Bisphenols and their derivatives in baby diaper samples

Aneta Chabowska[a, *], Natalia Jatkowska[a], Paweł Kubica[a], Justyna Płotka-Wasylka[a, b]

[a] *Department of Analytical Chemistry, Faculty of Chemistry, Gdańsk University of Technology, G. Narutowicza 11/12, 80-233 Gdańsk, Poland* ✉ *aneta.chabowska@pg.edu.pl*
[b] *BioTechMed Center, Research Centre, Gdańsk University of Technology, G. Narutowicza St. 11/12, 80-233 Gdańsk, Poland*

Keywords
bisphenols
disposable baby diaper
UPLC

Abstract
Many common products contain and leach hazardous chemicals, including endocrine-disrupting chemicals such as bisphenols that are harmful to human health. For toddlers, this dangerousness is higher because of their not fully developed detoxification system. Due to this, bisphenols content in products, such as baby diapers, should be monitored. Baby diapers not only remain in close contact with the skin, but are also used from the first hours of life. Baby diaper samples were prepared by ultrasound assisted solvent microextraction of porous membrane-packed solid sample (UASE-PMSS) and extracted analytes were determined by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Obtained recoveries value ranged from 60% to 115%. Bisphenol A was determined in 81% of the tested samples with a concentration in the range of 5.0–520 ng g^{-1}, while bisphenol A bis(3-chloro-2-hydroxypropyl)ether in 15% of the samples with a concentration ranged from 6.8 to 530 ng g^{-1}.

1. Introduction

Endocrine disrupting chemicals are nowadays widely investigated group of compounds due to their harmful properties for wildlife and humans. They can be characterized by structural similarities to naturally synthetized hormones in human organism. This property gives them possibility to react as natural hormones and in result may cause dysfunction of endocrine system such as cancer disease [1]. Not fully developed detoxification mechanism of newborns and infants may have even more dangerous effects in their organisms due to this exposure.

One of the first synthetic substances which was described as endocrine-disrupting chemical was bisphenol A (BPA) [2]. It is widely used in products made from polycarbonate plastic and epoxy resin coatings [3]. Several studies have highlighted that early exposure to BPA can cause many health effects such as

thyroid disrupting properties [4]. It has also been reported, that maternal exposure to BPA may influence to the offspring causing, e.g., genital malformations, testicular abnormalities, impairment in fertility or sexual functions [5]. Because another bisphenols have similar structure to BPA, it is highly possible to suppose, that they can cause similar health effects.

Due to the fact that the presence of bisphenols and its derivatives were reported in products such as tampons and pads [6] it seems highly recommended to monitor their content in disposable baby diaper. The aim of that project was to determine bisphenols and their derivatives in disposable baby diaper samples to estimate safety of everyday products dedicated for newborns and infants.

2. Experimental

2.1 Reagents and chemicals

Analytical standards of bisphenol A (BPA, CAS 80-05-7), bisphenol BP (BPBP, CAS 1844-01-5), bisphenol C (BPC, CAS 79-97-0), bisphenol F (BPF, CAS 620-92-8), bisphenol FL (BPFL, CAS 3236-71-3), bisphenol G (BPG, CAS 127-54-8), bisphenol M (BPM, CAS 13595-25-0), bisphenol P (BPP, CAS 2167-51-3), bisphenol S (BPS, CAS 80-09-1), bisphenol Z (BPZ, CAS 843-55-0), racemic mixture of bisphenol F diglicydyl ether (BFDGE, CAS 2095-03-06), bisphenol F bis(2,3-dihydroxypropyl) ether (BFDGE·$2H_2O$, CAS 72406-26-9), bisphenol F bis (3-chloro-2-hydroxypropyl) ether (BADGE·2HCl, CAS 4809-35-2), bisphenol A diglycidyl ether (BADGE, CAS 1675-54-3), bisphenol A (3-chloro-2-hydroxypropyl) glycidyl ether (BADGE·HCl, CAS 13836-48-1), bisphenol A bis(3-chloro-2-hydroxypropyl) ether (BADGE·2HCl, CAS 4809-35-2), bisphenol A (2,3-dihydroxypropyl) glycidyl ether (BADGE·H_2O, CAS 76002-91-0), bisphenol A bis(2,3-dihydroxypropyl) ether (BADGE·$2H_2O$, CAS 5581-32-8) and bisphenol A (3-chloro-2-hydroxypropyl)-(2,3-dihydroxypropyl) ether (BADGE·H_2O·HCl, CAS 227947-06-0) were purchased from Merck–Millipore, Germany. Internal standards, that is ^{13}C-labeled bisphenol A (CAS 263261-65-0) and d_{10}-labeled BADGE (CAS 1675-54-3), were purchased from Cambridge Isotope Laboratories Inc. (Cambridge, UK). Potassium chloride (CAS 7447-40-7) was bought from VWR, Poland, hypergrade purity methanol (CAS 67-56-1) from Merck (Germany) and 25% ammonia solution (CAS 1336-21-6) from Merck–Millipore, Germany. Salt solutions were prepared with ultrapure water, cleaned by HPL5 system (Hydrolab, Poland) equipped with an EDS-Pak cartridge (Merck–Millipore, Germany). For the extraction polypropylene membrane sheets purchased from the GVS Filter Technology (Rome, Italy) were used. Syringe filters filled with 0.2 μm pore size nylon core were obtained from Thermo Fisher Scientific, Poland.

