector: 250 °C, Gas flows H_2 40 ml/min and dry air 400 ml/min.

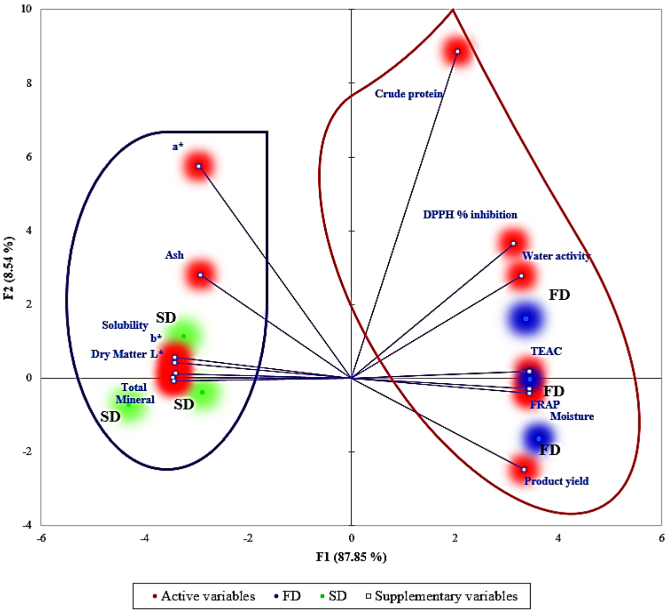

Figure 1. Multivariate analysis of physico-chemical, antioxidant activity and color of two types of instant tea. PC1 explaining 87.85%, versus PC2 explaining 8.54%, FD: Instant sage (*S. rosifolia* Sm) freeze-dried tea, SD: Instant sage (*S. rosifolia* Sm) spray-dried tea

2.10. Sensory analysis

Freeze-dried and spray-dried samples of brewed tea were prepared for sensory analysis according to ISO norms [21]. 0.4 g of each sample was taken and placed in porcelain pots with the addition of 10 ml of boiled water. The analysts were formed from 12 educated people (students of the food engineering department, the panel consisted of 6 male and 6 female, aged 18 to 24, nonsmoker panelists). Descriptive analysis of infused teas; Just the aroma, the color, the taste and smell, the appearance of the instant tea, and the overall acceptance were evaluated and the sensory scores ranged from 1 (1, 2, 3, and 4 = disliked extremely, disliked very much, disliked moderately, and disliked slightly, respectively, 5 = neither liked nor disliked) to 9 (6, 7, 8, and 9 = liked slightly, liked moderately, liked very much, and liked extremely, respectively).

2.11. Antimicrobial analysis

After determining the extractions conditions of the instant activity were assayed by the agar diffusion method.

Antimicrobial activity was tested on 31 different microorganisms. The agar diffusion test was performed according to the selected method [22]. Holes were made using sterile hole openers (6 mm in diameter) and, the sample was loaded in each hole (50 µL). Streptomycin sulfate and nystatin were used as a positive control. The prepared Petri dishes were kept at room temperature for 2 hours and placed face up in the incubator, incubated at +37 °C for 24 hours, and the inhibition zone diameters were measured with a scale.

2.12. Statistical analysis

PCA and AHC were performed to evaluate the possible relationship between the studied parameters using the software package (XLSTAT Addinsoft SARL 2019). All analyses were made triplicates and results were given as mean ± standard deviation (SD). To compare the significant differences in the mean values at $p < 0.05$ was used ANOVA.

3. Results and Discussion

3.1. Analyses of physicochemical, antioxidant activity and color

Results of the physicochemical analysis, and color are given in Table 1. PCA analysis of instant tea samples was performed on values of fourteen quality parameters aiming to identify similarities and differences among them. PCA, shown in Fig. 1 described 96.39% of the initial data variability of the total variance. On the observation plot, there was determined a clear separation between spray-dried and freeze-dried instant tea samples. This situation can be seen in Fig. 1, clustered in two different colored circles (red and blue colored). In order to describe these characteristics of applied physicochemical properties, antioxidant activity, and color parameters for freeze and spray-dried sage (*S. rosifolia* Sm) instant tea samples and obtain an overview of the main variations among them; product yield (PY), moisture (M), dry matter (DM), water activity (WA), solubility (S), crude protein (CP), ash (A), total mineral (TM), color ((C) (L*, a*, b*)), FRAP, TAC and DPPH% were subjected to PCA analysis together.

Table 1. Changes in the physicochemical properties and of the spray and freeze-dried *instant sage (S. rosifolia Sm.) tea*

Product Type	Product yield (g/100 g)	Moisture (g/100 g)	Dry Matter (g/100 g)	Water activity	Solubility (g/100 g)	Crude Protein (g/100 g)	Ash (g /100 g)	Color		
								L*	a*	b*
SD	20.0^{b} ± 0.8	4.0^{b} ± 0.1	96.0^{a} ± 1.0	0.26^{b} ± 0.01	97.4^{a} ± 1.9	5.5^{a} ± 0.5	12.7^{a} ± 0.7	66.6^{a} ± 0.8	3.2^{a} ± 0.2	29.6^{a} ± 0.18
FD	24.0^{a} ± 1.0	21.3^{a} ± 0.9	78.7^{b} ± 0.8	0.45^{a} ± 0.01	80.4^{b} ± 0.7	5.7 ^{a} ± 0.3	11.5^{b} ± 0.8	25.1^{b} ± 0.2	2.4^{a} ± 0.1	-0.9^{b} ± 0.1

Note: Results are presented as means; ± standard deviations. Instant sage was expressed as g/100 g dry weight protein and ash, except color. Different letters (a-b) in the same column are significantly different (p<0.05). FD: Instant sage (*S. rosifolia* Sm) freeze-dried tea, SD: Instant sage (*S. rosifolia* Sm) spray-dried tea.

Tea samples were characterized by significant correlations between the 14 variables analyzed while there were negative strong correlations between A, DM, S, TM, L*, a*, b* (blue colored circle) and FRAP, TEAC, DPPH, PY, WA, M (red-colored circle); on the other hand, strong positive correlations were found amongst themselves these clusters. According to PCA analysis, both negative and positive correlations were detected between CP and other analyzes. The correlation matrix is given in Table 2.

The moisture content of the products obtained by freeze dryer was found the moisture 21.3%, the dry matter content 78.7%, product yield 24.0%, and the water activity 0.45. The spray-dried products' moisture content was found the moisture 4.0%, dry matter content 96.0%, product yield 20.0%, and water activity 0.26. Moisture, dry matter content and water activity varied significantly ($p < 0.05$) with spray and freeze-dried instant sage tea (Table 1). It is mentioned that immediately tea powder having < 5 g/100 g moisture content material indicates a greater balance in packaging and storage [23]. Water activity content was found as compatible with the literature (0.23–0.29) for the SD sample [10]. Food processing techniques such as drying, freezing, curing, and baking affect water activity. On PCA analysis, except for moisture and CP (R = 0.558), there was a negative strong correlation between moisture and A, S, DM, and color (L*, a*, b*). On the other hand, a strong positive correlation was observed between moisture and WA, PY, and antioxidant capacities. These correlation values were given in Table 2.

When the water solubility ratios were examined, the solubility of the freeze-dried samples was found as 80.4% and it was found as 97.4% in the spray-dried samples. The solubility ratio of the spray-dried samples was found to be significantly ($p<0.05$) higher than the freeze-dried samples. In the literature; The solubility values of the sage (*Salvia fruticosa* Miller) obtained by spray-dried were found to be in the range of 97.4–99.2 g/100 g [10].

Ash and crude protein content is given in Table 1. The crude protein content of both obtained products was analyzed. Based on dry weight, there was not any significant ($p > 0.05$) difference between the two samples in the crude protein that were found to be 5.5% and 5.7% in the spray-dried and freeze-dried samples, respectively. In PCA analysis crude protein showed weak positive and negative correlations with other analyzes except for DPPH (R = 0.821 strong). In spray-dried and freeze-dried dried samples, ash content was found to be 12.7% and 11.5% respectively ($p > 0.05$).

In Table 1, it can be easily determined that there is a discrepancy in color for L*, a*, and b* difference values between spray-dried and freeze-dried samples. These values show that the spray-dried sample is lighter in color, slightly reddish, and much yellow in color than the freeze-dried sample. The brightness of spray-dried products is higher than freeze-dried products. There is no significant ($p>0.05$) difference between 'a*' value of the two methods. Spray-dried product has higher b* and L*value ($p<0.05$). The drying technique has an effect on the color of the final product. The total color difference between the two samples is ΔE*= +51.6±1.6. It seems that the difference in total color difference value is quite large and it is understood that the spray-dried sample is brighter than freeze-dried sample. These color changes in instant sage tea samples may result from not only

Table 2. Correlations (R values) between physico-chemical, antioxidant activity and color

Variables	**Product yield**	**Moisture**	**Dry Matter**	**Water activity**	**Solubility**	**Crude protein**	**Ash**	**TEAC**	**FRAP**	**DPPH% inhibition**	**L***	**a***	**b***	**Total Mineral**
Product yield	**1**	**0.951**	**-0.942**	**0.844**	**-0.937**	0.408	**-0.940**	**0.958**	**0.948**	**0.841**	**-0.914**	**-0.938**	**-0.946**	**-0.939**
Moisture		**1**	**-0.998**	**0.941**	**-0.998**	0.558	-0.808	**0.988**	**0.999**	**0.876**	**-0.993**	**-0.857**	**-1.000**	**-0.997**
Dry Matter			**1**	**-0.924**	**1.000**	-0.528	0.782	**-0.976**	**-0.999**	**-0.854**	**0.997**	**0.859**	**0.997**	**0.996**
Water activity				**1**	**-0.927**	0.763	-0.730	**0.962**	**0.932**	**0.913**	**-0.927**	-0.689	**-0.948**	**-0.937**
Solubility					**1**	-0.537	0.774	**-0.976**	**-0.999**	**-0.854**	**0.998**	**0.852**	**0.997**	**0.997**
Crude protein						**1**	-0.352	**0.611**	0.546	**0.821**	-0.547	-0.097	-0.579	-0.579
Ash							**1**	**-0.860**	-0.798	-0.787	0.734	**0.874**	0.805	**0.786**
TEAC								**1**	**0.983**	**0.911**	**-0.965**	**-0.846**	**-0.990**	**-0.981**
FRAP									**1**	**0.869**	**-0.995**	**-0.858**	**-0.999**	**-0.998**
DPPH% inhibition										**1**	**-0.843**	-0.604	**-0.885**	**-0.886**
L*											**1**	**0.828**	**0.992**	**0.993**
a*												**1**	**0.846**	**0.833**
b*													**1**	**0.997**
Total Mineral														**1**

Note: Values in bold are different from 0 with a significance level alpha = 0.05. Significant correlations are displayed **in bold**. Correlation coefficients vary -1 and 1. The closer is to 1 or -1. stronger is the link between two variables. **Negative** values indicate negative correlation and **positive** values indicate positive correlation. Values close **zero** reflect the absence of correlation

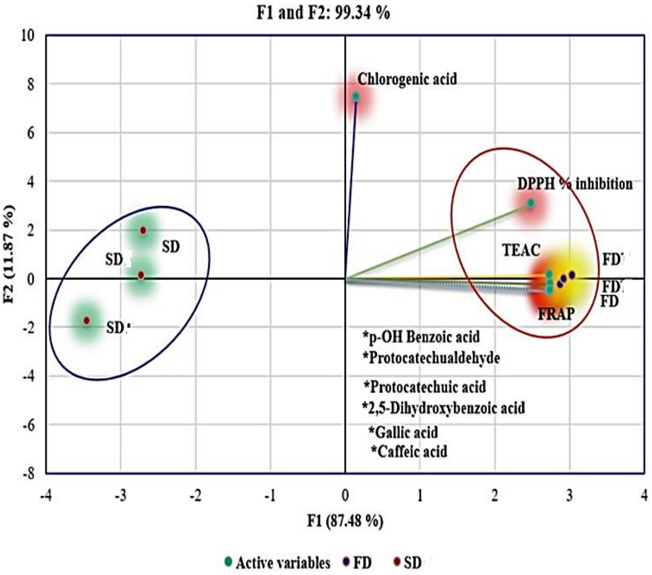

Figure 2. PCA of antioxidant activity and phenolic, flavonoid compounds of two types of instant sage tea. PC1 explaining 87.48%, versus PC2 explaining 11.87%. *Compounds in the bottom right quadrant. FD: Freeze-dried tea, SD: Spray-dried tea

thermal treatments and the non-enzymatic browning reaction but also the removal of volatile components in the spray-dried samples. 18 volatile compounds were detected in the spray-drying sample, while 61 volatile compounds were detected in the freeze-drying sample. In a study, significant differences were observed also for the color indices when comparing samples subjected to low-temperature and high-temperature drying. It was determined that there was a correlation matrix between color indices and volatile compounds [24]. Similar L*, a*, and b* results were also reported as 61.8-79.7, 0.1 to 4.0, and 15.8-20.5, respectively in previous studies [25].

3.2. TEAC, FRAP, DPPH, phenolic, and flavonoid substance analysis

Analysis of PCA, shown in Fig. 2 described 99.34% of the initial data variability of the total variance. The comparison between antioxidant, phenolic, and flavonoid profile of freeze-dried and spray-dried samples are given in (Fig. 2). In this evaluation, two different clusters occurred (red and blue colored circles) which were completely separate from each other.

Table 4. Phenolic and flavonoid contents for freeze-dried and spray-dried samples

No	Name	R.T.	FD	SD
			Concentration	
1	2,5-Dihydroxybenzoic acid	3.524	17.8^{a} ± 0.2	3.4^{b} ± 0.1
2	Benzoic acid	17.263	n.d.	n.d.
3	Caffeic acid	38.283	550.7^{a} ± 8.2	389.3^{b} ± 7.4
4	Catechin	29.646	n.d.	n.d.
5	Chlorogenic acid	26.122	491.5^{a} ± 9.3	388.9^{b} ± 7.9
6	Ferulik acid	28.334	n.d.	n.d.
7	Flavone	38.952	n.d.	n.d.
8	Galangin	30.123	n.d.	n.d.
9	Gallic acid	37.003	186.3^{a} ± 4.2	156.4^{b} ± 3.7
10	Myricetin	9.003	n.d.	n.d.
11	p-Coumaric acid	30.224	n.d.	n.d.
12	p-OH Benzoic acid	36.339	256.6^{a} ± 4.4	139.29^{b} ± 8.99
13	Protocatechualdehyde	23.371	35.1^{a} ± 2.1	19.2^{b} ± 3.3
14	Protocatechuic acid	20.399	174.3^{a} ± 5.7	88.1^{b} ± 4.2
15	Quercetin	15.993	n.d.	n.d.
16	Rutin hydrate	35.402	n.d.	n.d.
17	Sesamol	41.935	n.d.	n.d.
18	Sinapic acid	29.900	n.d.	n.d.
19	Syringaldehyde	39.822	n.d.	n.d.
20	Syringic acid	35.401	n.d.	n.d.
21	Vanillic acid	30.534	n.d.	n.d.
22	Vanillin	27.795	n.d.	n.d.

Note: Results are presented as means; ± standard deviations; all phenolic and flavonoid compounds are expressed as µg/g dry weight. Different letters (a-b) in the same rows lines are significantly different ($p < 0.05$). n.d: Not detected. FD: Instant sage (*S. rosifolia* Sm) freeze-dried tea, SD: Instant sage (*S. rosifolia* Sm) spray-dried tea

There was a strong positive correlation between the antioxidant analysis results (Table 2).

