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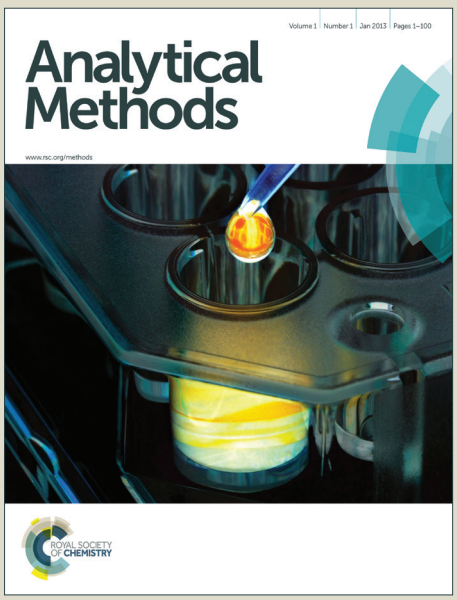

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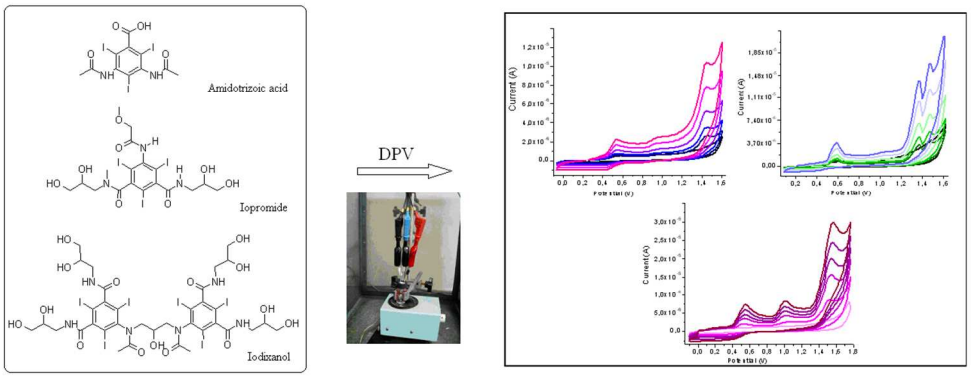

This manuscript shows the ability to quickly detect the concentration of three iodinated X–ray contrast agents (iopromide, iodixanol and amidotrizoic acid) using differential pulse voltammetry method.

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Determination of iodinated X–ray contrast agents in pharmaceutical formulations and artificial urine samples by differential pulse voltammetry technique

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Abstract

A new differential pulse voltammetry (DPV) technique was developed and validated for the determination of iopromide (IOP), iodixanol (ION) and amidotrizoic acid (DTZA), which belong to iodinated X–ray contrast agents group in pharmaceutical formulations and artificial urine samples. All measurements were performed in the three-electrode configuration with a glassy carbon electrode (GCE) as a working electrode, $Ag|AgCl|KCl_{(sat.)}$ as a reference and platinum wire as an auxiliary electrode. The supporting electrolytes for determination of selected contrast agents were mixture methanol / Britton–Robinson buffers with different pH values. Quantification was performed by the means of calibration curve and standard addition methods. The calibration curves for ION, IOP and DTZAwere linear over a concentration range of 0.032–0.258, 0.039–0.394 and 0.041–0.326 mM, respectively. Good linear behaviour over the investigated concentration ranges were observed with the values of r^2 higher than 0.994 for all the iodinated contrast agents (ICA). The limits of detection (LOD) and limits of quantification (LOQ) for all analysed contrast agents were calculated and recovery studies were also performed. The percentage recoveries varied from 94.44 to 101.05 %. Analytical methods for the preparation of urine samples before its voltammetry measurements (solid phase extraction − SPE) were worked out and optimized. The differential pulse voltammetry method described in this work is the first procedure allowing determination of three iodinated X–ray contrast agents (IOP, ION and DTZA) in pharmaceutical formulations and artificial urine samples.

Keywords: Iodinated X–ray contrast agents; iopromide; iodixanol; amidotrizoic acid; differential pulse voltammetry; solid-phase extraction.