2.2 Instrumentation

Experiment was conducted using ultra-performance liquid chromatograph Schimadzu Nexera X2 (Japan) coupled to Shimadzu LC-MS-8060 (Japan) tandem mass spectrometer. Separation of analysed substances took place in Kinetex® 1.7 µm EVO C18 100 Å, 100×2.1 mm chromatography column purchased from Phenomenex (Germany). Additionally UHPLC precolumn purchased from Phenomenex (Germany) was used. Moreover, instruments such as Mettler Toledo XP504 analytical balance (Poland), Z6667 impulse sealer (Poland), stainless steel net (Poland), ultrasonic bath UM 4-Badelin (Sonorex , Germany) and TurboVap LV evaporation system (Caliper LifeSciences, USA) were used.

2.3 Sample preparation

All samples of disposable baby diaper were purchased in local stores in Gdańsk, Poland. Firstly, they were homogenized by machinery cutting to small pieces and each sample was divided into absorbent core and supporting wings. Afterward weighted 0.1 g of sample was placed in prepared before polypropylene membrane bag. 20×10^{-3} mol dm^{-3} potassium chloride solution and two internal standards of concentration 10 µg mL^{-1} were then added to weighted sample. Prepared like that membrane bag was sealed by impulse sealer and placed in 15 mL vial, where 7 mL of methanol was added. Because of floating observed at the first moments of sample preparation cleaned stainless steel net was introduced to immobilize membrane bag inside of extractions solvent. Vials prepared this way was then placed in the beakers on ultrasonic bath were the ultrasound assisted solvent microextraction of solid samples contained in a porous membrane (UASE-PMSS) took place for 20 minutes at 25 °C . Subsequently, the membrane and stainless steel net was removed from extract. Afterward, it was ready to evaporate the methanol under gentle nitrogen stream on TurboVap LV evaporation system. Subsequently, 1 mL of methanol was added to dry residue of extract the analytes. Prepared solution was mixed on vortex, filtered by nylon syringe filters and placed in chromatographic vial. To exclude possible contamination blank samples were also prepared and analyzed.

2.3 Bisphenols determination

Obtained solution with internal standars and extracted analytes were analysed by UPLC-MS/MS. Calibration curve, necessary to quantify the amount of compounds in disposable baby diaper was prepared by dissolving stock solution of analytes and internal standars. During analysis internal standrads concentration was kept at 10 ng mL^{-1} each while analytes were prepared in concentration equal 0.5, 1, 2, 5, 10, 20, and 50 ng mL^{-1}. All analytes were detected and analysed in positive and negative ionization by multiple reaction monitoring mode. Separation took place

Table 1
Parameters of weighted regression, standard deviation of the slope of calibration curve, standard deviation of intercept of calibration curve, limit of detection, limit of quantification, and recoveries at three concentration levels.

Analyte	Regression equation	s_a	s_b	r	*LOD* / ng g^{-1}	*LOQ* / ng g^{-1}	Recovery / % ± SD (n = 9)		
							c = 20 ng g^{-1}	c = 50 ng g^{-1}	c = 100 ng g^{-1}
BPA	$y = 0.002317\,x + 0.00085$	0.000023	0.00011	0.9985	1.5	4.4	88.9 ± 5.5	60.6 ± 4.7	60 ± 12
BPS	$y = 0.04192\,x - 0.0084$	0.00037	0.0018	0.9990	1.4	4.1	45.8 ± 8.2	52 ± 12	58.6 ± 7.6
BPP	$y = 0.002852\,x + 0.00107$	0.000023	0.00011	0.9992	1.2	3.7	109.8 ± 7.6	105 ± 10	100.7 ± 2.2
BPG	$y = 0.002052\,x + 0.00196$	0.000033	0.00010	0.9946	1.5	4.5	103.3 ± 7.2	87.5 ± 2.1	90.8 ± 7.0
BPM	$y = 0.003364\,x + 0.000895$	0.000013	0.000064	0.9995	0.6	1.8	115 ± 14	103.7 ± 4.2	106.0 ± 4.6
BPBP	$y = 0.002485\,x + 0.00169$	0.000022	0.00010	0.9989	1.3	4.0	102 ± 13	66 ± 10	60 ± 13
BPZ	$y = 0.004991\,x + 0.001233$	0.000020	0.000096	0.9997	0.6	1.9	89 ± 14	73.3 ± 8.8	71 ± 19
BPFL	$y = 0.01013\,x + 0.0607$	0.00045	0.0021	0.9933	8.2	25	70 ± 13	70.9 ± 8.9	78.5 ± 7.9
BPC	$y = 0.0013883\,x + 0.000806$	0.0000034	0.000016	0.9992	0.4	1.2	111 ± 13	69.0 ± 3.6	71.4 ± 7.5
BPF	$y = 0.002831\,x + 0.001144$	0.000018	0.000088	0.9995	1.0	3.0	64 ± 20	72.6 ± 9.6	81.5 ± 6.9
BADGE·HCl	$y = 0.038057\,x + 0.00182$	0.000086	0.00041	0.9999	0.4	1.1	102.6 ± 9.5	85.1 ± 5.7	86.8 ± 4.0
BADGE	$y = 0.10232\,x + 0.0323$	0.00035	0.0017	0.9998	0.5	1.6	106.5 ± 5.2	90.0 ± 3.4	85.6 ± 7.5
BFDGE·2HCl	$y = 0.02598\,x + 0.01042$	0.00012	0.00057	0.9996	0.7	2.1	106.0 ± 2.7	87.2 ± 2.4	90.0 ± 5.7
BADGE·H_2O·HCl	$y = 0.03294\,x + 0.06268$	0.00016	0.00079	0.9997	0.8	2.4	105.1 ± 6.1	93.2 ± 7.8	92.8 ± 3.8
BFDGE	$y = 0.04814\,x + 0.00392$	0.00011	0.00051	0.9999	0.3	1.0	107.7 ± 3.6	91.7 ± 1.3	91.4 ± 5.7
BADGE·2HCl	$y = 0.004048\,x + 0.003677$	0.000011	0.000053	0.9987	0.4	1.3	80.8 ± 5.9	75 ± 10	83.7 ± 6.7
BADGE·H_2O	$y = 0.05415\,x + 0.01673$	0.00020	0.00097	0.9998	0.6	1.7	93.2 ± 3.8	86.0 ± 9.6	87.8 ± 1.5
BADGE·$2H_2O$	$y = 0.07478\,x + 1.3493$	0.00068	0.0032	0.9992	1.4	4.2	99 ± 14	81.8 ± 7.3	84.6 ± 7.5
BFDGE·$2H_2O$	$y = 0.05255\,x + 0.0124$	0.00024	0.0012	0.9996	0.7	2.1	87.8 ± 4.1	77.5 ± 6.6	85.0 ± 7.3