The antioxidant properties of natural products such as plant extracts are originating from the bioactive compound's nature and sometimes-synergistic effects between them. However, it is difficult and time-consuming to determine the contribution of each component to the total antioxidant activity. The general procedure is to measure the total antioxidant capacity of the whole sample. Lamiaceae family has high antioxidant properties [26,27]. They contain a variety of phenolic substances which have both reduction ability (FRAP) and radical sweeping (DPPH) effects. A variety of methodologies is commonly used to assess

Table 3. Mineral contents for freeze-dried and spray-dried samples

Sample	Be	Na	Mg	Al	P	S	K	Ca	V
SD	<LOQ	711.7^{a} ± 48.2	10337.5^{a} ± 299.2	603.5^{a} ± 79.8	5464.3^{a} ± 154.9	5971.1^{a} ± 128.7	72644.5^{a} ± 223.43	24597.4^{a} ± 126.1	2.2^{a} ± 0.5
FD	<LOQ	540.4^{b} ± 34.1	9054.7^{b} ± 187.9	583.3^{b} ± 78.65	4063.2^{b} ± 145.87	3964.7^{b} ± 156.6	63838.2^{b} ± 157.43	20596.5^{b} ± 870.9	1.1^{b} ± 0.2
	Cr	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**Ga**	**As**
SD	4.9^{a} ± 0.5	161.4^{a} ± 21.1	843.6^{a} ± 93.3	2.7^{a} ± 0.3	17.7^{a} ± 2.4	9.1^{a} ± 1.0	39.2^{a} ± 3.9	8.9^{a} ± 1.6	<LOQ.
FD	3.6^{b} ± 1.0	122.1^{b} ± 4.3	708.2^{b} ± 65.8	1.4^{b} ± 0.1	12.8^{b} ± 0.3	7.92^{b} ± 0.25	15.95^{b} ± 0.7	6.6^{b} ± 0.2	<LOQ
	Se	**Rb**	**Sr**	**Ag**	**Cd**	**Cs**	**Ba**	**Pb**	**Hg**
SD	0.2^{a} ± 0.1	24.4^{a} ± 0.8	102.04^{a} ± 4.56	0.56^{a} ± 0.09	<LOQ	0.3^{a} ± 0.1	116.1^{a} ± 8.8	<LOQ	<LOQ
FD	0.2^{a} ± 0.01	18.2^{b} ± 0.2	79.24^{b} ± 4.98	0.34^{b} ± 0.10	<LOQ	0.2^{b} ± 0.01	69.7^{b} ± 3.2	<LOQ	<LOQ

Note: Results are presented as means minerals mg/kg ± standard deviations, Different letters (a-b) in the same column are significantly ($P <0.05$) different FD: Instant sage (*S. rosifolia* Sm) freeze-dried tea, SD: Instant sage (*S. rosifolia* Sm) spray-dried tea.

antioxidant potential. The average total antioxidant capacities of instant tea obtained by spray-drying and freeze-drying methods are given in Table 5 in dry matter. The antioxidant capacity of the instant tea sample dried with the freeze dryer was found to be higher. The reason for this situation can be interpreted as the expulsion of certain compounds from the product due to the high-temperature treatment in the spray-drying process and this caused the determination of more compounds in the samples dried with a freeze dryer for the analysis of aromatic compounds.

A study demonstrated that the total phenolic amount in Salvia species in Türkiye ranged from 50.3 to 167.1 mg GAE/g in the dry matter [28]. DPPH% inhibition study was conducted by Senol and colleagues on 55 salvia species in 2010. They found that the DPPH% inhibition rate was 45.56, 77.25, and 90.61, respectively, at concentrations of 25 to 50 and 100 mg/ml prepared from the methanol extract of *S. rosifolia* [29]. In this study, ascorbic acid and Trolox were used as DPPH antioxidant standards. Inhibitions percentages of these standards were determined as 98.50% and 97.99% respectively. When compared to Trolox and Ascorbic acid, inhibition rates of both samples were found quite high in Table 5.

Phenolic and flavonoid compounds were analyzed by extraction of the samples and the HPLC-DAD method (Table 4). In the PCA analysis, except for the chlorogenic acid analysis result, there was a strong positive correlation between the other acid and aldehyde analysis results (Fig. 2, Table 2). In the literature, these phenolics (in Table 4) are found in salvia species [30–32]. Sage antioxidants are an alternative to the one of the common antioxidants of rosemary and can be used for the protection and preservation of certain food and nutraceutical products and can help the extension of their shelf life [44].

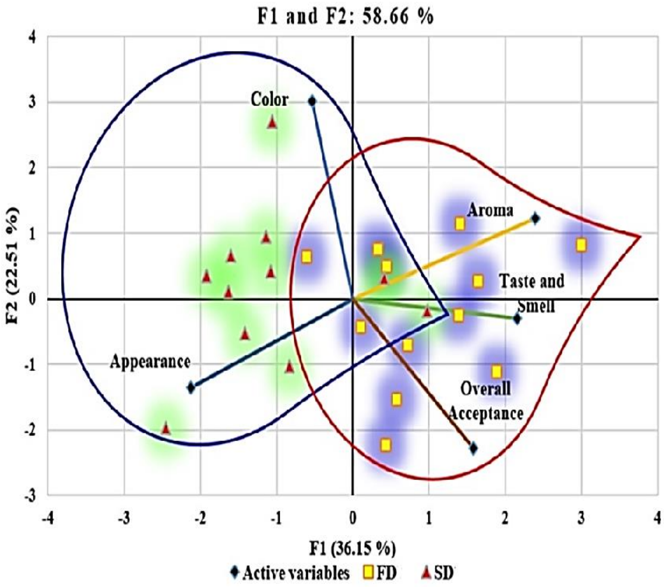

Figure 3. Two-dimensional principal component analysis: Representation of the sensory descriptors and the instant Sage tea samples evaluated by 12 panel members. PC1 explaining 36.15%, versus PC2 explaining 22.51%. FD: Instant sage (*S. rosifolia* Sm) freeze-dried tea, SD: Instant sage (*S. rosifolia* Sm) spray-dried tea

3.3. Mineral analysis

In the samples, twenty-seven mineral analyzes were performed by ICP-MS. The results are given in Table 3. The values of Na, Mg, Al, P, S, K, Ca, Mn, Fe, Sr, and Ba minerals are quite high in both samples. However, the contents of minerals were significantly higher in spray-dried sage tea ($p < 0.05$). This is due to the low probability of moisture content of the SD sample. When the results are given on the basis of dry matter, the results are close to each other. Heavy metal contents of Hg, Pb, Cd, and as were not detected (N.D.) (LOQ, Pb: 1 µg/kg, Hg: 5 µg/kg, Cd: 5 µg/kg, As: 5 µg/kg). The results are in agreement with the Turkish Food Codex and literature [33]. The results show that the sage contains essential minerals in human metabolism that have a vital preserve, prevention, and improvement of diseases in growth and development literature [34].

3.4. Analysis of the aroma content and sensory characteristics

PCA is the most well-known method to explore relationships between both variables and observations, which provide implicated information and can be analyzed graphically by considering all variables simultaneously. In the PCA analysis of instant tea groups, the diagram of PCA1 and PCA2 represented 58.66% of the cumulative variance. The two groups were separated partially, as shown by the score plot in Fig. 4 (red and blue colored circles). Scores were arranged in four areas. The separation between the samples pointed out the differences in certain investigated sensory parameters. Also, the score chart showed that from both groups surveyed, FD got scores for aroma, overall acceptance, taste, and smell while SD were got scores for

Table 5. Changes in antioxidant capacities of the spray and freeze-dried *instant sage (S. rosifolia Sm.) tea*

Product Type	TEAC	FRAP	DPPH % inhibition
SD	169.1^{b} ± 6.4	916.0^{a} ± 11.0	73.9^{b} ± 4.3
FD	231.9^{a} ± 9.1	1235.8^{a} ± 14.1	78.6^{a} ± 2.9

Note: Values for Trolox equivalent antioxidant capacity were expressed as mg TEAC/g dry weight, antioxidant activities of instant sage tea were expressed as mmol $FeSO_4$/g dry weight for FRAP, % inhibition for DPPH. Different letters (a-b) in the same column are significantly different ($p < 0.05$). In this study, ascorbic acid and trolox were used as antioxidant standards. Inhibitions percentages of these standards were determined as 98.50% and 97.99% respectively. TEAC: Trolox equivalent antioxidant capacity. DPPH: Sweeping Activity. FRAP Iron-reducing capacity. FD: Instant sage (*S. rosifolia* Sm) freeze-dried tea, SD: Instant sage (*S. rosifolia* Sm) spray-dried tea.

color and appearance by the panelists (red and blue colored circles). The five variables of sensory analysis are characterized by weak correlations in Fig. 3. Aroma and flavor indicators are based on volatile compounds. Aroma perception depends on the number of volatile substances present in food [27]. Therefore, the presence of aroma compounds affects the results of sensory analysis.

In this study, high scores of FD samples by panelists can be attributed to aroma compounds. A total of 61 compounds corresponded to 97.1% of the total area in the study of freeze-dried sample aroma analysis and 18 compounds were identified corresponding to 98.0% of the total area in the spray-dried sample aroma analysis. The compounds, their concentration ratios, and RI Index are given in Table 6 for both the experimental and the literature, according to the definition order of RI, MS. The reduced number of compounds in the spray-dried samples can be attributed to the removal of the compounds from the exhaust of the device due to the high temperature (200 °C) airflow during the applied process. The analysis of PCA obtained data of PCA1 and PCA2 represented 100.00% of the total aroma compound fractions variance. The freeze and spray dried instant teas were separated by PCA into two main groups in Fig. 4 (red and blue teardrop circles). The first group SD (blue teardrop circle) characterized a total of 18 compounds, and the second group FD (red teardrop circle) total of 61 compounds. According to the literature, Epimanool, Camphor, Aromadendrene, Borneol, Camphor, α-Thujone, caryophyllene oxide, and Carvone oxide were determined as volatile compounds of Sage samples [35,36,43]. On analysis of PCA, amounts of other compounds detected were < 1% lower and showed a negative correlation with compounds with high amounts of compounds.

3.5. Antimicrobial activities

The results of the antibacterial activity test freeze-dried and spray-dried samples according to the disk diffusion method are given in Table 7. Antibacterial activity of the tea extracts was compared with Streptomycin sulfate and Nystatin as positive control and used with a concentration of 10 µg/ mL. Instant tea of freeze-dried (1 mg/mL) showed antibacterial activities against *Bacillus cereus, Enterococcus faecalis, Escherichia coli, Listeria monocytogenes, Salmonella enteritidis, Salmonella typhimurium*, and Staphylococcus *aureus*.

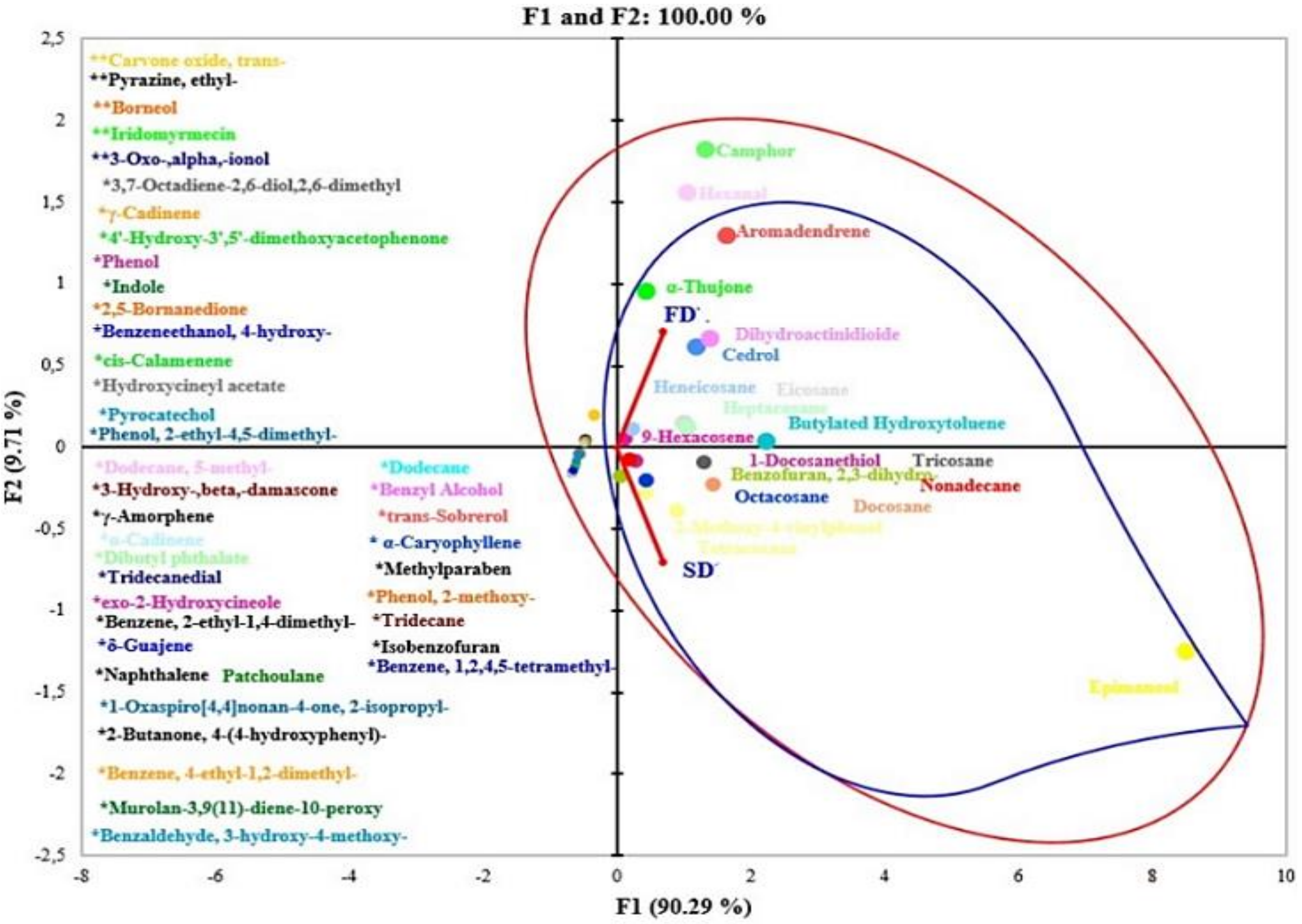

Figure 4. PCA score plot (PCA1/PCA2) of aroma compounds of spray and freeze drying instant sage tea PC1 explaining 90.29%, versus PC2 explaining 9.11% , *Compounds in the bottom left quadrant, **compounds in top left quadrant. FD: Instant sage (*S. rosifolia* Sm) freeze-dried tea, SD: Instant sage (*S. rosifolia* Sm) spray-dried tea.

Table 6. Volatile compounds of instant *salvia* teas, their concentration ratios, and RI Index