1 Introduction

Iodinated Contrast Agents (ICA) are commonly used in the clinical diagnosis in Computer Tomography (CT) and X–Ray Imaging (XRI) as a diagnostic compounds to enhance a soft tissues contrast, to evaluate a blood – flow abnormalities and to characterize all the lesions. The chemical structure of ICA is based on a benzene ring, three or six iodine atoms (responsible for enhancing X–Ray) and functional hydrophilic groups (responsible for ensuring water solubility of ICA). Over the last thirty years, the ICA has been shown to be a suitable marker for a glomerular filtration rate (GFR), because they do not bind to the plasma proteins, do not metabolize and they are 100 % filtered through the kidneys. GFR was determined with ICA in humans and in animals such as dogs and cats.[1-13]

The ICA with six iodine atoms is iodixanol (ION) 5-{*N*-[3-(*N*-{3,5-*bis*[(2,3-dihydroxypropyl)carbamoyl]-2,4,6-triiodophenyl}acetamido)-2-hydroxypropyl]acetamido}-1-*N*,3-*N*-*bis*(2,3-dihydroxypropyl)-2,4,6-triiodobenzene-1,3-dicarboxamide. Its trade name is VISIPAQUE™. ION is a non ionic, iso osmolar, dimeric ICA. It has been introduced in the clinical practice in 1996.[14] It is use as a contrast agent during coronary angiography and CT imaging of brain and body.[15,16] Determination of ION in human and animals plasma, urine and serum have been reported using high performance liquid chromatography (HPLC) with different detection: an ultraviolet detection (UV)[3,17-20] and tandem mass spectrometry (MS/MS).[21] Also the colorimetric method for determination of iodixanol in biological fluids was developed.[13,22] The other published methods used the spectrophotometric determination of ION in the mammalian cells[22] and HPLC method with inductively coupled plasma mass spectrometry as detection for determination of ION in Radiopaque Solution for Injection (RSI).[23]

Iopromide (IOP) and Amidotrizoic acid (DTZA) are an ICA based on three iodine atoms. IOP 1-*N*,3-*N*-*bis*(2,3-dihydroxypropyl)-2,4,6-triiodo-5-(2-methoxyacetamido)-1-*N*-methylbenzene-1,3-dicarboxamide is a non ionic, low osmolar contrast agent. It is commonly known as ULTRAVIST™. It was introduced in 1985, since then it is used in brain, abdominal CT and angiography.[24] On the other hand, DTZA 3,5-bis(acetylamino)-2,4,6-triiodobenzoic acid is an ionic, monomeric ICA known as HYPAQUE™ or UROGRAFIN™. Commonly use as contrast agent in CT imaging of digestive and urinary systems.[25] IOP and DTZA are both determinated in water sample using HPLC–MS/MS[26-30], gas chromatography (GC) with MS/MS detection[29,30] and measurement of organic iodine by ion chromatography (IC).[31] DTZA was also determinated in the RSI using capillary electrophoresis (CE).[32]

Samples preparation have been performed by deprotinization with various agents such as perchloric acid,[2,9,33] trichloroacetic acid (TCA),[2] trifluoroacetic acid (TFA),[11,12,34] acetone,[2] dichloromethane,[2] hydrochloric acid and methanol,[35] zinc sulfate and methanol,[36] acetonitryle.[6]

In addition, samples pretreatment have been performed using various extraction procedures such as: liquid – liquid extraction (LLE)[37] and solid phase extraction (SPE) with various columns such as: LiChrolut® EN,[21,27,38] Sep–Pak C_{18},[18] Isolut ENV+ [25,30] and Oasis HLB.[29] The extraction procedure developed by Agasøster uses aqueous two phase partitioning sample preparation.[39] The procedure developed by Jacobsen[20] requires an automated online dialysis system for sample preparation and procedure developed by Denis requires ultrafiltration.[21] Some of the published procedures for samples preparation of biological and water samples used direct dilution .[6,23,40]

Thus, the aim of a present work is to develop a simple and efficient analytical method for quantification of ICA in pharmaceutical formulations and artificial urine by differential

pulse voltammetry method. Secondly a validation of the procedure have been carried out, which provides accuracy and reliability.

In the literature there are no voltammetric methods for the determination of iopromide (IOP), iodixanol (ION) and amidotrizoic acid (DTZA) described. To our knowledge, this is the first electroanalytical method, that allows determination of IOP, ION and DTZA in pharmaceutical formulations and artificial urine. This method may be considered as a suitable alternative to the existing chromatographic methods.

2 Experimental

2.1 Chemicals, reagents and solutions

Iopromide (~ 96 % purity), iodixanol (~ 95 % purity) were purchased from Toronto Research Chemicals Inc. (2 Brisbane Rd., North York, Toronto, Canada). Amidotrizoic acid, uric acid (~ 99 % purity) and hippuric acid were purchased from Sigma–Aldrich (Schnelldorf, Germany). The structures and IUPAC names of analysed X–ray contrast agents are presented in Table 1.