by using two separate chromatographic method. For determination of BPA, BPS and BPA-C13 isocratic mode with water (component A) and methanol (component B) as mobile phases were used (55% of water and 45% of methanol) while for diglycidyl ethers with other bisphenols 0.01% ammonia solution in water (component A) and methanol (component B) were used.

3. Results and discussion

Obtained calibration curves were linear in tested concentration range. Limit of detection, limit of quantification and correlation coefficient were calculated by using weighted regression. Obtained data are shown in Table 1 together with recovery values. Results obtained for real samples analysis are shown in Table 2. BPA was quantified in the vast majority of tested samples (81%) with the concentration ranged between 5.0 ng g^{-1} to 520 ng g^{-1}. During the experiment also BADGE·2HCl was characterized by high concentration in the range from 6.8 to 530 ng g^{-1}, but it is frequency of determination was incomparably lower (15% of tested samples). The frequencies and bisphenols content in this research is comparable to determination of bisphenols in feminine hygiene products [6] and infant clothing [7]. Bisphenol A was quantified in infant clothing in 82% of tested samples [7] while in pads, panty liners and tampons frequency ranged from 69 to 92% [6].

Table 2
Concentrations and frequencies of determination bisphenols and their derivatives in disposable baby diaper samples (ND –not detected, NA – not analyzed).

Analyte	Concentration found / ng g^{-1}							
	Absorbent core				Supporting wings			
	Min	Median	Max	Frequency / %	Min	Median	Max	Frequency / %
BADGE·2H_2O	12	13	15	4.2	5.3	30	79	13
BADGE·2HCl	6.8	15	530	13	12	21	69	17
BFDGE	17	39	66	13	6.1	10	220	26
BADGE·H_2O·HCl	6.3	8.6	13	4.2	8.5	13	33	8.7
BADGE	20	49	96	17	4.9	43	98	17
BADGE·HCl	15	25	36	8.3	6.2	17	43	13
BPF	5.2	11	53	13	43	52	55	4.3
BPC	ND	ND	ND	ND	8.2	13	18	4.3
BPZ	8.6	11	18	4.2	ND	ND	ND	ND
BPBP	5.2	5.4	5.6	4.2	ND	ND	ND	ND
BPG	5.5	6.1	8.6	8.3	ND	ND	ND	ND
BPA	5.1	9.7	200	75	5	8	520	87
BPS	NA	NA	NA	NA	NA	NA	NA	NA
BPP	ND	ND	ND	ND	ND	ND	ND	ND
BFDGE·2H_2O	ND	ND	ND	ND	ND	ND	ND	ND

4. Conclusions

Bisphenol A was found in the vast majority of tested samples (81%). Other bisphenols were determined not more often than in 26% of tested samples. Although bisphenols and their derivatives were quantified in disposable baby diapers, it is impossible to judge if they can be harmful with such doses. To make sure about safety of personal care products dedicated for children, estimation of dermal exposure should be done and compared with present regulations.

Acknowledgments

This work was supported by the National Science Centre, Poland within the grant project number 2020/37/B/ST4/02886.

References

[1] Calaf G.M., Ponce-Cusi R., Aguayo F., Muñoz J.P., Bleak T.C.: Endocrine disruptors from the environment affecting breast cancer. *Oncol. Lett.* **20** (2020), 19–32.

[2] Eladak S., Grisin T., Moison D., Guerquin M.J., N'Tumba-Byn T., Pozzi-Gaudin S., Benachi A., Livera G., Rouiller-Fabre V., Habert R.: A new chapter in the bisphenol a story: Bisphenol S and bisphenol F are not safe alternatives to this compound. *Fertil. Steril.* **103** (2015), 11–21.

[3] Edginton A.N., Ritter L.: Predicting plasma concentrations of bisphenol A in children younger than 2 years of age after typical feeding schedules, using a physiologically based toxicokinetic model. Environ. *Health Perspect.* **117** (2009), 645–652.

[4] Boas M., Feldt-Rasmussen U., Main K.M.: Thyroid effects of endocrine disrupting chemicals. *Mol. Cell. Endocrinol.* **355** (2012), 240–248.

[5] Yi B., Kim C., Yang M.: Biological monitoring of bisphenol A with HLPC/FLD and LC/MS/MS assays. *J. Chromatogr. B* **878** (2010), 2606–2610.

[6] Gao C.J., Kannan K.: Phthalates, bisphenols, parabens, and triclocarban in feminine hygiene products from the United States and their implications for human exposure. *Environ. Int.* **136** (2020), 105465.

[7] Xue J., Liu W., Kannan K.: Bisphenols, benzophenones, and bisphenol A diglycidyl ethers in textiles and infant clothing. *Environ. Sci. Technol.* **51** (2017), 5279–5286.