No	Compounds	ED. RI	L. RI	GC-MS/FID % area		Identification
				FD	SD	
1	Hexanal	808	808	6.45^{a} ± 0.21	$-^{b}$	RI, MS
2	Phenol	992	992	0.10^{a} ± 0.01	$-^{b}$	RI, MS
3	Isobenzofuran	1026	1036	0.07^{a} ± 0.01	$-^{b}$	RI, MS
4	Benzyl Alcohol	1039	1039	0.34^{a} ± 0.01	$-^{b}$	RI, MS
5	Benzene, 4-ethyl-1,2-dimethyl-	1061	1062	0.07^{a} ± 0.01	$-^{b}$	RI, MS
6	Benzene, 2-ethyl-1,4-dimethyl-	1088	1089	0.05^{a} ± 0.01	$-^{b}$	RI, MS
7	Phenol, 2-methoxy-	1093	1093	0.05^{a} ± 0.01	$-^{b}$	RI, MS
8	α-Thujone	1118	1118	4.19^{a} ± 0.11	$-^{b}$	RI, MS
9	Benzene, 1,2,4,5-tetramethyl-	1123	1123	0.05^{a} ± 0.01	$-^{b}$	RI, MS
10	Camphor	1145	1145	7.50^{a} ± 0.12	$-^{b}$	RI, MS
11	Borneol	1170	1173	0.78^{a} ± 0.03	$-^{b}$	RI, MS
12	Naphthalene	1186	1186	0.03^{a} ± 0.01	$-^{b}$	RI, MS
13	3,7-Octadiene-2,6-diol,2,6-dimethyl	1192	1191	0.18^{a} ± 0.01	$-^{b}$	RI, MS
14	Dodecane	1200	1200	0.03^{a} ± 0.01	$-^{b}$	RI, MS
15	exo-2-Hydroxycineole	1215	1218	0.07^{a} ± 0.01	$-^{b}$	RI, MS
16	Benzofuran, 2,3-dihydro-	1226	1226	1.37^{a} ± 0.04	2.36^{b} ± 0.65	RI, MS
17	Dodecane, 5-methyl-	1249	1249	0.07^{a} ± 0.01	$-^{b}$	RI, MS
18	Hydroxycineyl acetate	1249	1247	0.20^{a} ± 0.01	$-^{b}$	RI, MS
19	2,5-Bornanedione	1268	1264	0.05^{a} ± 0.01	$-^{b}$	RI, MS
20	Carvone oxide, trans-	1273	1277	1.35^{a} ± 0.06	$-^{b}$	RI, MS
21	Indole	1298	1298	0.07^{a} ± 0.01	$-^{b}$	RI, MS
22	Tridecane	1300	1300	0.05^{a} ± 0.01	$-^{b}$	RI, MS
23	Phenol, 2-ethyl-4,5-dimethyl-	1307	1305	0.26^{a} ± 0.01	$-^{b}$	RI, MS
24	2-Methoxy-4-vinylphenol	1318	1318	1.87^{a} ± 0.09	3.84^{b} ± 0.91	RI, MS
25	1-Oxaspiro[4.4]nonan-4-one, 2-isopropyl-	1343	1350	0.07^{a} ± 0.01	$-^{b}$	RI, MS
26	Pyrazine, ethyl-	1350	1352	0.72^{a} ± 0.04	$-^{b}$	RI, MS
27	trans-Sobrerol	1385	1384	0.10^{a} ± 0.02	$-^{b}$	RI, MS
28	Benzaldehyde, 3-hydroxy-4-methoxy-	1404	1401	0.47^{a} ± 0.03	$-^{b}$	RI, MS
29	Iridomyrmecin	1426	1422	0.77^{a} ± 0.04	$-^{b}$	RI, MS
30	Benzeneethanol, 4-hydroxy-	1434	1431	0.33^{a} ± 0.01	$-^{b}$	RI, MS
31	Methylparaben	1454	1459	0.46^{a} ± 0.01	$-^{b}$	RI, MS
32	α-Caryophyllene	1461	1461	0.21^{a} ± 0.01	$-^{b}$	RI, MS
33	γ-Cadinene	1482	1482	0.11^{a} ± 0.01	$-^{b}$	RI, MS
34	Pyrocatechol	1492	1493	0.33^{a} ± 0.01	$-^{b}$	RI, MS
35	δ-Guajene	1497	1498	0.10^{a} ± 0.01	$-^{b}$	RI, MS
36	Butylated Hydroxytoluene	1518	1518	5.87^{a} ± 0.04	8.64^{b} ± 0.82	RI, MS
37	γ-Amorphene	1521	1521	0.11^{a} ± 0.01	$-^{b}$	RI, MS
38	cis-Calamenene	1530	1530	0.28^{a} ± 0.01	$-^{b}$	RI, MS
39	Dihydroactinidioide	1539	1539	5.87^{a} ± 0.07	3.97^{b} ± 0.78	RI, MS
40	α-Cadinene	1544	1544	0.03^{a} ± 0.01	$-^{b}$	RI, MS
41	2-Butanone, 4-(4-hydroxyphenyl)-	1561	1555	0.05^{a} ± 0.01	$-^{b}$	RI, MS
42	Cedrol	1592	1589	4.99^{a} ± 0.21	3.50^{b} ± 0.77	RI, MS
43	Aromadendrene	1601	1606	7.06^{a} ± 0.12	2.74^{b} ± 0.76	RI, MS
44	Patchoulane	1619	1618	0.13^{a} ± 0.01	$-^{b}$	RI, MS
45	3-Hydroxy-.beta.-damascone	1621	1627	0.11^{a} ± 0.01	$-^{b}$	RI, MS
46	3-Oxo-.alpha.-ionol	1654	1656	0.82^{a} ± 0.01	$-^{b}$	RI, MS
47	Tridecanedial	1692	1690	0.08^{a} ± 0.01	$-^{b}$	RI, MS
48	Murolan-3,9(11)-diene-10-peroxy	1729	1729	0.16^{a} ± 0.01	$-^{b}$	RI, MS
49	4'-Hydroxy-3',5'-dimethoxyacetophenone	1741	1740	0.05^{a} ± 0.01	$-^{b}$	RI, MS
50	Nonadecane	1899	1900	1.79^{a} ± 0.02	2.45^{b} ± 0.69	RI, MS
51	Dibutyl phthalate	1967	1967	0.16^{a} ± 0.01	$-^{b}$	RI, MS
52	Eicosane	2001	2000	3.75^{a} ± 0.11	4.39^{b} ± 0.87	RI, MS
53	Epimanool	2068	2068	15.16^{a} ± 1.23	32.87^{b} ± 2.02	RI, MS
54	Heneicosane	2101	2100	2.27^{a} ± 0.07	2.07^{b} ± 0.56	RI, MS
55	Docosane	2201	2208	3.85^{a} ± 0.04	6.96^{b} ± 0.75	RI, MS
56	Tricosane	2307	2307	3.83^{a} ± 0.04	6.03^{b} ± 0.45	RI, MS
57	Tetracosane	2400	2400	2.53^{a} ± 0.02	5.70^{b} ± 0.77	RI, MS
58	1-Docosanethiol	2511	2512	1.96^{a} ± 0.01	2.79^{b} ± 0.43	RI, MS
59	9-Hexacosene	2611	2614	1.91^{a} ± 0.01	1.81^{b} ± 0.54	RI, MS
60	Heptacosane	2701	2705	3.78^{a} ± 0.03	4.61^{b} ± 0.76	RI, MS
61	Octacosane	2801	2804	2.00^{a} ± 0.01	3.63^{b} ± 0.54	RI, MS

Note: Results are presented as means; ± standard deviations; all volatile compounds are expressed as % area. Different letters (a-b) in the same rows lines are significantly ($P < 0.05$) different. FDT: Freeze-dried tea, SDT: Spray-dried tea, ED: Experimental determined, L: Literature, a: experimental RI, b: Literature RI

Table 7. Antimicrobial analysis results of 31 microorganisms

Microorganisms	**FD** **1 mg/mL**	**SD** **1 mg/mL**	**Streptomycin sulfate** **10 μg/mL**	**Nystatin** **30 μg/mL**
Bacteria sp.	**Diameter of inhibition zones (mm)**			
Aeromonas hydrophila ATCC 35654	-	-	17	ND
Bacillus cereus ATCC 9634	6 ± 1	4 ± 1	16 ± 3	ND
Bacillus subtilis ATCC 6633	-	-	19 ± 3	ND
Citrobacter freundii ATCC 3624	-	-	13 ± 3	ND
Enterococcus faecalis ATCC 29212	12 ± 2	-	4 ± 1	ND
Enterococcus sakazakii ATCC 29544	-	-	21 ± 4	ND
Escherichia coli ATCC 25922	5 ± 1	-	7 ± 1	ND
Escherichia coli O157:H7 35150	-	-	15 ± 3	ND
Klebsiella pneumoniae ATCC 13883	-	-	16 ± 3	ND
Lactobacillus bulgaricus KCTC 3188	-	-	11 ± 3	ND
Lactobacillus plantarum NCDO 343	-	-	10 ± 3	ND
Listeria monocytogenes ATCC 7644	6 ± 1	-	19 ± 3	ND
Micrococcus luteus KCTC 10240	-	-	10 ± 3	ND
Proteus vulgaris FMC 1	-	-	14 ± 3	ND
Pseudomonas aeruginosa ATCC 27853	-	-	17 ± 3	ND
Salmonella enteritidis ATCC 13076	4 ± 1	-	16 ± 3	ND
Salmonella typhimurium ATCC 23566	6 ± 1	4 ± 1	13 ± 3	ND
Shigella flexneri ATCC 12022	-	-	8 ± 2	ND
Staphylococcus aureus ATCC 25923	7 ± 1	5 ± 1	12 ± 3	ND
Staphylococcus epidermidis KCTC 1917	-	-	18 ± 3	ND
Streptococcus pneumoniae ATCC 49619	-	-	9 ± 2	ND
Vibrio parahaemolyticus ATCC 17802	-	-	11 ± 3	ND
Yersinia enterocolitica ATCC 1501	-	-	7 ± 1	ND
Yeast and Molds				
Aspergillus flavus ATCC 46283	-	-	-	11 ± 3
Aspergillus niger ATCC 9142	-	-	-	14 ± 3
Candida albicans ATCC 10231	-	-	-	12 ± 3
Fusarium oxysporum ATCC 44187	-	-	-	12 ± 3
Penicillum expansum ATCC 7861	-	-	-	10 ± 3
Rhizopus oryzae ATCC 4858	-	-	-	20 ± 3
Sacc. cerevisiae S288C	-	-	-	18 ± 3
Zygosaccharomyces bailii ATCC 66825	-	-	-	13 ± 3

FD: Instant sage (*S. rosifolia* Sm) freeze-dried tea, SD: Instant sage (*S. rosifolia* Sm) spray-dried tea

Instant tea of spray-dried (1 mg/mL) showed antibacterial activities against *Bacillus cereus, Enterococcus faecalis, Salmonella typhimurium, and Staphylococcus aureus* (Table 7). Quantitative chemical differences due to differences in production during the samples showed the effect on antimicrobial activity of samples; antimicrobial activity of freeze-dried samples was higher. This is because the freeze-dried sample has excess volatile oil compounds. The tea samples did not show any effect on the yeast and the mold. Analyzed flavonoids, phenols, terpenes, alkaloids, etc. components are responsible for biological activity [37]. There have been many studies in the literature on the antimicrobial properties of salvia species [38]. In literature, the oils of *S. rosifolia, S. fruticosa, S. tomentosa, S. ringens and S. officinalis* were reported to have strong antimicrobial activity [35,39–42].

4. Conclusions

The present work was carried out to produce freeze-drieid and spray-dried instant tea from *S. rosifolia* Sm. The obtained results showed that instant teas dried by freezing and spraying have some properties different from physicochemical, antioxidant, and antimicrobial aspects, at the same time these results revealed negative effects of heat treatment on tea compounds. Drying techniques used affected the composition, aroma, sensory properties, antioxidant activity, and antimicrobial activity and physical properties of instant sage. This is a step in the technological development of herbal-based tea products freeze-drying and spray-drying. According to our overall results, instant sage tea represents an important dietary source of minerals and antioxidants. It may be concluded that the spray and freeze-dried instant sage can be used as a functional drink.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Declaration of Competing Interest

None

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Research Article

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Synthesis and determination of pK_a values of some new di-{2-ethoxy-6-[(3-substitue-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)azomethine]phenyl} terephthalates

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Abstract

In this study, seven novel di-{2-ethoxy-6-[(3-substitue-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)azomethine]phenyl} terephthalates (**4a–g**) were synthesized from the reaction of 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**2a-g**) with di-(2-formyl-6-ethoxyphenyl) terephthalate (**3**). The compounds **4a–g** were characterized using IR, ^{1}H-NMR, ^{13}C-NMR, and UV spectral data. In addition, **4** types of compounds were titrated potentiometrically with tetrabutylammonium hydroxide in four non-aqueous solvents such as isopropyl alcohol, *tert*-butyl alcohol, acetone, *N,N*-dimethylformamide and the half-neutralization potential values and the corresponding pK_a values were determined for all cases.

Keywords: 1,2,4-triazole, Schiff base, acidity, potentiometric titrations, pK_a

1. Introduction

It is known that 4,5-dihydro-1*H*-1,2,4-triazol-5-one ring has weak acidic properties so some 1,2,4-triazole and 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives were titrated potentiometrically with TBAH in non-aqueous solvents [1–6]. Determination of pK_a values of the active constituent of definite pharmaceutical preparations is significant because of the distribution, transport behavior, bonding to receptors, and contributions to the metabolic behavior of the active constituent molecules depend on the ionization constant [7–9].

1,2,4-Triazole derivatives are documented to have a broad spectrum of biological activities such as antitumor, antibacterial, antioxidant, and anti-inflammatory properties [2–4,10–13]. Several articles, involving the synthesis of some Schiff bases having 4,5-dihydro-1*H*-1,2,4-triazol-5-one ring have been published up to date [1–4,10–13].

In this paper, we present the synthesis of seven new di-{2-ethoxy-6-[(3-substitue-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)azomethine]phenyl} terephthalates (**4a–g**) were synthesized from the reaction of 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**2a–g**) with di-(2-formyl-6-ethoxyphenyl) terephthalate (**3**), which were synthesized by the reactions of 3-ethoxy-2-hydroxybenzaldehyde with terephthaloyl chloride by using triethylamine (Scheme 1). The starting compound 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **(2)** was prepared with the reactions of the related ester ethoxycarbonyl-hydrazones **(1)** by using an aqueous solution of hydrazine hydrate according to the literature [14,15]. Additionally, the potentiometric titrations of the synthesized compounds **4** were also carried out with tetrabutylammonium hydroxide (TBAH) in four non-aqueous solvents such as isopropyl alcohol, tert-butyl alcohol, N,N-dimethylformamide (DMF), and acetone to determine the half-neutralization potential (HNP) and the corresponding pK_a values.

2. Experimental

2.1. Chemistry

Chemical reagents used were provided by Merck AG, Aldrich, and Fluka. Melting points were determined in open glass capillaries using a Stuart SMP30 melting point apparatus and were not corrected. The infrared spectra were taken on an Alpha-P Bruker FT-IR

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Spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were determined in deuterated dimethyl sulfoxide with TMS as an internal standard using a Varian Mercury spectrophotometer at 400 MHz and 100 MHz, respectively.

2.1.1. General procedure for the synthesis of di-{2-ethoxy-6-[(3-substitue-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]phenyl} terephthalates (4a–g):

3-Ethoxy-2-hydroxybenzaldehyde (0.01 mol) dissolved in ethyl acetate (15 mL) was treated with terephthaloyl chloride (0.01 mol), and to this solution was added triethylamine (0.02 mol) slowly with stirring at 0–5 °C. Stirring was continued for 2 h; then the mixture was refluxed for 3 h and filtered. The filtrate was evaporated in vacuo and the crude product was washed with water and recrystallized from ethanol to afford compound **3**, yield 81%, mp 45 °C; IR (KBr) (υ, cm^{-1}): 2845 and 2760 (CHO); 1730, 1695 (C=O); 1255 (COO). UV (ethanol) λ_{max} (ε, L mol^{-1} cm^{-1}): 3602 (8045), 254 (25940), 242 (27370) nm. The corresponding compound **2** (0.01 mol) was dissolved in acetic acid (15 mL) and treated with di-(2-formyl-6-ethoxyphenyl) terephthalate (**3**) (0.01 mol). The mixture was evaporated at 50–55 °C in vacuo after it refluxed for 1.5 h. Several recrystallizations of the residue from DMSO-H_2O (1:3) gave pure compounds **4** as colorless crystals.

2.1.2. Di-{2-ethoxy-6-[(3-methyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]phenyl} terephthalates (4a):

Yield: 99%, m.p. 209 °C. IR (KBr, *υ*, cm^{-1}): 3181 (NH), 1737, 1709 (C=O), 1602 (C=N), 1246 (COO), 820 (1,4-disubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.21 (t, 6H, $2CH_2\underline{CH_3}$; J = 6.8 Hz), 2.11 (s, 6H, $2CH_3$), 4.12 (q, 4H, $2\underline{CH_2}CH_3$; J = 6.8 Hz), 7.36-7.45 (m, 4H, ArH), 7.58 (d, 2H, ArH; J=8.0 Hz), 8.38 (s, 4H, ArH), 9.91 (s, 2H, 2N=CH), 11.80 (s, 2H, 2NH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 11.35 ($2CH_3$), 14.90 ($2CH_2\underline{C}H_3$), 64.92 ($2\underline{C}H_2CH_3$), [116.94 (2C), 118.92 (2C), 127.52 (2C), 127.86 (2C), 130.98 (4C), 133.39 (2C), 139.50 (2C), 151.03 (2C)] (arom-C), 144.06 (2Triazole C3), 149.06 (2N=CH), 151.63 (2Triazole C5), 163.59 (2COO). UV (ethanol) λ_{max} (ε, L mol^{-1} cm^{-1}): 294 (25450), 242 (37990), 234 (38730) nm.

2.1.3. Di-{2-ethoxy-6-[(3-ethyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]phenyl} terephthalates (4b):

Yield: 97%, m.p. 244 °C. IR (KBr, *υ*, cm^{-1}): 3174 (NH), 1739, 1702 (C=O), 1595 (C=N), 1276 (COO), 820 (1,4-disubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.07 (t, 6H, $2CH_2\underline{CH_3}$; J = 7.2 Hz), 1.21 (m, 6H, $2OCH_2\underline{CH_3}$), 2.45 (t, 4H, $2\underline{CH_2}CH_3$; J = 7.2 Hz), 4.10 (m, 4H, $2O\underline{CH_2}CH_3$), 7.35-7.42 (m, 4H, ArH), 7.54-7.55 (m, 2H, ArH), 8.39 (s, 4H, ArH), 9.91 (s, 2H, 2N=CH), 11.81 (s, 2H, 2NH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 9.77 ($2CH_2\underline{C}H_3$), 14.37 ($2OCH_2\underline{C}H_3$), 18.28 ($2\underline{C}H_2CH_3$), 64.41 ($2O\underline{C}H_2CH_3$), [116.39 (2C), 118.81 (2C), 127.01 (2C), 127.35 (2C), 130.46 (4C), 132.93 (2C), 138.86 (2C), 150.58 (2C)] (arom-C), 147.81 (2Triazole C3), 148.80 (2N=CH), 151.27 (2Triazole C5), 163.06 (2COO). UV (ethanol) λ_{max} (ε, L mol^{-1} cm^{-1}): 296 (25285), 234 (39840), 222 (36820) nm.