Urea and creatinine (≥ 99 % purity) were bought from Merck (Darmstadt, Germany). Potassium phosphate monobasic (KH_2PO_4), sodium phosphate dibasic (Na_2HPO_4), *o*-Boric acid (H_3BO_3), acetic acid (CH_3COOH), *o*-phosphoric acid (H_3PO_4), sodium hydroxide (NaOH) and methanol, all of analytical grade, were purchased from POCH S.A. (Gliwice, Poland). All other chemicals and reagents (acetonitrile and ethyl octane) were used of good commercially quality available and obtained from POCH S.A. (Gliwice, Poland). Aqueous solutions were prepared with double-distilled water. Britton-Robinson (BR) buffer solutions were prepared employing standard laboratory procedures.

2.2 Buffers preparation

Britton–Robinson (BR) buffer solutions at different pH values were prepared by mixing appropriate amounts of the 200 μM NaOH solution with 25 mL of a mixed acid that contains 40 μM of each of *o*-boric, *o*-phosphoric and acetic acids (H_3BO_3 − H_3COOH − H_3PO_4). Phosphate buffer at pH 6.81 was prepared by mixing 25 mL 67 μM KH_2PO_4 solutions with 25 mL 67 μM Na_2HPO_4 solution. 67 μM KH_2PO_4 and Na_2HPO_4 solutions were prepared by dissolving 0.9 g each compound in 100 mL water.

All chemicals used were analytical grade and were used without further purification. The pH of the solutions was adjusted by mixing buffer components and was verified before each measurement.

2.3 Stock solutions

Separate stock solutions of analysed iodinated X-ray contrast agents at different concentrations were prepared in 10 mL volumetric flasks by dissolving the appropriate amount of reference substance in a mixture of methanol/water (1/1, v/v). Stock solutions were prepared at the beginning of the study and were stored at 4 ºC. Solutions of lower concentrations were prepared by dilution of stock solution with water.

2.4 Artificial urine samples

Normal urine is a aqueous mixture of organic and inorganic substances. The majority of the constituents are either waste products of cellular metabolism or products derived directly from certain foods. The most important organic substances are an urea, an uric acid and a creatinine. In one embodiment, the artificial urine includes between about 55 – 900 mg L^{-1} of urea. The concentration of creatinine, when present, is preferably above about 50 mg L^{-1}, and more preferably, between about 350 and 3000 mg L^{-1}.

The artificial urine includes an appropriate amount of individual components of human urine such that the sample can appear to be a genuine human urine sample.

Further, a artificial urine of laboratory grade chemicals is safe for handling because there is no risk of disease. These urine samples were stored at 4 ˚C before analysis.

2.5 Instrumentation

All voltammetric measurements were carried out using a potentiostat μAUTOLAB Type III (Eco-Chemie, The Netherlands); with glassy carbon electrode (GCE) as a working electrode (1.5 mm diameter), that was polished with 0.03 μm alumina (Buehler), then ultrasonicated in a distilled water and finally rinsed with methanol. A platinum rod was used as a counter electrode, Ag|AgCl|$KCl_{(sat.)}$ electrode was used as a reference electrode (all electrodes purchased from Cypress Systems, Lawrence, USA). Voltammetric measurements were carried out in a 3 mL glassy electrochemical cell. All the measurements were automated and controlled through the programming capacity of the apparatus.

Examined samples were carried through solid-phase extraction (SPE) on J.T. Baker System (Deventer, The Netherlands) using Waters HLB® cation-exchange (*N*-vinylpyrrolidone – *m*-divinylbenzene copolymer) SPE columns (500 mg, 6 mL) (Milford, U.S.A.). The SPE 0.45 μm Nylon Hydrophilic Membrane Disposable Filters were purchased from J.T. Baker (Deventer, The Netherlands). All pH measurements were made with a ELMETRON (Zabrze, Poland) pH meter Model CP-401 using a combined glass electrode and calibrated with standard buffers. A cenrtrifuge HERMILE Z 323 K (Gosheim, Germany) was used. Argon was used for the removal of dissolved oxygen from the measured solutions.

2.6 Analytical procedure for ICA determination

All of the electrochemical experiments were carried out at ambient laboratory temperature (22 ± 3 ºC). All of the measurements were carried out in mixture BR buffer at different pH valuves /methanol (9/1, v,v).