Investigation of burdock tea: Spectroscopic and electrochemical study

Sali Muriqi[a,*], Libor Červenka[a], Irina Matveeva[a]

[a] *University of Pardubice, Faculty of Chemical Technology, Department of Analytical Chemistry, Studentská 573, 532 10 Pardubice, Czech Republic* ✉ *sali.muriqi@student.upce.cz*

Keywords
burdock
color
pH
spectroscopy
voltammetry

Abstract
The aqueous extract of burdock root exhibits variability in color development over time. Spectrophotometric and electrochemical characterization of these changes in the aqueous extracts of burdock root were examined in distilled water, very hard water, phosphate buffers pH = 7.0, 7.5, 8.0, and Britton-Robinson buffer pH = 8.0. Furthermore, the effects of EDTA and inorganic ions in the aqueous extracts of burdock root were investigated.

1. Introduction

Tea plays a vital role in most individual's daily routines, making it an integral part of their lives. Whether used for its pleasant taste or its numerous health benefits, tea is one of the most consumed beverages worldwide [1]. Two prominent characteristics of tea as a beverage are its taste and color. The composition of tea is influenced by various factors, such as the region in which they are cultivated and the time of harvesting while the antioxidant activities of tea infusions are influenced by their preparation methodologies [2–3]. Previous studies have shown that a correlation between the quality of tea and multiple factors, including the environment, the variety of tea plants, the cultivation practices employed, and the technology used during processing, collectively influence the fundamental characteristics of tea, such as color, aroma, and taste [4].

Arctium lappa L., commonly called burdock, is a medicinal plant from the Asteraceae family known for a diverse range of biological activities and has been widely used in folk medicine to treat different conditions such as skin diseases, digestive and genitourinary tract disorders [5]. Moreover, root extracts have shown hepatoprotective effects in murine models of liver injuries, preventing mucous injuries caused by alcohol [6]. Arctium species have many scientifically proven biological properties related to phenolic compounds, including a wide range of benefits including reduction of inflammation, antitumor activity, antidiabetic, antimicrobial, antifungal, and antioxidant. The primary metabolites found in burdock extracts include some carbohydrates, such as inulin, galactose,

rhamnose, glucose, mannose, and fructans. Polysaccharides used as an inulin source are present mainly in burdock roots [6–7]. In addition to its traditional medicinal uses, burdock has also found its utility in the food industry and the cosmetic industry, particularly in shampoos and conditioners, due to its anti-dandruff properties and hair health benefits. [8].

The aqueous extract of Burdock root exhibited variability in color development over time, the color changing from golden dark to bright green. While this manifestation was not studied before, in order to understand the cause of this transformation and the qualities of burdock root tea, spectroscopic and electrochemical studies were carried out.

2. Experimental

2.1 Reagents and chemicals

Deionized water, potassium dihydrogen phosphate (Lach-Ner), ethanol 96% (Lach-Ner), potassium phosphate anhydrous (J.T. Baker), magnesium chloride hexahydrate (Lachema), manganese chloride tetrahydrate (Lachema), calcium chloride anhydrous (Penta), sodium bicarbonate (Lachema), sodium hydroxide (Penta), barium chloride (Lachema), potassium chloride (Penta), acetic acid 99% (Lach-Ner), methanol ≥99% (Honeywell), sodium acetate (Sigma), magnesium sulfate (Sigma), calcium sulfate (Sigma), ethylenediaminetetraacetic acid (Penta).

2.2 Sample preparation

2.2.1 Preparation of buffers

For the preparation of infusions, several types of phosphate buffers were used, with a pH of 7.0, 7.5, and 8.0, which were prepared by mixing respectively 9.343 g L^{-1} K_2HPO_4 and 6.309 g L^{-1} KH_2PO_4; 12.813 g L^{-1} K_2HPO_4 and 3.598 g L^{-1} KH_2PO_4; 16.282 g L^{-1} K_2HPO_4 and 0.8878 g L^{-1} KH_2PO_4.

Very hard water was prepared, by mixing the exact amount of $NaHCO_3$ (0.384 g), $CaSO_4$ (0.24 g), $MgSO_4$ (0.24 g), KCl (0.016 g) in distilled water.

Britton-Robinson buffer was prepared by measuring 1.16 mL CH_3COOH (0.04 mol L^{-1}), 1.35 ml of H_3PO_4 (0.04 mol L^{-1}), 1.2366 g of H_3BO_3 (0.04 mol L^{-1}). Next, a 0.2 mol L^{-1} NaOH solution was prepared. A pH = 8.0 buffer was prepared by mixing appropriate amounts of Britton-Robinson buffer and NaOH solution into a 250 mL flask. The pH values were always checked using a pH meter with a glass electrode.

2.2.2 Preparation of EDTA solutions and inorganic salts

EDTA solutions were prepared by mixing a certain amount of loose disodium EDTA salt (0.05 g, 0.1 g, 0.2 g, 0.3 g, 0.5 g, 0.8 g, 1 g) with 20 ml of burdock extract

solution in phosphate buffer pH = 8.0 into glass flasks with closable lids. For control, a blank experiment with only burdock extract solution without the addition of EDTA was prepared.

Furthermore, 0.2 mol L^{-1} and 0.1 mol L^{-1} EDTA solutions were prepared in 250 mL flasks. The solutions were diluted with distilled water to the required concentrations (0.05 mol L^{-1}, 0.033 mol L^{-1}, 0.025 mol L^{-1}, 0.05 mol L^{-1}, 0.005 mol L^{-1}, 0.0005 mol L^{-1}), then 1 mL of each concentration was mixed in a glass flask with a closable lid with 20 mL of burdock extract in phosphate buffer pH = 8.0. A blank was prepared in the same way, but distilled water was used instead of the EDTA solution. Chlorides (Mn^{2+}, Mg^{2+}, Ca^{2+}), and sulfates (Mg^{2+}, Ca^{2+}), were accurately weighed on analytical balances and transferred to a 100 mL flask to make up a final concentration of $MgSO_4$ 0.5 mol L^{-1} and $CaSO_4$ 1.84×10^{-2} mol L^{-1}. Furthermore, solutions with a concentration of 0.25 mol L^{-1}, 0.33 mol L^{-1}, 0.1 mol L^{-1} $CaSO_4$ and solutions with a concentration of 9.2×10^{-3} mol L^{-1}, 3.07×10^{-3} mol L^{-1} were prepared in the same way.