2.1.4. Di-{2-ethoxy-6-[3-n-propyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]phenyl} terephthalates (4c):

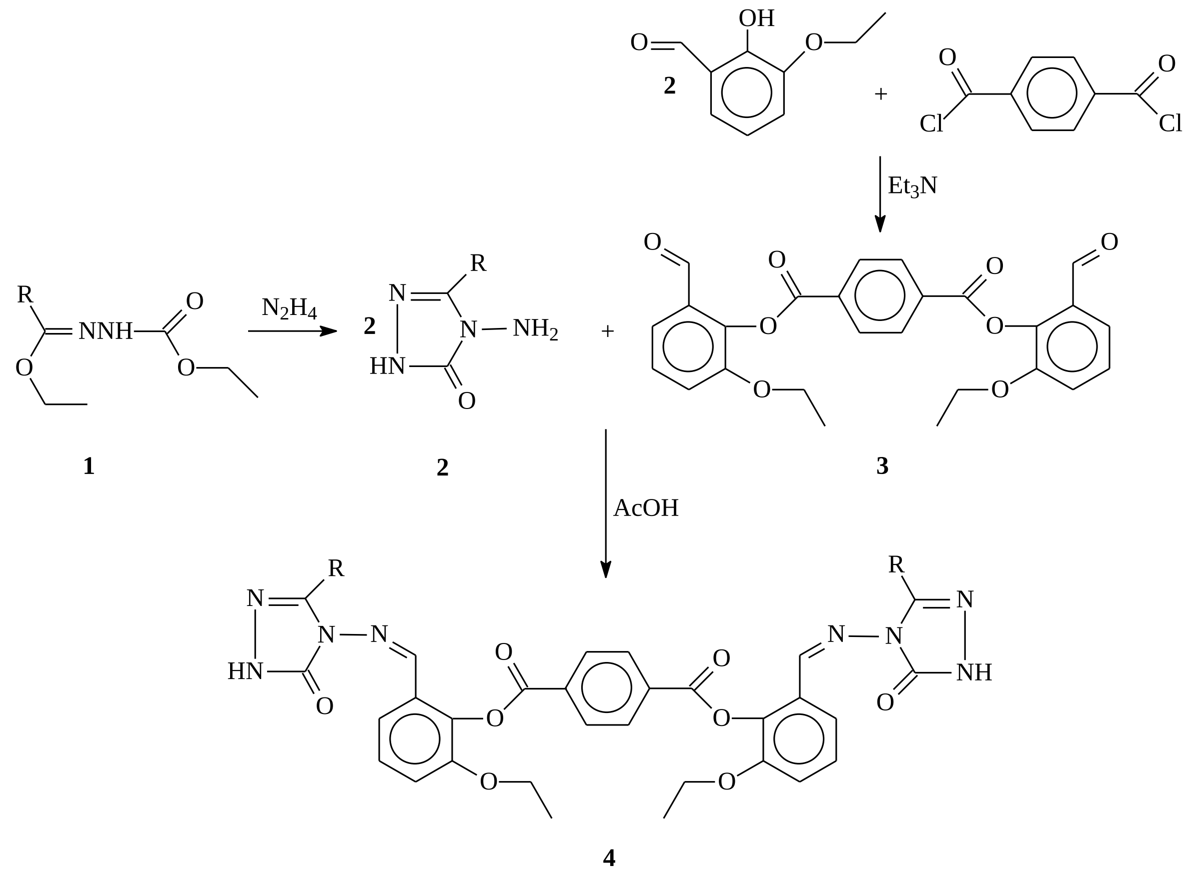
Yield: 95%, m.p. 249 °C. IR (KBr, *υ*, cm^{-1}): 3172 (NH), 1735, 1703 (C=O), 1595 (C=N), 1270 (COO), 820 (1,4-disubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 0.83 (m, 6H, $2CH_2CH_2\underline{CH_3}$), 1.20 (m, 6H, $2OCH_2\underline{CH_3}$), 1.56 (m, 4H, $2CH_2\underline{CH_2}CH_3$), 2.42 (m, 4H, $2\underline{CH_2}CH_2CH_3$), 4.09 (m, 4H, $2O\underline{CH_2}CH_3$), 7.34-7.40 (m, 4H, ArH), 7.53 (m, 2H, ArH), 8.39 (s, 4H, ArH), 9.92 (s, 2H, 2N=CH), 11.83 (s, 2H, 2NH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 13.27 ($2CH_2CH_2\underline{C}H_3$), 14.34 ($2OCH_2\underline{C}H_3$), 18.57 ($2CH_2\underline{C}H_2CH_3$), 26.45 ($2\underline{C}H_2CH_2CH_3$), 64.39 ($2O\underline{C}H_2CH_3$), [116.30 (2C), 118.76 (2C), 127.04 (2C), 127.29 (2C), 130.45 (4C), 132.97 (2C), 138.89 (2C), 150.58 (2C)] (arom-C), 146.67 (2Triazole C3), 148.85 (2N=CH), 151.23 (2Triazole C5), 163.06 (2COO). UV (ethanol) λ_{max} (ε, L mol^{-1} cm^{-1}): 294 (20640), 230 (38820), 218 (34985) nm.

2.1.5. Di-{2-ethoxy-6-[(3-benzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]phenyl} terephthalates (4d):

Yield: 99%, m.p. 268 °C. IR (KBr, *υ*, cm^{-1}): 3179 (NH), 1738, 1713 (C=O), 1604 (C=N), 1273 (COO), 815 (1,4-disubstituted benzenoid ring), 778 and 715 (monosubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.20 (t, 6H, $2CH_2\underline{CH_3}$; J = 6.8 Hz), 3.95 (s, 4H, $2CH_2Ph$), 4.10 (q, 4H, $2\underline{CH_2}CH_3$; J = 6.8 Hz), 7.21-7.43 (m, 14H, ArH), 7.55 (d, 2H, ArH; J=7.6 Hz), 8.35 (s, 4H, ArH), 9.92 (s, 2H, 2N=CH), 11.96 (s, 2H, 2NH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 14.37 ($2CH_2\underline{C}H_3$), 30.83 ($2CH_2Ph$), 64.41 ($2\underline{C}H_2CH_3$), [116.41 (2C), 117.76 (2C), 126.67 (2C), 127.04 (2C), 127.35 (2C), 128.38 (4C), 128.71 (4C), 130.43 (4C), 132.83 (2C), 135.57 (2C), 139.27 (2C), 150.48 (2C)] (arom-C), 146.07 (2Triazole C3), 148.08 (2N=CH), 151.14 (2Triazole C5), 163.10 (2COO). UV (ethanol) λ_{max} (ε, L mol^{-1} cm^{-1}): 280 (17880), 230 (45040), 218 (40390) nm. *Anal.* Calculated for $C_{44}H_{38}N_8O_8$: C, 65.50; H, 4.75; N, 13.99. Found: C, 64.77; H, 4.44; N, 13.32.

2.1.6. Di-{2-ethoxy-6-[(3-p-methylbenzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl} terephthalates (4e):

Yield: 98%, m.p. 244 °C. IR (KBr, *υ*, cm^{-1}): 3191 (NH), 1740, 1708 (C=O), 1595 (C=N), 1273 (COO), 820 (1,4-disubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.20 (m, 6H, $2CH_2\underline{CH_3}$), 2.23 (s, 6H,



a) R = CH_3, b) R = CH_2CH_3, c) R = $CH_2CH_2CH_3$, d) R = $CH_2C_6H_5$, e) R = $CH_2C_6H_4CH_3$ (*p*-), f) R = $CH_2C_6H_4Cl$ (*p*-), g) R = C_6H_5

Scheme 1. Synthetic route of di-{2-ethoxy-6-[(3-substitue-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)azomethine]phenyl} terephthalates

2PhCH$_3$), 3.89 (s, 4H, 2CH$_2$Ph), 4.10 (m, 4H, $2\underline{CH}_2CH_3$), 7.10-7.14 (m, 8H, ArH), 7.36-7.42 (m, 4H, ArH), 7.56 (d, 2H, ArH; *J*=7.6 Hz), 8.34 (s, 4H, ArH), 9.91 (s, 2H, 2N=CH), 11.94 (s, 2H, 2NH). ^{13}C-NMR (100 MHz, DMSO-*d*$_6$): δ 14.36 ($2CH_2\underline{C}H_3$), 20.55 (2PhCH$_3$), 30.43 (2CH$_2$Ph), 64.41 ($2\underline{C}H_2CH_3$), [116.40 (2C), 117.76 (2C), 127.05 (2C), 127.35 (2C), 128.56 (4C), 128.95 (4C), 130.42 (4C), 132.45 (2C), 132.83 (2C), 135.74 (2C), 139.27 (2C), 150.48 (2C)] (arom-C), 146.21 (2Triazole C3), 148.00 (2N=CH), 150.48 (2Triazole C5), 163.11 (2COO). UV (ethanol) λ_{max} (ε, L mol^{-1} cm^{-1}): 294 (12600), 254 (19510), 224 (35400) nm.

2.1.7. *Di-{2-ethoxy-6-[(3-p-chlorobenzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl} terephthalates (4f):*

Yield: 97%, m.p. 208 °C. IR (KBr, *υ*, cm^{-1}): 3194 (NH), 1750, 1704 (C=O), 1597 (C=N), 1274 (COO), 822 (1,4-disubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): δ 1.20 (m, 6H, $2CH_2\underline{CH}_3$), 3.95 (s, 4H, 2CH$_2$Ph), 4.10 (m, 4H, $2\underline{CH}_2CH_3$), 7.29-7.35 (m, 12H, ArH), 7.53 (m, 2H, ArH), 8.34 (s, 4H, ArH), 9.91 (s, 2H, 2N=CH), 11.96 (s, 2H, 2NH). ^{13}C-NMR (100 MHz, DMSO-*d*$_6$): δ 14.35 ($2CH_2\underline{C}H_3$), 30.17 (2CH$_2$Ph), 64.41 ($2\underline{C}H_2CH_3$), [116.40 (2C), 117.78 (2C), 127.00 (2C), 127.33 (2C), 128.30 (4C), 130.41 (4C), 130.61 (4C), 131.44 (2C), 132.84 (2C), 134.48 (2C), 139.28 (2C), 150.48 (2C)] (arom-C), 145.72 (2Triazole C3), 148.15 (2N=CH), 151.14 (2Triazole C5), 163.10 (2COO). UV (ethanol) λ_{max} (ε, L mol^{-1} cm^{-1}): 294 (19730), 230 (41080), 224 (40500) nm.

2.1.8. *Di-{2-ethoxy-6-[(3-phenyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]phenyl} terephthalates (4g):*

Yield: 95%, m.p. 236 °C. IR (KBr, *υ*, cm^{-1}): 3176 (NH), 1739, 1697 (C=O), 1605 (C=N), 1254 (COO), 820 (1,4-disubstituted benzenoid ring), 772 and 691 (monosubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): δ 1.22 (m, 6H, $2CH_2\underline{CH}_3$), 4.12-4.13 (m, 4H, $2\underline{CH}_2CH_3$), 7.40-7.43 (m, 10H, ArH), 7.49 (m, 6H, ArH), 8.22 (s, 4H, ArH), 9.85 (s, 2H, 2N=CH), 12.34 (s, 2H, 2NH). ^{13}C-NMR (100 MHz, DMSO-*d*$_6$): δ 14.40 ($2CH_2\underline{C}H_3$), 64.46 ($2\underline{C}H_2CH_3$), [116.68 (2C), 118.37 (2C), 126.40 (2C), 126.90 (2C), 127.44 (2C), 127.89 (4C), 128.42 (4C), 130.06 (2C), 130.23 (4C), 132.71 (2C), 139.29 (2C), 151.25 (2C)] (arom-C), 144.60 (2Triazole C3), 150.60 (2N=CH), 151.45 (2Triazole C5), 163.11 (2COO). UV (ethanol) λ_{max} (ε, L mol^{-1} cm^{-1}): 238 (43820), 226 (41780), 220 (40570) nm. *Anal.* Calculated for $C_{42}H_{34}N_8O_8$: C, 64.78; H, 4.40; N, 14.39. Found: C, 64.37; H, 4.55; N, 14.03.

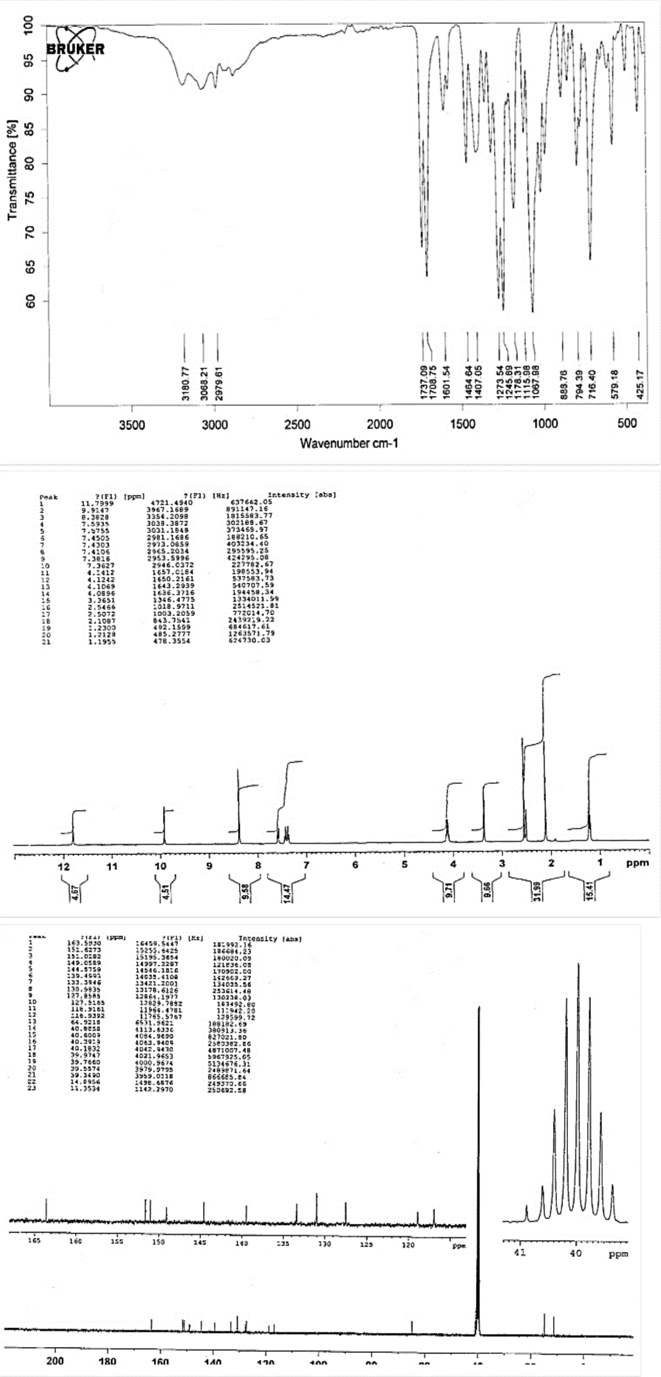


Figure 1. IR, ^{1}H-NMR, and ^{13}C-NMR spectra of compound **4a**

2.2. Determination of Acidity Constants

A Jenway 3040-model ion analyzer was employed for potentiometric titrations. An Ingold pH electrode was used because of the advantage. The 0.001 M solution was separately prepared in each non-aqueous solvent for each compound titrated. The 0.05 M solution of TBAH in isopropyl alcohol, which is widely used in the titration of acids, was employed as the titrant. The mV values obtained in pH meter were recorded. Then, the HNP values were calculated by drawing the mL (TBAH)-mV graphic. The acidity constants and HNP values of compounds were calculated using the half-neutralization method [16–20].

3. Results and discussion

In this study, seven new di-{2-ethoxy-6-[(3-substitue-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)azomethine]phenyl} terephthalates (**4a–g**) were synthesized and characterized with IR, ^{1}H-NMR, ^{13}C-NMR and UV spectral data.

Table 1. The HNP and the corresponding pK_a values of compounds 4 in isopropyl alcohol, tert-butyl alcohol, DMF, and acetone at 25 °C

Compound. no	**DMF**		**Acetone**		**tert-Butyl alcohol**		**Isopropyl alcohol**	
	HNP (mV)	**pK_a**	**HNP (mV)**	**pK_a**	**HNP (mV)**	**pK_a**	**HNP (mV)**	**pK_a**
4a	-332	15,37	-573	-	-202	12,15	-	-
4b	-320	13,46	-175	10,92	-241	11,48	-	-
4c	-291	13,74	-259	11,8	-189	9,8	-	-
4d	-345	15,16	-296	13,37	+171	5,88	-318	13,57
4e	-345	15.08	57	5,49	-	-	-406	16,52
4f	-334	14,89	-245	12,16	-	-	-346	15,09
4g	-368	15,50	-209	11,52	-	-	-11	7,42

As an example, the IR, ^{1}H-NMR, and ^{13}C-NMR spectra of Compound **4a** are presented in Fig. **1**.

Then, synthesized **4** type compounds were titrated potentiometrically with TBAH in four non-aqueous solvents (isopropyl alcohol, *tert*-butyl alcohol, *N,N*-dimethylformamide (DMF), acetone), and the mV values from each titration were plotted against TBAH volumes used (mL), and the potentiometric titration curves were formed for all the cases. The HNP values were measured from the titration curves and the corresponding pK_a values were calculated.