Each measurement was repeated six times using fresh sample solution to ensure reproducibility of the results. 2 mL mixture methanol/BR buffer (9/1, v,v) at different pH range as a supporting electrolyte was transferred into the 3 mL glass voltammetric cell. With the aim of removing oxygen, the solution was purged with a pure argon for 10 min and for 30 s before each measurement. After measurement of the electrolyte, the appropriate amount of the relevant compounds was added and voltammograms were recorded for different concentrations of standard solutions.

Before measurements, the GCE was polished manually to a mirror finish using an alumina (1.0, 0.3 and 0.05 μm particle size) paste and thoroughly rinsed with purified water and methanol. Each measurement was repeated six times using fresh sample solutions to ensure reproducibility of the results. Between experiments, the cell was treated with concentrated nitric acid and then washed with water. Parameters for the cyclic and differential pulse voltammetry were presented in Tables 2 and 3.

2.7 Calibration curves

The calibration curves were evaluated by the least squares linear regression method. Calibration curves and ranges of determinations for all examined X-ray contrast agents in model solutions presented in $y = ax + b$ equation, where "y" indicates intensity of current (A), "a" and "b" constants, and "x" concentrations of analysed compounds. The calibration curves were evaluated by the least squares linear regression method. The height of peak current vs. concentration dependence was recorded in concentrations range 0.032–0.394 mM.

The regression line was used to calculate concentrations of all compounds in the standard solutions based on the peak area ratio. The calibration curves were measured six times.

2.8 Sample preparation

Artificial urine samples preparation and extraction method are described below step by step. Urine sample (2.5 mL) was placed into clean centrifuge tube (10 mL), and then suitable amounts of IOP, ION or DTZA solutions were added to each tube. The solution was mixed with 3 mL phosphate buffer at pH 6.81 and 3 mL methanol. After shaking for 1 min, the obtained mixture was centrifuged for another 10 min in 5000 r.p.m. at room temperature (*ca*. 22 °C).

Then, the sample was transferred to a volumetric flask (10 mL), and water was added to the mark. The obtained sample was filtered through a syringe nylon Bakerbond filter (0.45 μm). After that, the clear supernatant was transferred into Oasis® HLB (500 mg, 6 mL) column. Earlier this column was conditioned by pulling 4 mL of methanol and 4 mL of water. X–ray contrast agents were eluted with 5 mL of acetonitrile. The sample was evaporated to dryness under the stream of nitrogen at room temperature. The residues were dissolved in 3 mL of BR buffer at pH = 1.81 and 0.5 mL transferred to the voltammetric cell. The same procedure was repeated for urine without an addition of analytes in order to register a blank test. Sample preparation procedure for determination of IOP, ION and DTZA in artificial urine presented Fig.1.

3 Results and discussion

DPV was used in the voltammetric measurement due to its good sensitivity and resolving power. The peak current depends on pH of the medium, concentration and chemical composition of the buffer solution and instrumental parameters. We have studied optimization of the proposed procedure and examined conditions, which could affect the results. The current was measured and recorded for the sample solution.

As a working electrode for IOP, ION and DTZA determination glassy carbon electrode was used, where analysed iodinated X–ray contrast agents were oxidated. For each compound were obtained three oxidation peaks. As an analytical peaks selected: 1.46, 1.40 and 1.32 (vs. Ag|AgCl|$KCl_{(sat.)}$) for IOP, ION and DTZA, respectively. BR buffer solutions at different pH values were used as supporting electrolytes. When BR buffer at pH 1.81 was used, the peaks of IOP, ION and DTZA were all well defined.

3.1 Development of CV and DPV methods

The aim of the presented study was to evaluate an analytical method with the optimized parameters for determination of four selected X–ray contrast agents in pharmaceutical formulations and artificial urine samples. The current was measured and recorded for the sample solution.

The first step in the investigation of IOP, ION and DTZA was to check examined compounds for electroactive in studied conditions (GCE as a working electrode, BR buffer as a supporting electrolyte) and determined by voltammetric methods.

Cyclic voltammetry technique was applied as a diagnostic tool to get information about electrochemical oxidation of IOP, ION and DTZA at a glassy carbon electrode in the B–R universal buffer of pH (1.81–11.20).

Fig. 2 shows the representative cyclic voltammograms of the solution of IOP, ION and DTZA in mixture of BR buffer at pH 1.81 and methanol (9/1, v/v), where three well – distinguished peaks proportionally increasing with concentration are observed. For each analyte there are only three peaks appearing in the entire potential range between 0.0 V and 1.75 V resulting from oxidation processes.

IOP, ION and DTZA are electroactive compounds, which give well–defined three oxidation peaks at GCE in the working potential range from – 1.0 to 1.5 V in acidic media by DPV (Fig. 3).