2.3 Instrumentation

Spectrophotometric measurements were conducted using the Shimadzu UV-VIS UV-2600 instrument. Samples were measured in quartz cuvettes (10 mm). The measurement range was set to 800–450 nm. The measurements were performed at a scanning speed of "medium" with a sensitivity of 0.1 nm.

Both freshly prepared extracts and extracts stored in darkness for 24 hours were measured. The results were processed using UVProbe software version 2.7 (Shimadzu).

All electrochemical measurements were carried out in a conventional three-electrode system. It consisted of one working electrode, carbon paste electrode; saturated calomel electrode as the reference electrode, and Pt-plate as the counter electrode. This electrode setup was connected to a potentiostat/galvanostat Autolab PGSTAT101 operated via the Nova software (version 1.11; Metrohm). The measurements were conducted in a glass voltammetric cell containing 16 mL of the sample under investigation. Prior to each measurement, it was necessary to renew the surface of the carbon paste electrode by polishing it for 10 seconds to remove any residual water and surface contaminants.

Changes in color for samples were measured by the UltraScan VIS spectrophotometer (Hunter Associates Laboratory, USA) in a reflectance mode against a white tile. The color was expressed as CIELAB color space values L^* [dark (0) to light (100)], a^* [red (+) to green (−)], b^* [yellow (+) to blue (−)].

3. Results and discussion

3.1 Effect of pH

At first, it was necessary to find out whether the pH values influence the color of the burdock extract, and in that case, several burdock extracts were prepared and monitored under different conditions with three types of water and buffers. The results show that the pH values of freshly prepared solutions and solutions after 24 hours differ for some extracts. The burdock extract in demineralized water and the burdock extract in very hard water experienced a rise in pH value after 24 h from 6.1 to 6.3 and 7.3 to 7.4 respectively. Furthermore, for some extracts, the pH value decreased after 24 hours, for the burdock extract in Britton Robinson buffer pH = 8.0, where the pH value dropped to 7.7, and for the burdock extract in phosphate buffer pH = 7.0, where the pH value decreased from 6.9 to 6.8. It is possible that these minimal changes in pH values after 24 hours may affect the stability of extracts, especially for extracts with unstable pH; therefore, only phosphate buffer (pH = 8.0) was used in further experiments.

Table 1 shows the color parameters of various infusions, where the L^* value ranges from 59.9 to 87.9. The burdock extract in phosphate buffer with pH = 8.0 showed a significantly darker color (L^* = 76.05). After 24 hours, there was a significant decrease in L^* for the extract in very hard water (a decrease of approximately 25 units) and for the extract in phosphate buffer pH = 8.0 and Britton-Robinson buffer pH = 8.0 there was a decrease of 10 units.

The burdock extract in very hard water with a value of 59.9 and the burdock extract in phosphate buffer pH = 8.0 with a value of 65.3 are significantly different and are closer to the middle of the scale. The parameter a^* (transition between green and red color) was positive for the freshly prepared solution of burdock root in deionized water, while the other fresh extracts were slightly greener. After 24 hours, the a^* value decreased, and the samples were greener. The greatest change was observed in the very hard water extract (change of 25 units) and in the extract in phosphate buffer pH = 8.0 (change of 10 units).

Parameter b^* (transition between yellow and blue color) was for all samples in the positive quadrant, where the yellow color prevailed in the samples. It can be seen from the table that the samples in very hard water and phosphate buffer pH = 8.0 were more yellow than the other samples. After 24 hours, the value of the b^* parameter was lower (less yellow) in these samples.

According to the C^* parameter, all samples, except for the extract in Britton-Robinson buffer, are close to 100, indicating a small representation of gray color and high saturation. The position on the standard color wheel, expressed as the h value, usually ranges between 22.9 and 49.2. The extract in Britton-Robinson buffer pH = 8.0 is in a completely different region (89.7 to 98.1). The h value was the highest for freshly prepared Britton-Robinson buffer pH = 8.0 but it decreased by 10 degrees over time. The burdock extracts prepared in very hard water and

Table 1
Measured pH, color values (expressed as CIE color space values: L^* for perceptual lightness, a^* and b^* for the four unique colors of human vision: red, green, blue and yellow, C^* for chroma, and h for hue), and the actual color of the sample detected according to the parameters $L^*a^*b^*$. Values expressed as the arithmetic mean ± standard deviation.

Burdock extract prepared in	pH	L^*	a^*	b*	C^*	h / °	Color
Deionized water	6.1	87.9±3.4	1.8±0.5	23.9±4.4	87.9±4.4	23.9±1.7	
Deionized water after 24 h	6.3	85.85±1.5	−2.25±0.1	22.5±1.4	85.9±1.4	22.6±0.5	
Very hard water	7.3	85.1±2.4	6±1.4	37.8±1.9	85.1±1.7	38.3±2.4	
Very hard water after 24h	7.4	59.9±3.1	−30.5±2.4	23.7±4.3	59.9±1	38.9±7	
Phosphate buffer pH = 8.0	8.0	76.05±2	−4.5±2.3	48.9±3	76.1±2.9	49.2±2.8	
Phosphate buffer pH = 8.0 after 24h	8.0	65.3±1.1	−14±2.3	42.1±1.4	65.3±1.2	44.4±3.1	
Phosphate buffer pH = 7.5	7.3	84.3±0.4	3.9±0.4	34.8±0.1	84.4±0.1	35±0.6	
Phosphate buffer pH = 7.5 after 24h	7.3	81.2±0.4	−5.2±0.7	37.6±0.4	81.2±0.9	38±1.2	
Phosphate buffer pH = 7.0	6.9	82.9±1.3	−2.2±0.5	35.7±2.2	82.9±2.2	35.8±1.1	
Phosphate buffer pH = 7.0 after 24h	6.8	83.5±2.1	−0.9±0.8	40.1±3	83.5±3	40.1±1.3	
Britton-Robinson buffer	8.0	81.9±3.5	−4.6± 0.7	32.6 ±1.6	32.9 ±1.5	98.1± 1.6	
Britton-Robinson buffer after 24h	7.7	71±9.4	0.1± 2.7	36.4± 1.8	36.4 ±1.8	89.7 ±4.3	