The half-neutralization potential values and the corresponding pK_a values of the compounds **4**, determined from the potentiometric titrations with 0.05 M TBAH in isopropyl alcohol, *tert*-butyl alcohol, DMF, and acetone are given in Table 1. The pH of weak acids can be calculated using the following equation: $pH = pK_a + \log[A^-] / [HA]$ where $pH = pK_a$ when $[A^-]$ is equal to $[HA]$ at the half-neutralization points. Therefore, the pH values at the half-neutralization points were taken as pK_a. According to the dielectric permittivity of the solvents, the acidity ranking might be expected to be as follows: *N,N*-dimethylformamide (ε = 37) > acetone (20,6) > isopropyl alcohol (ε = 19.4) > *tert*-butyl alcohol (ε = 12.0).

In amphiprotic solvents, the data obtained for compound 4d do not conform to the theoretical ordering. HNP values and corresponding pK_a values could not be obtained for compounds **4a**, **4b**, and **4c** in isopropyl alcohol and for compounds **4e**, **4f**, and **4g** in *tert*-butyl alcohol. So, the acidity strength of the compounds between solvents could not be compared.

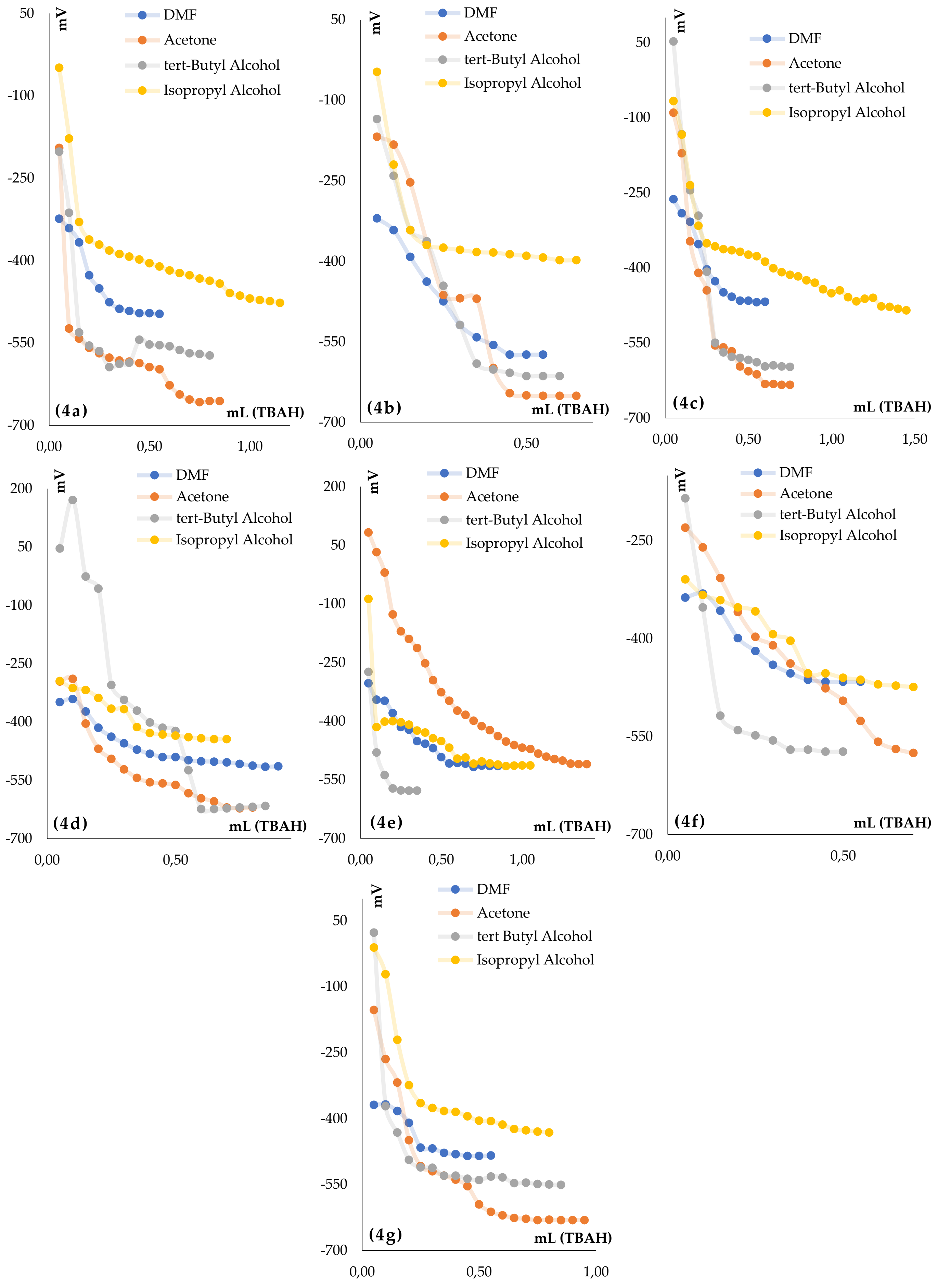


Figure 2. Potentiometric titration curves of 0.001 M solutions of compounds **4a-4g** titrated with 0.05 M TBAH in isopropyl alcohol, *tert*-butyl alcohol, acetone, and *N*,*N*-dimethylformamide at 25 °C.

Dipolar aprotic solvents give SH_2^+ ions but not S^- ions.

$$\underset{(a)}{HA + S \leftrightarrow \cdots HA} \leftrightarrow \underset{(b)}{SH^+A^-} \leftrightarrow \underset{(c)}{SH^+ + A^-} \qquad (1)$$

(HA: Acid (Molecular) and S: Solvent) When the equilibrium (1) is examined, the equilibrium of (a) and (b) occur more in protophilic (DMF) solvents than in protophobic (Acetone) solvents. The equilibrium of (c) is very low in protophilic solvents, but in trace amounts in protophobic solvents. The SH^+ in the protophobic solvent is a much stronger acid. This explains why compounds **4b–4g** are more acidic in acetone. Compound **4a** conforms to the theoretical sequence.

Considering the autoprotolysis constant, it was seen that the Hnp values of the compounds and the potential measured ranges of the solvents in tert-butyl alcohol (1200), isopropyl alcohol (1000), DMF (1300), and acetone (1550) medium are weakly acidic compounds **4d** and **4e** were leveled in DMF medium. It has been differentiated in other solvents.

The half-neutralization potential (HNP) values and the corresponding pK_a values of compounds **4a-4g**, founded from the potentiometric titrations with 0.05 M TBAH in isopropyl alcohol, *tert*-butyl alcohol, acetone, and DMF, are given in Table 1.

The potentiometric titration curves for 0.001 M solutions of compouds **4a–4g** titrated with 0.05 M TBAH in isopropyl alcohol, tert-butyl alcohol, *N*,*N*-dimethylformamide, and acetone are given in Fig. 2.

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Electrochemical determination of antiviral drug Famciclovir in human serum samples at boron-doped diamond electrode

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Abstract

A new electrochemical method using differential pulse voltammetry at a boron-doped diamond electrode (BDDE) was developed for the quantitative determination of famciclovir in the pH range of 2.0–10.0. The experimental results from the pH and scan rate studies showed that the oxidation behavior of famciclovir at BDDE was irreversible and diffusion-controlled. The anodic peak current was also observed to be linear over the concentration range of 0.5–12 μM and 6–100 μM in pH 4.7 acetate buffer solution (ABS) for standard drug solution and human serum, respectively, as obtained using the differential pulse voltammetry (DPV) at BDDE. Limits of detection were found as 0.022 μM and 0.42 μM for standard drug solution and human serum, respectively. The repeatability, reproducibility, selectivity, precision, and accuracy of the developed method in all media were investigated and calculated. This method was successfully applied for the analysis of famciclovir in human serum samples.

Keywords: Boron-doped diamond electrode, differential pulse voltammetry, Famciclovir, human serum samples

1. Introduction

Nucleoside analogs are a pharmacological classes of compounds with cytotoxic, immunosuppressive, and antiviral properties [1]. Thus, nucleoside analogs can be used as therapeutic drugs, including several antiviral products used to inhibit viral replication in infected cells. In addition to these, drug resistance can develop rapidly in a short time as a result of a mutation during the treatment of various viruses [2]. To overcome the resistance that develops against one of the nucleoside analog drugs, a new drug (e.g., famciclovir, penciclovir, and valaciclovir) from the same drug group is employed throughout treatment. Famciclovir (Fig. 1) is an antiviral drug that distinguishes out among nucleoside analog treatments due to its low toxicity and high selectivity [3]. Famciclovir is also a penciclovir prodrug with increased oral bioavailability of approximately 77% [4]. It is used to treat the symptoms of herpes zoster (commonly known as shingles), a herpes virus infection of the skin, and recurrent herpes virus infections of the mucous membranes (lips and mouth) [5]. Famciclovir's structure and mechanism of activity are quite similar to other nucleoside analogs, such as the more widely used acyclovir.

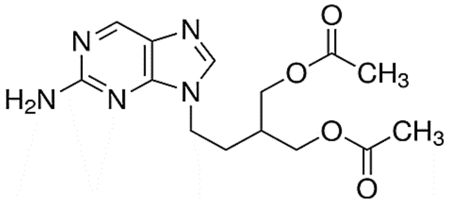

Figure 1. Chemical structure of famciclovir

Electrochemical techniques have been proved to be excellent procedures for monitoring organic compounds in pharmaceutical forms and biological samples [6]. In these techniques, the quantitative analysis method is developed by taking advantage of the fact that many active compounds in dosage forms can be easily oxidized or reduced unlike excipients [7]. The interest in experimental electrochemical techniques in the field of drug detection is due to their relatively short analysis time, simplicity, and low cost compared to other techniques [8].

While developing a new electrochemical-based analytical method in drug detection, studies were performed by modifying carbon electrodes with various nanomaterials such as metal/metal oxide [9], metal-

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organic framework [10,11], polymer [12], graphene [9,13], carbon nanotube [14], carbon fiber [15], and fullerene [16] are the most current research topics. Nevertheless, due to the use of extra chemical materials in both the synthesis and modification stages of these materials, the development of electrochemical methods using modified electrodes is far from the development of green analytical chemistry methods that will contribute to the reduction of the human footprint on Earth [17]. Moreover, obtaining the same modified surface, creating stable surfaces for a long time, and acquiring low reproducible results are the biggest difficulties encountered in the use of modified electrodes.

Due to their inherent high thermal conductivity, chemical inertness, and wide electrochemical potential range, boron–doped diamond electrodes (BDDE) outperform other carbon-based electrodes such as glassy carbon and carbon paste [18–21]. As a result, a method with a low limit of detection (LOD) might be developed and used in drug analysis without the requirement for costly and time-consuming modifications to improve sensitivity [22–26].

In the literature, there are only a few analytical methods used for determining famciclovir such as RP-HPLC [27–31], UPLC-MS/MS [31,32], UV-VIS spectroscopy [33,34], MIP-voltammetry [35], potentiometry [36], and spectrofluorimetry [37,38] from pharmaceutical preparations. When the sensitivity of the method developed in this study was compared to the voltammetric approach as previously described using carbon paste electrode (CPE), the suggested study has provided the lower LOD values [35]. Furthermore, the proposed novel method's sample and electrode preparation procedure are simpler, more practical, and less expensive. Moreover, although the previously described method was utilized for the analysis of pharmacological forms, this work showed that it could also be performed with human serum samples. Therefore, this study aims to propose the development of an electrochemical method for famciclovir quantification in human serum samples using the differential pulse voltammetric method at BDDE. The electrochemical behavior of the drug was also investigated at BBDE using cyclic voltammetry (CV) and DPV techniques in terms of buffer solution and pH. Moreover, the change of the electrochemical behavior depending on the scan rate was examined. The proposed method could be an eco-friendly alternative to chromatographic techniques in therapeutic drug monitoring without any time-consuming evaporation, adsorption, extraction, and separation steps before drug assay.

2. Experimental

2.1. Apparatus

All the electrochemical measurements were carried out with an AUTOLAB 204 PGSTAT electrochemical analyzer (Eco Chemie, Utrecht, The Netherlands), equipped with a three-electrode system that included the Ag/AgCl (BAS, 3 M NaCl) reference electrode, the BDDE (Windsor Scientific Ltd.; 3 mm diameter) working electrode, and the platinum wire auxiliary electrode. The clean electrode surface was obtained using alumina powder and a polishing cloth before each measurement. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were employed for investigating the oxidation behavior of famciclovir. The parameters of step potential: 10 mV; modulation amplitude: 50 mV; modulation time: 50 ms; interval time: 500 ms were chosen for the measurement of famciclovir with DPV.

2.2. Chemicals

Famciclovir was kindly supplied by Neutec Drug A.S., (Istanbul, Turkey). The stock solution of 1×10^{-2} M famciclovir was prepared in double-distilled water. H_2SO_4 solutions (0.1 M and 0.5 M) and Britton-Robinson (BR) buffer solutions were prepared using H_3BO_4, CH_3COOH, and H_3PO_4 (0.04 M, pH 2.0–10.0), phosphate buffer solutions were prepared using K_2HPO_4 and KH_2PO_4 (PBS, 0.1 M, pH 2.0, 3.0, 5.0, 6.0, 7.0, 8.0), and acetate buffer solutions were prepared using CH_3COOH and $CH_3COONa.3H_2O$ (ABS, 0.1 M, pH 3.7, 4.7, 5.7), which were preferred as supporting electrolytes and prepared in distilled water. Synthetic human serum samples were purchased from Sigma-Aldrich. Required solutions were prepared with high-purity chemicals.

2.3. Analysis of human serum samples

Human serum samples were stored frozen till analysis in a -20 °C refrigerator. 1.0 mL of famciclovir from the stock solution (1.0×10^{-2} M), 3.6 mL of serum, and 5.4 mL of acetonitrile were added to the test tube for precipitating the protein to obtain the 1.0×10^{-3} M standard serum solution. To eliminate protein residue, tubes were sonicated for 15 minutes and then centrifuged at 5000 rpm for 20 minutes. For electrochemical experiments, the supernatant was prepared in a selected buffer and then added to the cell. Within a given concentration range, a calibration plot was obtained. The serum recovery test was performed using the standard addition method and repeating the procedure at least five times from the point on the calibration chart with the best reproducibility.

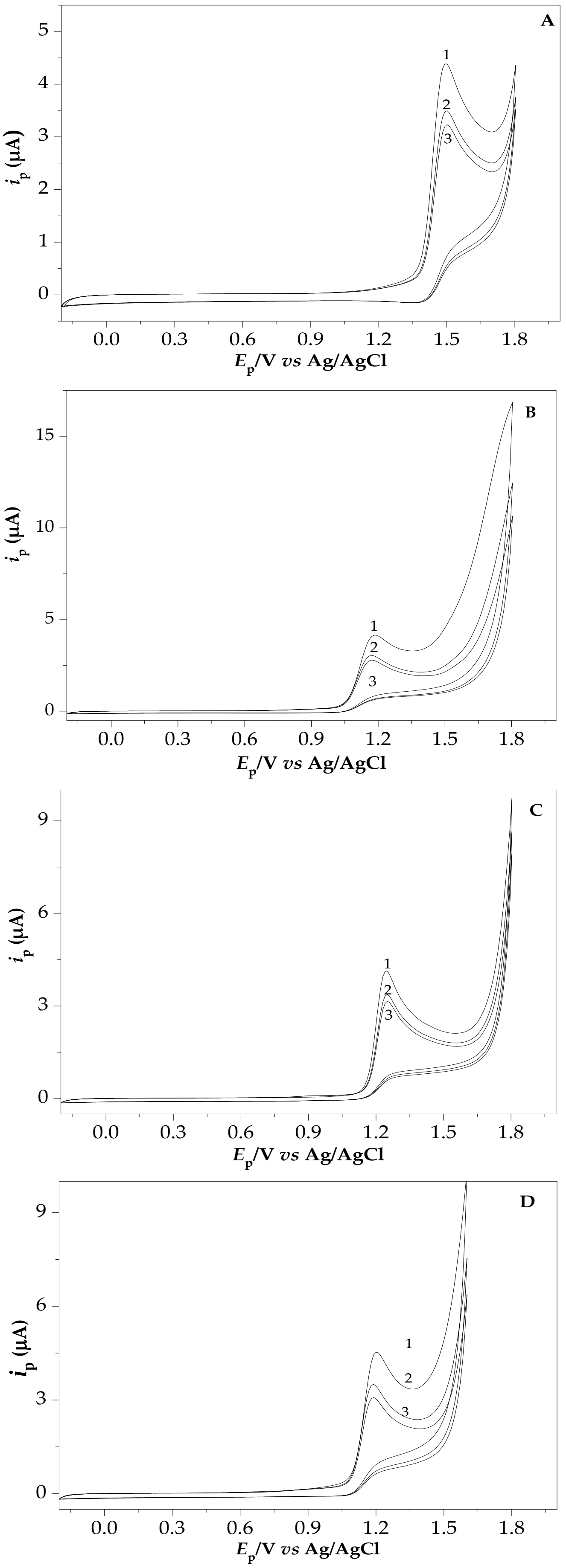

Figure 2. Repetitive cyclic voltammograms of 1.0 × 10^{-4} M famciclovir solutions in (a) 0.1 M H_2SO_4; (b) pH 8.0 BR buffer solution; (c) pH 4.7 ABS (d) pH 7.0 PBS, as obtained at a scan rate of 100 mVs^{-1}.

3. Results and discussion

3.1. Electrochemical behavior of famciclovir on the BDDE

Experiments were carried out using CV and DPV to study the redox process of famciclovir on the BDDE surface.

In order to determine the anodic oxidation behavior of famciclovir, repetitive cyclic voltammograms of 1.0 × 10^{-4} M famciclovir solutions in (a) 0.1 M H_2SO_4; (b) pH 8.0 BR buffer solution; (c) pH 4.7 ABS (d) pH 7.0 PBS were obtained at a scan rate of 100 mVs^{-1} (Fig. 2). Famciclovir exhibited a single well–defined oxidation peak for anodic direction while no peak was obtained for cathodic direction in all electrolyte mediums, which proved the irreversible oxidation reaction on BDDE. Moreover, as depicted in Fig. 2, when the intensity of the peaks obtained as a result of the scans was examined, it was found that the intensity of the peaks in the first scan was higher compared to other scans, and electrode surface contamination may be the reason for the decrease in the peak density.

Since the best peak shape and highest current were obtained in pH 4.7 AB solution, scanning rate studies and quantification of the drug analysis were carried out in this buffer solution. To evaluate whether the surface interaction mechanism is under the control of diffusion or adsorption or both, scan rate studies were carried out by CV using 1.0 × 10^{-4} M famciclovir in pH 4.7 ABS buffer solution in the scan rate range of 5–1000 mVs^{-1} (Fig.3). Linear response for the BDDE was found with the square root of the scan rate as follows:

$$I_P(\mu A) = 0.31 v^{\frac{1}{2}} (mVs^{-1}) + 0.018 \; (r = 0.997) \qquad (1)$$

For BDDE, a plot of the logarithm of the peak current versus the logarithm of the scan rate revealed a linear line with a slope of 0.47. The obtained value of the slope is close to the theoretical value of 0.5, which is assumed the diffusion-controlled process for an ideal reaction of solution species. The equation found in experimental data was;

$$\log I_P(\mu A) = 0.48 \log v \; (mVs^{-1}) - 0.464 \; (r = 0.996) \qquad (2)$$

Considering the change in scan rate values from 5 mV/s to 1000 mV/s, it was observed that there was a 60-mV shift towards positive potential values related to the irreversibility of the electrode reaction.

In the pH range of 2.0–10.0, changes in the graph of pH values against the measured current values (Fig. 4A) and the potential values of the anodic oxidation peak (Fig. 4B) were indicated.

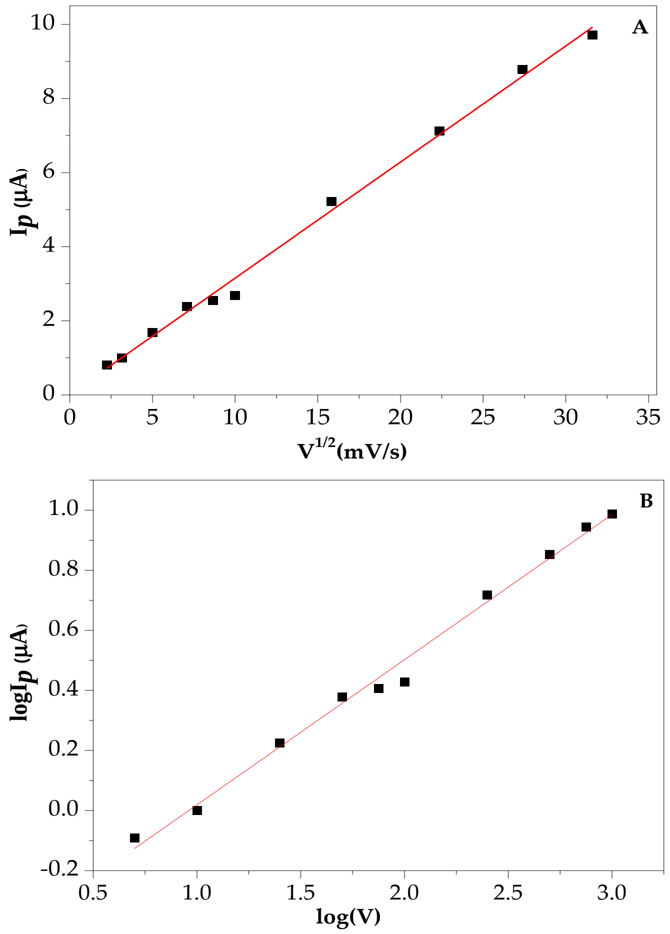

Figure 3. I_P *vs* $v^{1/2}$ (A) and log I_P *vs* log v (B) graphs of 1.0 × 10^{-4} M famciclovir in pH 4.7 ABS obtained in the range of 5–1000 mV/s

When the peak heights were obtained in different pH solutions, it was seen that the values obtained in pH 3.0 PBS, pH 4.7 ABS, pH 6.0 PBS, and pH 6.0 BR buffer solutions were very close to each other. However, the peak obtained in pH 4.7 ABS was slightly higher but has more reproducible values. The peak potential value of pH 4.7 was also found lower than other pH values, which indicated that famciclovir oxidizes more easily at this pH medium. Thus, pH 4.7 ABS was used as working pH in further studies. The peak potential of famciclovir seems to be pH-independent between pH 8.0 and 10.0 means that there are no proton transfer steps before the electron transfer rate-determining step. Furthermore, the plot of the peak potential (E_P) vs. pH gave two straight lines (Fig. 4A). The first linear line of Ep vs pH 2.0 to 5.0 was observed to be linear with a slope indicating that the electrochemical reaction contained an equivalent number of protons and electrons. On the other hand, the slope of the change in drug peak potential between pH 5.0–8.0 indicates that the number of protons in the electrochemical reaction is half the number of electrons. In addition, the intersection point of Ep-pH lines was found to be around 5, and this value was determined to be compatible with the monograph pKa value of the drug [39].

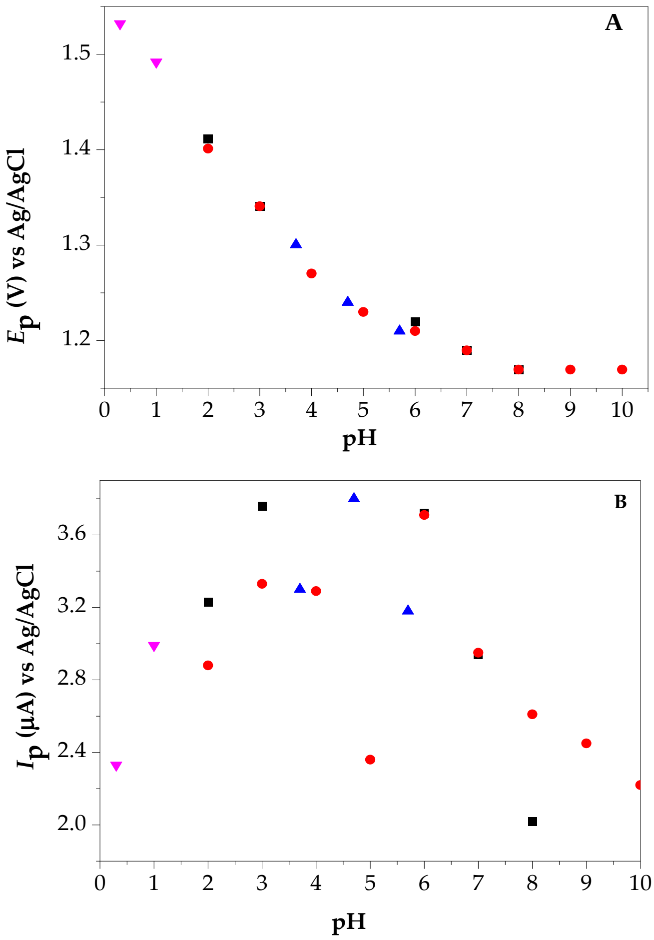

Figure 4. Effects of the pH on the famciclovir peak potential (A) and peak current (B) in different supporting electrolytes, (●) Britton–Robinson buffer; (▲) phosphate buffer; (■) acetate buffer; (▼) H_2SO_4 solutions (0.1 M and 0.5 M). These experiments were performed using DPV with the famciclovir concentration of 1 × 10^{-4} M

$$E_P(mV) = -64.3\ pH + 1535.8; \quad r = 0.9960\ (pH\ 2.0 - 5.00\ with\ DPV) \tag{3}$$

$$E_P(mV) = -22.3\ pH + 1348.5; \quad r = 0.9914\ (\text{pH } 5.00 - 8.00 \text{ with DPV}) \tag{4}$$

3.2. Analytical performance parameters

To evaluate the analytical performance of the developed method, parameters such as specificity, linearity, linear dynamic range (LDR), precision, accuracy, the limit of detection (LOD), and limit of quantification (LOQ) were calculated under optimized conditions. Thus, the linear relationship between changing the drug molecule concentration and the measured current value was used to determine these parameters. The experiments were carried out in pH 4.7 ABS, where the best peak symmetry and the highest peak current were obtained. The linearity was achieved in the concentration range of 0.5–12 μM (Fig. 5). The following was the related equation between peak current and concentration:

$$i_P\,(\mu A) = 0.045\ C\,(\mu M) - 0.00894\ (n{:}\,10,\ r = 0.9987) \tag{5}$$

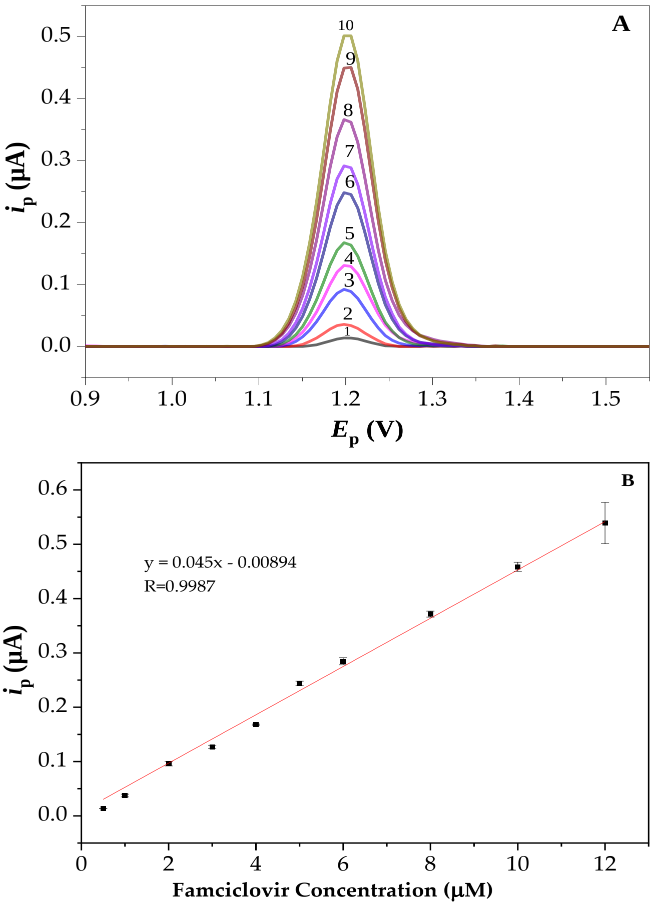

Figure 5. Calibration curve of DP voltammograms for famciclovir (standard solution) in different concentration ranges from 0.5 μM to 12 μM (A) and calibration curve of standard famciclovir (B) in pH 4.7 ABS

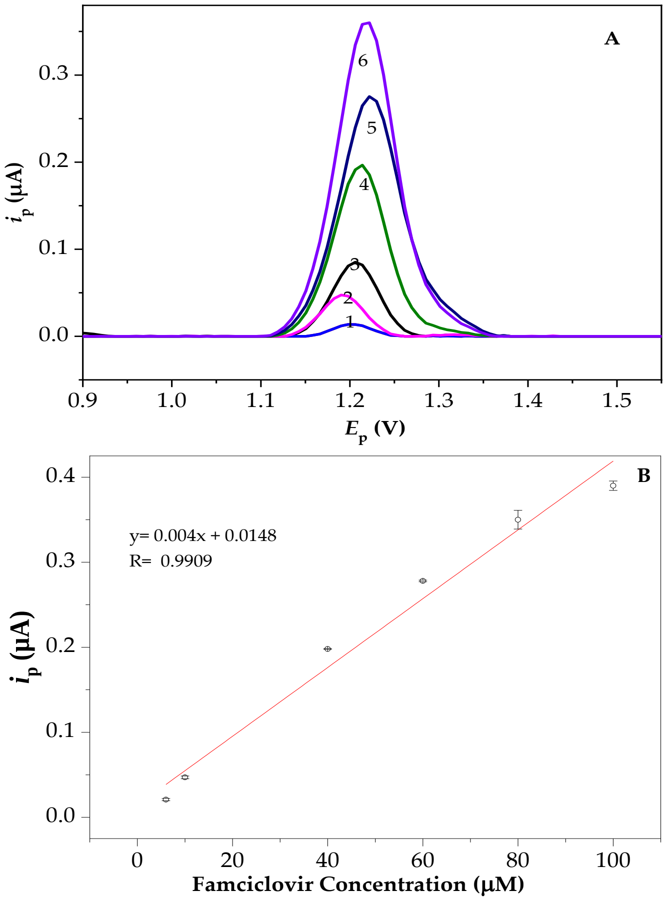

Figure 6. DP voltammograms of serum samples in different concentration ranges from 6 μM to 100 μM (A) and calibration curve of serum samples (B) in pH 4.7 ABS

Statistical data of the calibration are given in Table 1. Repeated measurements of the famciclovir peak potential and peak current within and between days illustrate the developed method's precision. The formulas of 3 s/m and 10 s/m were used to determine the LOD and LOQ values, where "s" represents the standard deviation of the response and "m" indicates the slope of the calibration curve. The LOD and LOQ values (Table 1) indicated the sensitivity of the method. The performance of the developed method is also compared with the previous analytical methods.

As can be seen from Table 2, the developed method is the most superior in terms of sensitivity while most other methods include time-consuming sample preparation, high consumption of harmful solvents, and expensive equipment.

Table 1. Correlation data for famciclovir calibration was produced using DPV at BDDE from standard solution and serum samples

Parameters	Standard	Serum
Anodic potential (mV)	1206	1210
Linearity dynamic range (μM)	0.5–12	6–100
Slope (μA M^{-1})	0.045	0.004
Intercept (μA)	–0.00894	0.0148
Correlation coefficient (*r*)	0.9987	0.9909
LOD (μM)	0.022	0.42
LOQ (μM)	0.066	1.38
Intra-day precision of peak current (RSD%)*	1.51	1.09
Inter-day precision of peak current (RSD%)*	1.63	2.36

* Each value is the mean of five experiments

3.3. Determination of famciclovir in spiked biological samples

Drug detection from serum samples is a time-consuming process that necessitates the use of expensive organic solvents and other reagents. To demonstrate the applicability of the proposed techniques to human serum samples, the calibration equation was obtained for spiked biological samples.

Table 2. Comparison of the analytical performance of BDDE electrode for the determination of famciclovir with other analytical methods reported previously

Analytical Method	Linearity range	LOD	Sample	Ref.
HPLC	5–40 g/mL	0.18 μg/mL	tablets	[27]
HPLC	5–40 μg/mL	0.19 μg/mL	tablets	[28]
HPLC	20–240 μg/mL	0.60 μg/mL	tablets	[29]
UPLC-MS/MS	0.1–10 μg/L	0.02 μg/kg	chicken muscle samples	[31]
UV-spectrophotometry	2–10 μg/mL	-	tablets	[33]
MIP-voltammetry	2.5 μM–1.0 mM	0.75 μg/mL	tablet	[35]
Spectrofluorimetry	100–1000 ng/mL	0,051 g/mL	tablets	[37]
Spectrofluorimetry	2–100 ng/mL	0.56 ng/mL	drug	[38]
DPV	0.5 – 12 μM 6 – 100 μM	0.022 μM 0.42 μM	Human serum	This study

The preparation of the samples and measurements of famciclovir are explained in Section 2. Calibration equation parameters and related validation parameters are indicated in Table 1. The DP voltammograms obtained from spiked serum samples at different concentrations of famciclovir with BDDE is shown in Fig. 6. No oxidation substances, as well as extra noise peak, was monitored from human serum samples in the potential range where the analytical peak emerged (Fig. 6).