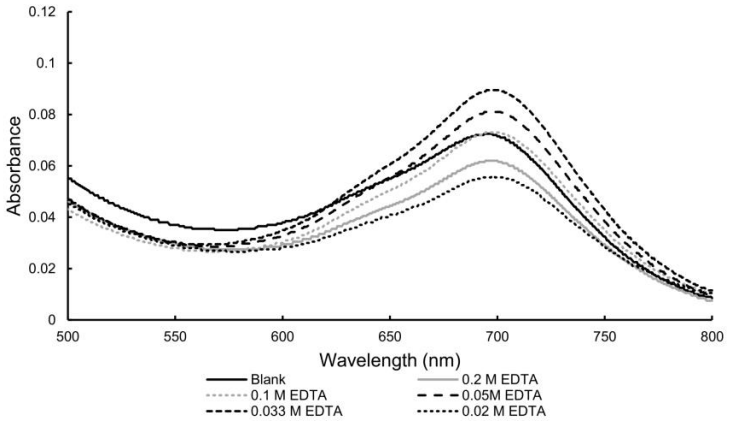

Fig. 1 Effects of the addition of EDTA solutions in different concentrations on burdock extract in phosphate buffer pH= 8.0.

phosphate buffer with pH = 8.0 differed the most, where their values significantly decreased compared to other samples. There was a change in the parameter a^* where from yellow completely changed to green. Since the greatest color changes occurred with burdock extract in very hard water and phosphate buffer pH = 8.0, the influence of pH can be assumed for these samples. Therefore, further experiments were used to monitor the effect of the addition of EDTA and metal ions.

3.2 Effects of addition of EDTA and inorganic ions

The change in the color and spectral properties of burdock extract in very hard water and in phosphate buffer pH = 8.0 can be caused by the formation of a complex of phenolic substances with some mineral substances. Assuming that the changes in the color of the burdock extract are due to the presence of a complex, it can be expected that the addition of a chelating agent may decompose the complex and lead to the loss of the green color. This assumption applies if the stability of the complex present in the burdock root extract is less than the stability of the complex of mineral substances with EDTA. In the first experiment, different amounts of EDTA were added to freshly prepared burdock root extracts in phosphate buffer pH = 8.0.

After the addition of EDTA solutions (Fig. 1) there was a decrease in absorbance at 699 nm, indicating the possibility that the original unknown complex was disrupted and a new complex with EDTA was formed. Unfortunately, no direct dependence on the EDTA concentration has been demonstrated. Originally, a clear connection between the concentration of EDTA and the change in absorbance was expected (the higher the concentration, the greater the change), this fundamental discrepancy suggests that the possible formation of a complex in the burdock root

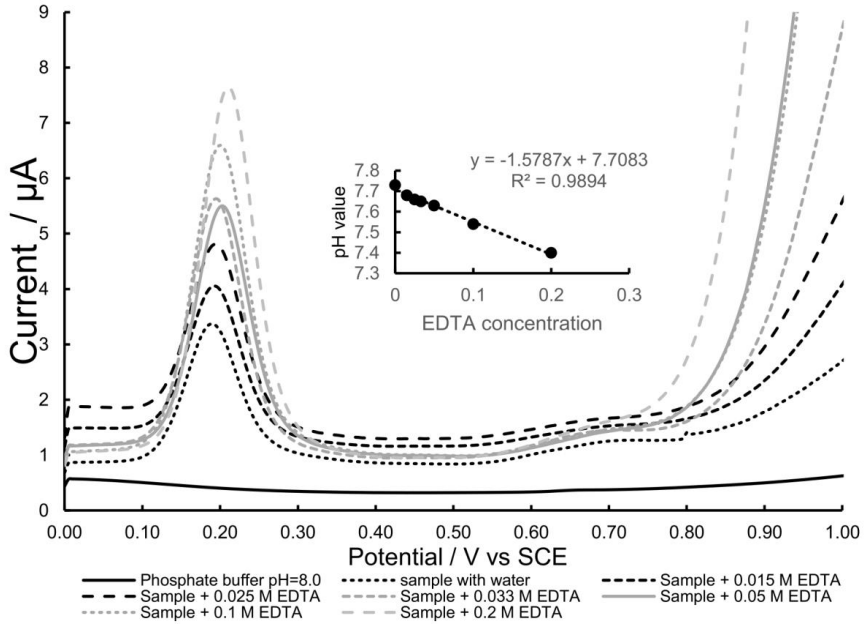

Fig. 2 Voltammetric measurement of the addition of EDTA in different concentrations with burdock extract in phosphate buffer pH = 8.0 and the dependence of EDTA added concentration on the pH of the solutions.

extract may not be related only to the concentration of mineral and organic substances present. It is possible that the color formation of the complex occurs in connection with other unknown factors, such as the concentration of oxygen, carbon dioxide, etc.