The peak current was linearly related to famciclovir concentrations over the range of 6 μM to 100 μM (A) for DPV measurements.

$$I_p\ (\mu\text{A}) = 0.004C\ (\mu\text{M}) + 0.0148\ (\text{n:}6,\ r = 0.9909)$$

The proposed procedures achieved repeatable results that were simple to use, and sensitive enough to detect famciclovir in human serum samples (Table 3).

Table 3. Results of recovery studies in developed DPV method in BDDE from serum samples

Parameters	DPV
Added concentration (μM)	20
Found concentration (μM)	21.69
Average recovered %	108.47
Number of experiments	5
RSD% of recovery	4.04
Bias%	8.47

4. Conclusion

It was shown for the first time in this study that famciclovir is oxidized irreversibly on a boron-doped diamond electrode. The obtained results indicated that the electrochemical method developed in this study could be applied for the determination of famciclovir in conventional electrolytes as well as in more complex matrices such as human serum samples. Thus, the developed DPV technique enables a more convenient and efficient application of famciclovir analysis in human body fluids. The proposed method for drug analysis has several advantages, including simplicity, sensitivity, reproducibility, ease of sample preparation, and quick analysis.

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Geochemical investigation of the potability of surface water in Çit Stream and related creeks in Avliyana Basin (Gümüşhane, NE Türkiye)

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Abstract

In the study, physicochemical parameters of Çit Stream and related creeks in Avliyana Basin (Gümüşhane, NE Türkiye) were determined. In terms of water quality parameters, it has been observed that Çit Stream may have drinking water quality. According to the piper diagram, all of the samples except one have fallen into regions of a class of $CaCO_3$ and $MgCO_3$ waters. The different one is the mixed water class. It has been determined that the metal content that may pose a risk to water is below the permissible limits. It has been seen that there may be only one point of risk for lead. As a result, the physicochemical aspect of water quality of Çit Stream and related creeks in Avliyana Basin has been seen to be suitable for drinking water standards.

Keywords: Water quality, surface waters, trace element, piper diagram

1. Introduction

As throughout the world, Türkiye is a poor country in terms of freshwater resources [1]. According to data from the General Directorate of State Hydraulic Works of Türkiye (DSI), the total renewable water potential of Türkiye is estimated to be 234 billion m^3 gross and the total surface water potential can be consumed for various purposes within the framework of today's technical and economic conditions is an average of 98 billion m^3 per year. Therefore, it must be more rigorous in the context of the conservation and efficient use of water resources, and it should make water resources planning much more rigorous. Especially since the beginning of the 20th century, environmental problems brought about by rapid industrialization have been one of the most important issues that have given humanity a headache [2]. Human-induced heavy metal/trace element pollution has become a serious threat to terrestrial and aquatic systems [3–7]. The approach of utilizing natural resources in an environmentally friendly-sustainable development perspective continues with an increasing awareness after the 1980s [2,7]. Sustainable utilization of water, which is one of the most important natural resources, is among the priorities of societies in this context, and serious efforts are made for the protection and management of underground and surface water resources.

The study area in Gümüşhane is located in the northeastern Black Sea region, Türkiye (Fig. 1). The region has a typical continental climate. The average annual rainfall in the region is 470.1 mm (Turkish State Meteorological Service 2016) and lower than Türkiye's average annual rainfall (643 mm/year). Therefore, Gümüşhane's water resources, especially river resources should be evaluated in the most efficient way. The Büyük Çit Stream is one of the important rivers of Gümüşhane. The stream is fed groundwater and rains and after reaching 30–35 km, it connects to the Harşit River and then, flows to the Black Sea. There is no settlement in the vicinity of the source of the river and

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on the creek route except for the connection point to the Harşit river. The stream is used in the irrigation of gardens and a few trout production facilities, as well as in drinking water, especially the upstream. So, the stream is very important to the local people.

Surface waters suitable for drinking or recreational use, along with their feeding tributaries, should be monitored for a long time and frequently sampled and analyzed for water quality. For this purpose, water quality can be monitored in two stages: (*i*) First, pre-control samples are taken, and some basic parameters of the water are analyzed. Thus, the instantaneous status of the water is determined. (*ii*) In the light of the results obtained from the instantaneous control of the water, several samples are taken from the stations determined during certain periods of the year, and then a more comprehensive analysis is performed.

The main purpose of this study is to determine the instantaneous values of some physicochemical and heavy metal contents of the Büyük Çit Stream, one of the most important rivers in the region, to shed light on new studies to be carried out and to reveal whether it has potable water potential.

2. Sampling, Materials and Methods

Büyük Çit Stream (Torul, Gümüşhane, NE Türkiye) is located in the southern part of the eastern Black Sea region (Fig.1). The basement of rocks of the study region consists of granitoid of the Middle to Late Carboniferous Period [8]. These rocks are unconformably overlain by volcani-clastic unit Zimonköy formation [9], which passes upward conformably to the platform carbonate rocks called as “Berdiga formation” [10]. The Late Cretaceous clastic unit, conformably covers those carbonates. The Eocene volcanic and volcani-clastic unit called “Alibaba formation” overlies all of these rocks with an angular unconformity [11,12] and was intruded by calc-alkaline granitoids of a possible similar age (Avliyana granitoids, Fig. 1) [13,14].

“Büyük Çit Stream” passes through three settlements along the valley where it is located: Avliyana, Haviyana, and Altınpınar. A total of 17 water samples were collected in September 2016 from the stream along with these settlements and their tributaries. Twice sampling was done with 500 mL PP bottles from each sampling station. Since the first sampling was done for minor-trace element and heavy metal analysis, ultrapure concentrated HNO_3 was added to these bottles at a pH of approximately 2 (approximately 0.5 mL). No reagent was added to the other bottle. After the bottles were appropriately labeled, they were transported to the laboratory at the end of the day and stored in a refrigerator at +4 °C until the analysis.

As September is generally the dry season in the region, this month was preferred for sampling. Acidified and natural samples were analyzed for minor-trace elements and heavy metals at Gümüşhane University Labs (Türkiye), by microwave plasma atomic emission spectrometer (Agillent 4200 model MP-AES, Santa Clara, US) and inductively coupled plasma mass spectrometer (Agilent 7700e model ICP-MS, Santa Clara, US). Electrical conductivity (EC), temperature (T), total dissolved solids (TDS), dissolved oxygen (DO), Chloride (Cl^-), Nitrate (NO_3^-), ammonium (NH_4^+), and pH values were determined by electrochemical methods. Portable multiparameter devices, YSI Procomm II Portable meter (YSI Incorporated, Yellow Springs, OH, USA) and Hach-Lange HQD Series Portable Meter (Hach-Lange, Loveland, Colorado, US) with suitable electrode systems were used for this purpose.

Alkalinity and subsequent carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) analyzes were performed according to the titrimetric method reported by the American Public Health Association [15]. Hardness analysis was carried out according to the titrimetric method with EDTA (ethylenediaminetetraacetic acid) specified in [16]. Nitrite (NO_2^-) analyzes were performed according to the colorimetric method reported in [17]. The color intensity formed at the end of the reaction is in the spectrophotometer. Sulfate (SO_4^{2-}) contents were determined according to the turbidimetric method reported by [18]. The intensity of the turbidity formed at the end of the reaction was measured in a UV-Vis spectrophotometer (Hach-Lange DR3900) whose wavelength was adjusted to 420 nm, and sulfate concentrations were determined with the help of the standard calibration graphs [18].

3. Quality Control

Some statistical tests were applied to the results for the reliability and quality of the analysis results. Each sample was repeated at least 3 times and the mean values and standard deviations of the results were calculated. The LOQ, which is the lowest concentration that can be determined quantitatively, was calculated by taking 10 times the standard deviation of the data obtained as a result of the analysis of a series of blank solutions.

The presence of possible interferences was tested by applying spiked/recovery tests to the samples for all physicochemical parameters. The accuracy of the metal analysis was also tested with a standard reference material "CRM-TMDW-A Trace Metals in Drinking Water". Satisfactory results were obtained from the accuracy tests for all parameters.

A very effective accuracy parameter for water analysis is the charge-balance error (CBE) test. Surface waters contain mostly the following ions, which are almost 95% of the ions present in the water; HCO_3^-, CO_3^{2-}, SO_4^{2-}, NO_3^-, Cl^-, Ca^{2+}, Mg^{2+}, Na^+, and K^+. In waters, the total ion charge must be zero, and the closer the result obtained from the CBE equation given in Formula 1 to zero, the less error there is in the analysis [19]. The CBE values of the water samples examined in this study were found to be between 0.7 and 7.9%.

$$CBE\ (\%) = \frac{\sum cations\ (meq/L) - \sum anions\ (meq/L)}{\sum cations\ (meq/L) + \sum anions\ (meq/L)} \times 100 \qquad (1)$$

CBE: Charge-balance error
meq: milliequivalent

4. Results and Discussion

The descriptive statistics of physicochemical parameters of the samples are listed in Table 1. Fig. 2 and Fig. 3 show the distribution of water quality parameters of the water samples taken from 17 points. The standard deviation values given in Table 1 are quite high for many parameters. This is due to widespread results. It can be seen more clearly from Fig. 3 that many parameters are heterogeneously distributed over a wide range.

Water temperature can cause changes in the physical and chemical properties of water by affecting many parameters dissolved in the water. In this context, the water temperature should also be considered when determining other parameters. In terms of health, the temperature value of drinkable water should be between 4 and 12. It is expected that the temperature values of the samples taken in September are generally higher than this range. What is important here is the temperature conditions under which other water quality parameters are present.

Perhaps the most critical parameter for water quality is pH. Perhaps the most critical parameter for water quality is pH. If the pH of the water is too high or too low, the aquatic organisms living in it will eventually die. pH also affects the water solubility and toxicity of chemicals and heavy metals. For health, the pH content of the waters should not be outside the range of 6.0–9.0. The pH values of the water samples examined in this study varied between 6.07 and 8.12 (mean of 7.53). Looking at the standard values in Table 2, according to TS 266, the pH value of drinking water should be between 6.5–9.0. According to WHO, this range is 6.5–8.5. Considering the pH results obtained from this study, only one sample was outside this range with a value of 6.07.

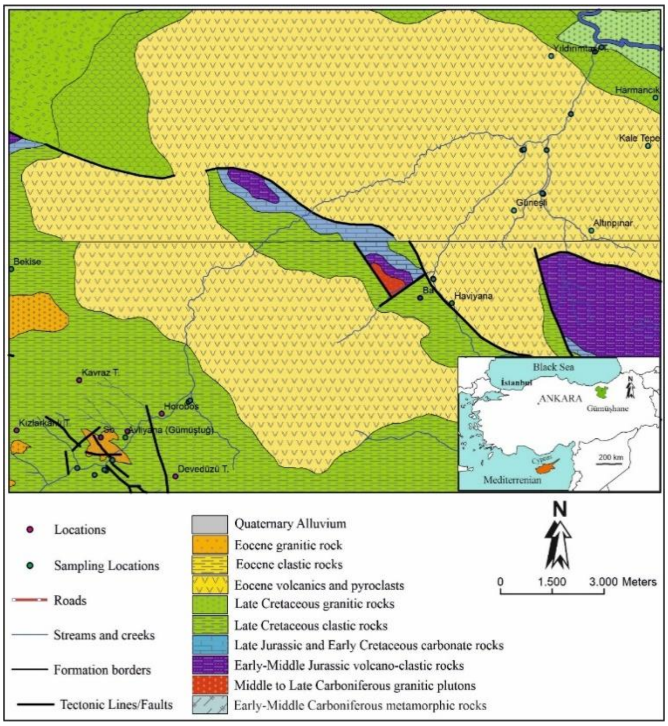

Figure 1. Geological map of the area and sample locations (modified from [13,20]).

According to WHO and EC, the electrical conductivity value in water should be < 2500 µS/cm. Although the electrical conductivity values in the examined waters are in accordance with the standards, it is seen that the values are distributed in a very wide range (31.6–379.3 µS/cm). Although the samples were taken from the same stream and its branches, the fact that the stream passes through different settlements and different geological geographies along the valley is reflected in the results.

In the directive published by the Turkish Ministry of Forestry and Water Affairs in the Official Gazette in 2016, in the table "Quality Criteria for General Chemical and Physicochemical Parameters of Inland Surface Water Resources by Class", it is reported that dissolved oxygen content should be > 8 mg/L for 1st class waters and between 6–8 mg/L for 2nd class waters. When the results are analyzed in terms of these criteria, the dissolved oxygen content in only one sample is critical (3.88 mg/L). The dissolved oxygen contents of the other samples are quite satisfactory.

TDS values of the samples are under permissible level. The scatter plot is similar to EC as the TDS values are calculated with a formula based on the results from the EC measurements. In other words, the comments made for EC values are also valid for TDS values.

The chloride contents of only two samples (w-14 and w-17) taken from the Altınpınar settlement were above the relevant LOQ value, and no chloride was detected in the other samples.

Table 1. Descriptive statistics of water quality parameters and trace elements concentration in water samples

Descripitive statistics	T (°C)	[1]pH	[2]EC (µS/cm)	DO (mg/L)	TDS (mg/L)	Hardness (°F)	NO_2^- (µg/L)	NO_3^- (mg/L)	Alkalinity (mg/L)	CO_3^{2-} (mg/L)	HCO_3^- (mg/L)	SO_4^{2-} (mg/L)	NH_4^+ (mg/L)	Cl^- (mg/L)
Mean	16.8	7.53	190.5	7.74	151.1	12.6	4.4	0.90	79.1	16.8	79.1	15.2	0.05	2.7
Median	17.0	7.84	188.7	7.99	143.0	11.3	2.0	0.46	78.6	13.8	78.6	10.7	0.02	2.7
Std. deviation	2.0	0.63	120.5	1.02	96.1	8.3	9.7	0.84	31.5	14.0	31.5	10.1	0.05	0.1
Minimum	13.5	6.07	31.6	3.88	25.4	1.5	1.0	0.23	25.3	0.0	25.3	6.4	0.02	2.7
Maximum	21.4	8.12	379.3	8.36	289.9	24.3	40.0	3.04	157.1	46.0	157.1	42.8	0.13	2.8
LOQ (mg/L)	–	–	–	–	–	1.0	2.0	0.23	0.1	0.1	0.1	0.1	0.01	1.0

Descripitive statistics	Na (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)	Fe (mg/L)	Cu (µg/L)	Co (µg/L)	Ni (µg/L)	Mn (µg/L)	Zn (µg/L)	Cd (µg/L)	Al (µg/L)	Pb (µg/L)	Cr (µg/L)
Mean	7.1	17.2	78.9	9.9	0.21	17.5	–	15.4	30.6	79.5	8.50	79.3	47.2	–
Median	5.6	17.5	78.7	9.5	0.13	13.79	–	15.4	30.6	74.7	8.50	83.6	13.9	–
Std. deviation	3.7	8.5	22.9	4.2	0.24	12.2	–	–	–	64.2	–	51.7	68.7	–
Minimum	3.4	5.6	28.0	3.0	0.05	8.3	–	15.4	30.6	15.1	8.50	21.8	10.8	–
Maximum	16.6	45.8	138.3	22.1	1.01	50.0	–	15.4	30.6	285.5	8.50	155.3	150.2	–
LOQ (µg/L)	10.7	7.3	7.3	4.7	10.3	8.3	10.0	10.3	10.3	9.0	6.7	11.7	10.0	10.0

* Respectively Class I and IV values

[1,2] The pH and electrical conductivity values are measured at 20 °C and 25 °C, respectively.

EC: Electrical conductivity, T: Temperature, DO: Dissolved oxygen, TDS: Total dissolved solids, °F: French unit of hardness

LOQ: Limit of quantification

There is no problem with chloride for either sample, as the reported guideline value (250 mg/L) for chloride is relatively high.

Hardness is an important water quality parameter. Water hardness is mainly caused by Ca^{2+} and Mg^{2+} ions.

Water containing these ions in large amounts becomes hard. The hardness values of the waters show a heterogeneous distribution in soft and medium hard classes between 1.5–24.3 °F. The WHO guideline value reported in 2017 is 20 °F. There are 5 samples that exceed this value, albeit slightly: However, according to TS266 records, water hardness is allowed up to 50 off. As a result, it can be said that the hardness values of the samples are within acceptable limits.

Alkalinity gives information about its ability to neutralize or buffer water against the ingress of any acidic substance. This function of water enables the living organisms it contains to continue their lives. The total alkalinity contents of the waters vary in a wide range between 27.6–133.4. Carbonate-rich rocks such as limestone increase the alkalinity of waters. Gümüşhane region has an important limestone reserve. It is quite possible for carbonate and bicarbonate ions to enter the waters not only with anthropogenic activities but also with geological formations. No limit value for alkalinity is given in the guide values.

Nitrite is a highly toxic substance that enters the waters as a result of microbiological contamination, and it is undesirable, especially in drinking water. Nitrite is an unstable substance and turns into nitrate, which is much less harmful after a while. The main sources of nitrite and nitrate compounds are fertilizer and sewage systems used especially in rural areas. Failure to control them can lead to serious problems. Guideline values for nitrite and nitrate are 0.5 mg/L and 50 mg/L, respectively, according to TS266. However, nitrite is allowed up to 3 mg/L in the WHO 2017 guideline. When the nitrite distributions of the analyzed water samples are examined, it is seen that the values are generally very low, but the w-13 coded sample does not exceed the limit value despite the relatively higher nitrite content (40 µg/L).

The presence of ammonia in water causes negative effects on human health, as in nitrate and nitrite. Ammonium enters the waters from domestic wastes, industrial wastes, and fertilizers. In short, due to anthropogenic effects, a significant amount of ammonium mixes into the waters and taste, and odor problems occur in the waters. The presence of NH_4^+ ions in the water indicates the possibility of mixing feces, ie sewage or animal wastes into the water. The ammonium ion is then converted to nitrite and then to nitrate by oxidation by bacteriological activities. Guideline values for ammonium are 0.5 mg/L according to TS266 and 1.5 mg/L according to WHO. Looking at the analyzed samples, ammonium was not observed in most samples. The results are in the range of 0.012–0.13 mg/L in ammonium detected samples. The presence of high sulfate in the water means high hardness, high sodium salt, and high acidity. For this reason, it is a pollutant whose analysis is routinely requested. Although the sulfate contents of the samples show a very heterogeneous distribution, it is seen that there is no problem based on the guide value.

One of the water quality parameters is undoubtedly the metal content of the waters and especially heavy metals are extremely important for health. Heavy metals can enter the waters through natural geological formations (especially from mining areas), as well as, of course, industrial, and anthropogenic activities. The metals given in Table 1 can be classified into 3 categories: minor elements, trace metals, and heavy metals. In this sense, Na, K, Ca, and Mg can be categorized as minor elements, Fe, Cu, Mn, and Zn as trace metals, and Al, Co, Ni, Pb, Cd, and Cr as heavy metals.

Na, K, Ca, and Mg, which are classified as minor elements, are also known as mineral elements and must be taken into the body regularly every day through a certain amount of water and other nutrients. However, excess of these minerals may not be healthy for the body. In drinking water, 200 mg/L Na is accepted as the upper limit. The water samples examined in this study show a Na distribution between 3.4–16.6 mg/L. Therefore, there is no danger for Na. No threshold values are specified in the guidelines for K, Ca, and Mg in waters. However, as the amount of Ca and Mg increases, the water becomes hard and undrinkable. While the K contents of the waters are homogeneously distributed between 5–25 mg/L, only the w-15 coded sample has a relatively high K of 48.8 mg/L. The Ca concentrations of the samples are distributed relatively homogeneously, like K. With a concentration of 138.3 mg/L, the sample w-15 has the highest Ca content.

Fe, Cu, and Zn, which are in the trace element class, are generally at normal levels in the samples examined. The upper limit of Fe is reported as 0.2 mg/L in the TS266 standard, and 0.3 mg/L in EPA and WHO. 1.01 mg/L Fe was detected in the w-2 coded sample and 0.51 mg/L Fe was detected in the w-12 coded sample. If 0.5 mg/L is taken as the threshold value, it would be appropriate to reanalyze these two samples. Zn values of 5 mg/L according to EPA and 4 mg/L according to WHO is reported as threshold values for waters. Among the samples, the w-1 coded sample has the highest Zn value with 0.29 mg/L. As a result, there is no problem for Zn. The standard values are given in Table 2 generally indicate 2 mg/L for Cu. While the Cu contents of the samples are generally between 0.008–0.023 mg/L, the w-12 coded sample contains 0.05 mg/L Cu (Fig. 2). However, no value exceeds the declared limit value.

WHO reports a 0.1 mg/L limit value for Al in drinking water. However, the limit value in other standards (Table 2) is 0.2 mg/L. When the samples are evaluated according to the WHO critical value, there are three samples exceeding 0.1 mg/L (Fig. 3).

While Co and Cr, which are classified as heavy metals in water, could not be detected in any sample, Mn, Ni, and Cd were detected in only one sample each. According to the WHO guideline values, Mn, Ni, and Cd in waters should be at maximum 0.5 mg/L, 0.07 mg/L, and 0.005 mg/L limit values, respectively. The w-15 coded sample contains 0.03 mg/L Mn, the w-12 coded sample contains 0.02 mg/L Ni, and the w-13 coded sample contains 0.008 mg/L Cd. Although the w-13 coded sample seems to be troublesome in terms of Cd, this sample needs to be analyzed again.

For Pb, WHO and other standards report a limit value of 0.01 mg/L. When the Pb distributions of the samples are examined in Fig. 2, it is seen that lead can be detected in only four samples, but these values are above the guide value. The w-13 coded sample exceeds the guide value by 15 times with a Pb content of 0.15 mg/L. It is interesting that Pb, which could not be detected in many samples, was detected above the guide value in 4 samples. Since these values are instantaneous values measured for a single time, a more detailed examination should be made by resampling.

Also, the chemical classification of the water samples from the study area was conducted using a Piper diagram (Fig. 4). All of the samples except one (w-186) have fallen into region 5 in the Piper diagram and these waters are in the class of $CaCO_3$ and $MgCO_3$ waters [21]. In the case of w-186 falls to region 9 in the Piper diagram and is in the class mixed water. This data shows compatibility with the geological structure of the region.

Table 2. Comparison of drinking water quality standards (mg/L)

Parameter	**RWIHC (2005)**	**TS266 (2005)**	**WHO (2017)**	**EPA (2002)**	**EC (1998)**
pH	6.5–9.5	6.5–9.5	6.5–8.5	6.5–8.5	6.5–9.5
EC	–	2500	2500	–	2500
NO_2^-	–	0.5	3	–	0.5
NO_3^-	50	50	50	45	50
SO_4^{2-}	250	250	250	250	250
NH_4^+	0.5	0.5	1.5	–	0.5
Hardness (°F)	–	50	20	–	–
TDS	–	–	600	500	–
Cl^-	250	250	250	250	250
Na	200	200	200	–	200
K	–	–	–	–	–
Ca	–	–	–	–	–
Mg	–	–	–	–	–
Fe	0.2	0.2	0.3	0.3	0.2
Cu	2	2	2	1	2
Mn	0.05	0.05	0.5	0.05	0.05
Zn	–	–	4	5	–
Cd	0.005	0.005	0.005	0.005	0.005
Al	0.2	0.2	0.1	0.2	0.2
Pb	0.01	0.01	0.01	0.015	0.01
Ni	–	–	0.07	–	–
Cr (Total)	0.05	0.05	0.05	1	0.05

RWIHC: Regulation on Water Intended for Human Consumption (Turkish Standard)
TS: Turkish Standards
WHO: World Health Organization
EPA: Environmental Protection Agency
EC: European Commission

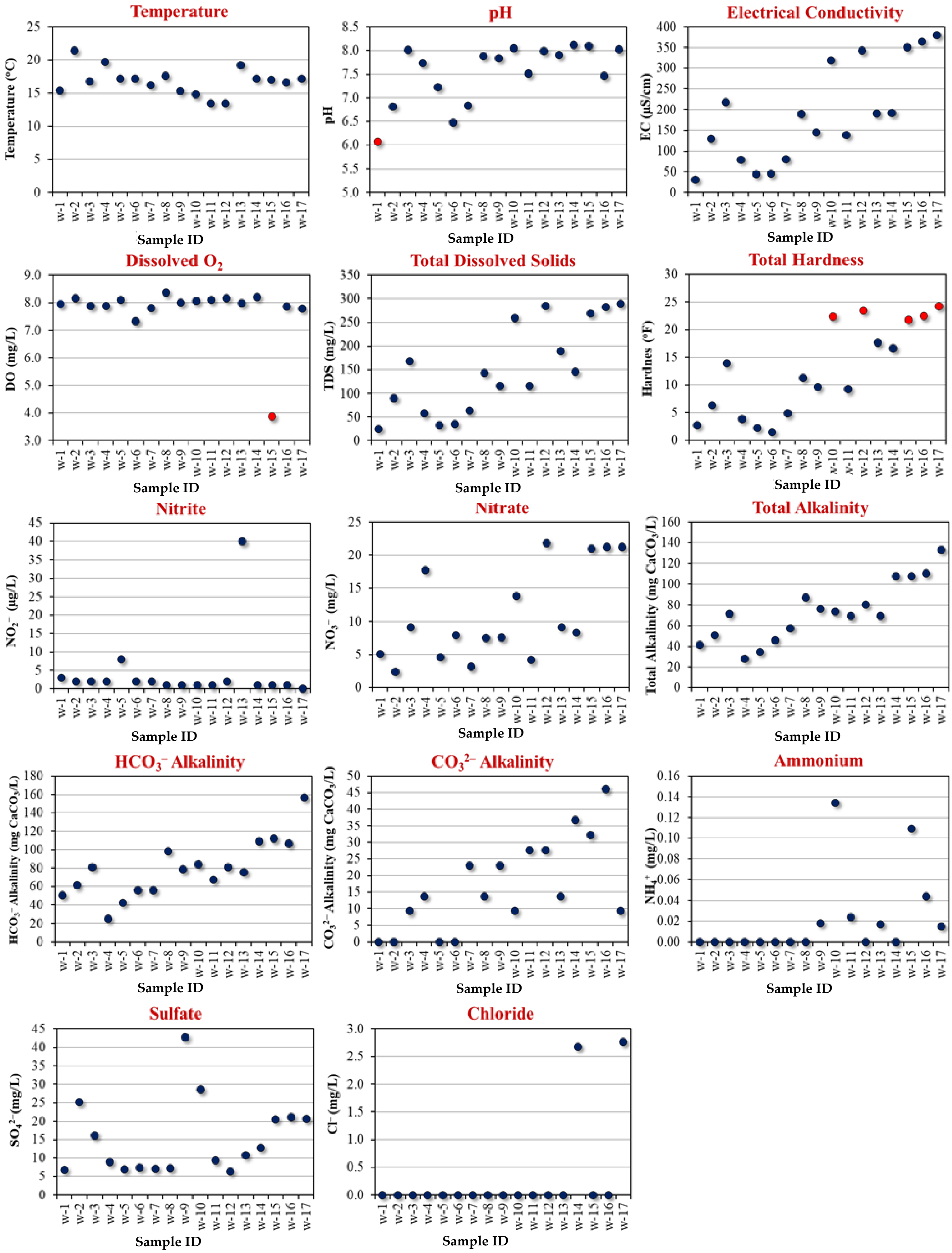

Figure 2. Distributions of some anions and physicochemical contents of the water samples for water quality

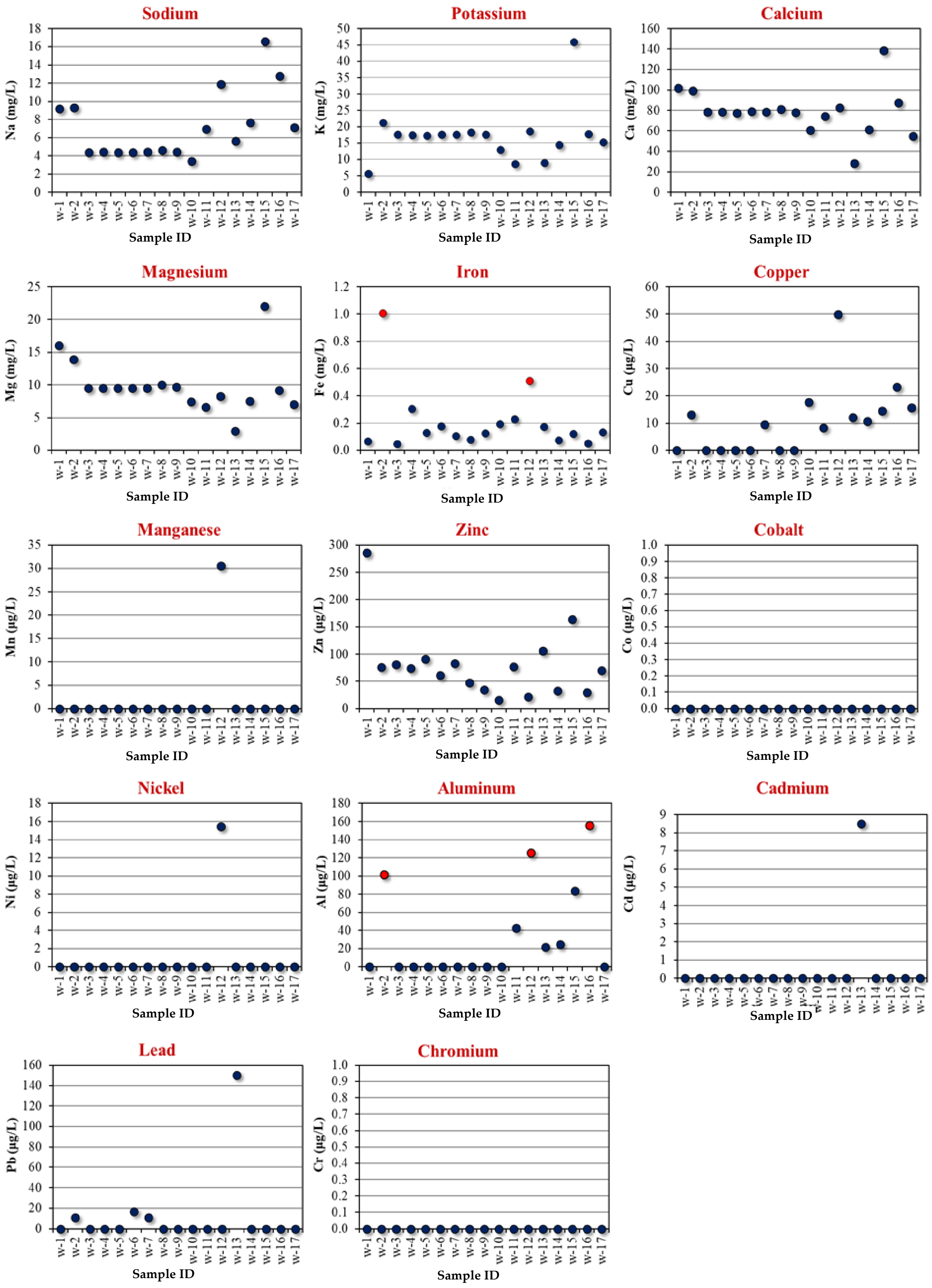

Figure 3. Some minor-trace element and heavy metal distributions in the water samples for water quality

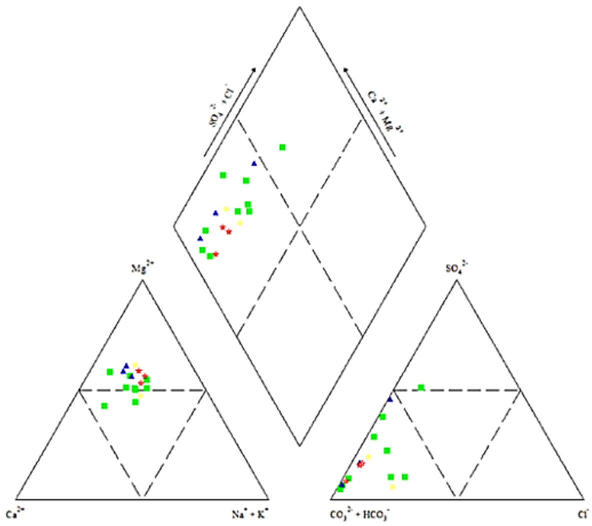

Figure 4. Piper diagram of the Büyük Çit Stream

5. Conclusion

In this study, analyses of some physicochemical water quality parameters were carried out by taking samples from the parts and branches of the Büyük Çit river, which is located within the borders of Gümüşhane province, along the valley. This study was designed to examine the effects of settlement areas in the valley, natural geological formations, and mining activities in the region on the water quality of the Büyük Çit river and to determine whether this river has a potable water potential in the future. In this study, only one sampling was made, revealing the instantaneous situation of the Büyük Çit river, and shedding light on new studies to be planned in the future.

Increasing pollution of clean drinking water sources points to a big problem in the future. For this reason, investigating the potential of uncontaminated surface waters as drinking water draws attention as an important study area.

As a result of the analyzes made, it was concluded that although some samples taken along the valley exceeded the guideline values in terms of some parameters, the Büyük Çit stream may have the potential to be used as drinking water in the future. It is thought that other additional studies to be made with these instantaneous values will gain more meaning. In addition, the microbiological evaluation of the examined waters, apart from the parameters applied in this study, will help to reach a healthier decision.

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