After selecting the working electrode and working conditions (initial potential (0.0 V), end potential (+1.2 V), frequency (10 Hz), amplitude (25 mV s^{-1}), and step potential (5 mV)), voltammetric measurements of the same infusions as the previous experiment were performed (Fig. 2). All extracts with the addition of an EDTA solution showed higher current yields with a potential of 0.2 V compared to the extract in distilled water. As the EDTA concentration decreased, so did the current value. The highest current was measured in the extract with the addition of 0.2 mol L^{-1} EDTA (7.64 µA), the lowest current was measured in the extract with the addition of water (3.31 µA). At a potential of 0.20 V, almost no significant shift was observed. The pH values of the solution range from 7.4 to 7.68.

Figure 2 also shows the overall dependence of the concentration of added EDTA on the pH of the solutions, and the result is that the higher the concentration of EDTA in the extract, the greater the pH value decreased from the original 8.0 to 7.4 Although this difference may appear to be negligible, previous experiments have shown that simply changing the pH of the extract from 8.0 to 7.5 resulted in a significant change in color.

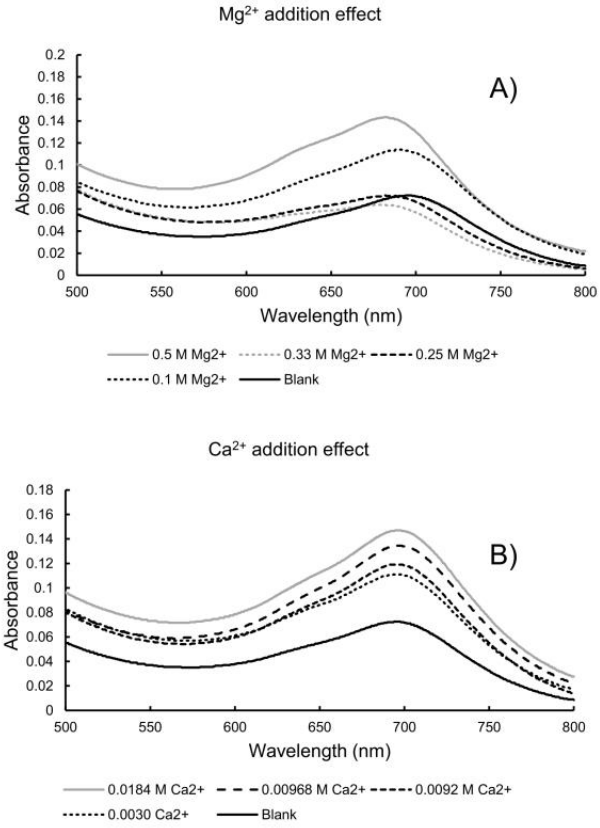

Fig. 3 Addition effect of (a) $MgCl_2$, and (b) $CaCl_2$ solution in different concentrations on burdock extract.

As shown in Fig. 3, $MgSO_4$ and $CaSO_4$ solutions were used to test their effect on the extract. It was found experimentally that Mg^{2+} and Ca^{2+} sulfate cations have an effect on the extract. The addition of different concentrations of cations caused an increase in absorbance compared to the blank. This means that Mg^{2+} and Ca^{2+} ions can influence the formation of a colored complex in the extract. In this case, even using a lower solution concentration increased the absorbance. The most significant increase in absorbance was observed for the Mn^{2+} cation, for which a maximum value of 0.44 was reached. The Ca^{2+} cation follows with a maximum of 0.275. These observations confirm the assumption that the color change of burdock extract in phosphate buffer (pH = 8.0) can be caused by complexes with some divalent ions.

Figure 4 shows a clear influence of the current response on cation concentration. The blank current value is 11.57 μA while the current value for Mg^{2+} ions is 14.92 μA for 0.005 mol L^{-1} and 21.43 μA for 0.0005 mol L^{-1}. Even in this case, it was confirmed that a lower concentration of the ions has a greater effect on the current peaks. The potential was stable at 0.21 V. There is a large effect on the addition of Ca^{2+} cation in both concentrations. The blank current value is 11.57 μA versus 16.25 μA for 0.0005 mol L^{-1} and 16.26 μA for 0.005 mol L^{-1} Ca^{2+}. The differences in

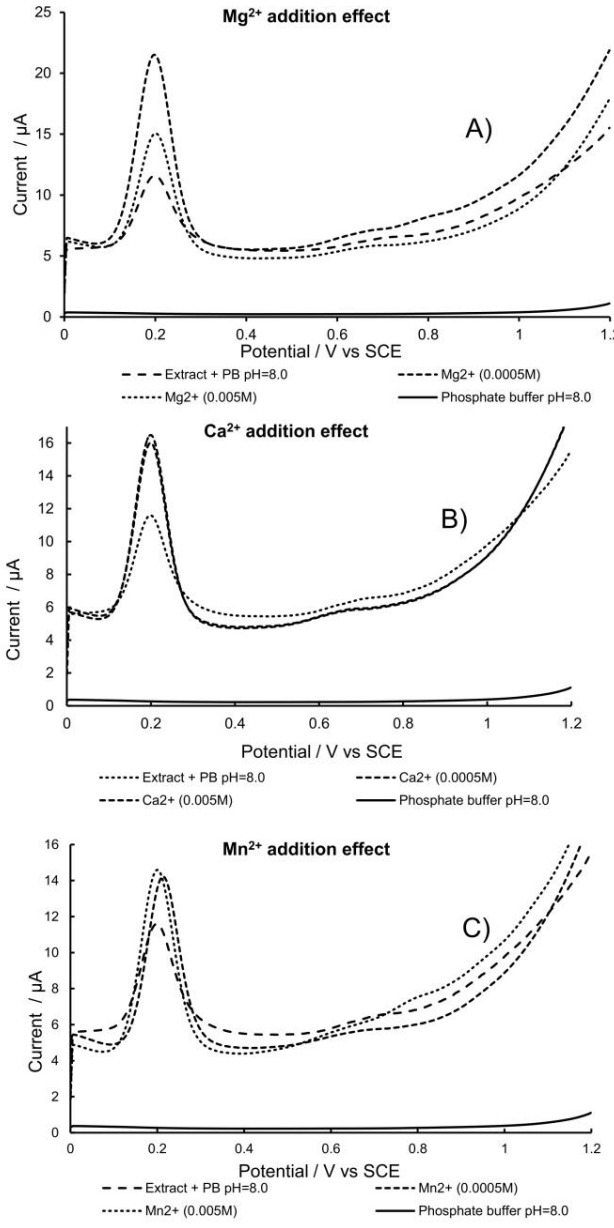

Fig. 4 Voltammetric measurement of (a) Mg^{2+}, (b) Ca^{2+}, and (c) Mn^{2+} ions addition in different concentrations of burdock extract in phosphate buffer pH = 8.0.

Mn^{2+} ion addition current yield are not significant. Thus, the current values for the blank are 11.82 µA compared to 14.11 µA for 0.0005 mol L^{-1} and 14.44 µA for 0.005 mol L^{-1} Mn^{2+}. The current yield is not related to the concentration of Mn^{2+}.

The voltammetric measurements were performed as a supplement to the UV/Vis measurement of burdock extract in phosphate buffer pH 8.0 in order to verify the results of this measurement and obtain additional information about

the electrochemical behavior of ions in the solution. It was observed that the addition of any concentration of Mg^{2+} to the burdock extract had an effect on the current response, but a linear relationship between the concentration of added Mg^{2+} and the current response was not demonstrated. The addition of Ca^{2+} ions had also a positive effect on the current yield, overall, the most significant effects were investigated at concentrations of 0.003 mol L^{-1} Ca^{2+} and 0.25 mol L^{-1} Mg^{2+}.

4. Conclusions

It can be concluded from the research that the color of the extracts is related to the resulting pH value, in the basic environment (pH = 8.0), a pronounced green color component predominates in terms of color.

Experiments showed that the addition of EDTA caused a decrease in the absorbance of the color of the extract in the 680–690 nm region (responsible for the green color), which was more related to a change in the value of pH at high EDTA concentrations (shift to the neutral region).

The electrochemical properties of burdock root extracts differed in the shift of the oxidation potential and the increase in the oxidation potential current. This effect is rather a consequence of the change in the pH of the solution. In this part of the research, it is not possible to unequivocally state that EDTA had an effect on the formation of a complex between metal and phenolic ions substances present in burdock extract.

As part of the investigation, selected cations were added and their influence on the spectral was evaluated. It was found experimentally that only Mg^{2+}, Ca^{2+}, and Mn^{2+} have a real influence on the color of the extract. The most significant increase in absorbance was observed for the Mn^{2+} addition, followed by the Ca^{2+} addition, and the smallest increase in absorbance was observed for the Mg^{2+} cation addition.

The whole experiment was accompanied by excessive variability in color development over time, which could have been caused by different sample particle sizes. Different concentrations of oxygen may also have played a role here, which must be excluded/confirmed in further experiments.

Acknowledgments

This research was financially supported by the student's grant no. 12/2022 provided by the Faculty of Chemical Technology, University of Pardubice.

References

[1] Dhaouadi K., Belkhir M., Raboudi F., Mecha E., Ghommeme I., Bronze M.D.R., Ammar H., Fattouch S.: Pomegranate and mint syrup addition to green tea beverage stabilized its polyphenolic content and biofunctional potentials during refrigerated storage. *J. Food Sci. Technol.* **53** (2016), 1164–1177.

[2] Dias P.M., Changarath J., Damodaran A., Joshi M.K.: Compositional variation among black tea across geographies and their potential influence on endothelial nitric oxide and antioxidant activity. *J. Agric. Food Chem.* **62** (2014), 6655–6668.

[3] Lantano C., Rinaldi M., Cavazza A., Barbanti D., Corradini C.: Effects of alternative steeping methods on composition, antioxidant property and colour of green, black and oolong tea infusions. *J. Food Sci. Technol.* **52** (2015), 8276–8283.
[4] Li Y., Ran W., He C., Zhou J., Chen Y., Yu Z., Ni D.: Effects of different tea tree varieties on the color, aroma, and taste of Chinese Enshi green tea. *Food Chem.: X* **14** (2022), 100289.
[5] de Souza A.R.C., de Oliveira T.L., Fontana P.D., Carneiro M.C., Corazza M.L., de Messias Reason I.J., Bavia L.: Phytochemicals and biological activities of burdock (*Arctium lappa* L.) extracts: A review. *Chem. Biodivers.* **19** (2022), e202200615.
[6] Ya-Chen W., Lian-Feng L., Ching-Sheng Y., Ya-Ling L., Hui-Jen Ch., Shiu-Ru L., Mei-Yin Ch., Chao-Peng H., Shih-Chiang L.: Burdock essence promotes gastrointestinal mucosal repair in ulcer patients. *Fooyin J. Health Sci.* **2** (2010), 26–31.
[7] Rodriguez J.M.F., de Souza A.R.C., Krüger R.L., Bombardelli M.C.M., Machado C.S., Corazza M.L.: Kinetics, composition and antioxidant activity of burdock (*Arctium lappa*) root extracts obtained with supercritical CO_2 and co-solvent. *J. Supercrit. Fluids* **135** (2018), 25–33.
[8] Li C., Xuan Z., Danni Z., Yuan L., Yuxing G., Jin F., Wuyang H., Ying L.: Isolation and identification of lactic acid bacteria and their effects on the off-odor of burdocks. *J. Agricult. Food Chem.* **71** (2023), 7485–7494.