The mechanisms of oxidation were connected with the presence of imino and hydroxyl groups in the IOP, ION and DTZA molecules. No peaks were observed in the cathodic scan, pointing to the irreversible nature of the oxidation process.

3.2 Calibration curves and linearity

The calibration curves were measured and evaluated by the least squares linear regression method. Calibration curves and ranges of determinations for analysed iodinated X-ray contrast agents in model solutions are presented in $y = ax + b$ equation, where „*a*" is the slope, „*b*" is the intercept, „*y*" indicates intensity of current (A) and „*x*" concentrations (mM) of analysed compounds. The calibrations were linear for IOP, ION and DTZA in the studied concentration ranges. The high correlation coefficients of the all calibration curves were between 0.995 and 0.999. The calibration curves show linear response over the whole range of concentration used in the assay procedure. The equations associated with the calibration are summarized in Table 4.

3.3 Effect of pH

The peak current depends on pH of the medium, concentration and chemical composition of the buffer solution, and instrumental parameters. The electroxidation researches of analysed X-ray contrast agents were performed in the BR buffers at different pH values. The pH effect of the electrolyte was examined between pH values of 1.81 – 11.20 for IOP and ION, and 1.81 – 7.24 for DTZA.

Position of peak current obtained by reduction of compounds was strongly pH depended. The effect of pH for IOP, ION and DTZA can be seen at Fig. 4. Peaks potential of analysed drugs moved into direction of more positive potentials, with the growth of supporting electrolyte acidity. In acidic media the peak of reduction process was narrow and well-defined. Therefore pH 1.81 was chosen as the best to analytical applications. For the lowest value of pH the highest peaks were observed at voltammograms.

3.4 Effect of scan rate

The influence of potential scan rate on the peak current of 50 x 10^{-6} M contrast agents in the BR buffer in pH 1.81 at GCE was investigated in the range 0.2 – 1.0 V s^{-1} (Fig.5). As shown in Fig. 5 oxidative peaks current of IOP (Fig. 5A), ION (Fig. 5B) and DTZA (Fig. 5C) showed linear dependence on potential scan rate in range 0.2 – 1.0 V s^{-1} with quite good correlation coefficient indicating that the oxidative reaction is surface – diffusion controlled.

3.5 Effect of conditioning potential and time

The effect of conditioning potential and time on the oxidative peak current of 20 x 10^{-5} M IOP, ION and DTZA was investigated in BR buffer pH = 1.81 in the potential range – 1.1 ÷ –2.0 V and in the time range 30 s – 120 s at GCE. Fig. 6 shows the dependence of conditioning potential on the peak current of IOP (A), ION (B) and DTZA (C).

As shown (Fig. 6A) the response of GCE for IOP decrease with increasing negative conditioning potential to – 1.3 V, then the peak current increase to potential – 1.5 V. The response of the working electrode decrease with adding the higher negative potential. In the case of ION (Fig. 6B) the peak current decrease from – 1.2 V with applied higher negative potential. The peak current of DTZA (Fig. 6C) decrease with the increasing negative potential up to – 1.5 V, then it increase during the conditioning with the potentials in the range – 1.6 V ÷ – 1.8 V, and again decrease with applied potential – 1.9 V.

As shown on Fig. 7A the peak current of IOP decrease with increasing of conditioning time. The dependence of peak current of ION is different (Fig. 7B) – the peak potential increase with increasing conditioning time to reach the maximum at 75 s, and then decrease with conditioning time. Similar dependence of peak current was observed for determination of DTZA (Fig. 7C). At the beginning the peak current increase with the increasing of conditioning potential beyond 60 s and then increasing conditioning potential cause the decrease in the peak current of DTZA. Hence, – 1.5 V was taken as optimized.

Hence, – 1.5 V and 30 s; – 1.2 V and 75 s; – 1.1 V and 60 s were taken as conditioning potential and time as the optimized parameters for the determination of IOP, ION and DTZA, respectively.

3.6 Analytes recoveries

Recoveries of analysed X-ray contrast agents during their separation from urine were analysed. Extraction experiments were first performed using standard solutions, and then the procedure was checked with artificial urine samples. The determination of the recovery rates was carried out from spiked artificial urine samples.

The mean recovery of analytes ranged from 94.4 to 101.1 %. Recoveries for the studied analytes (IOP, ION and DTZA) in the other protocols ranged from about 69 to 109 %.

The coefficient of variation (C.V.) for three successive determinations of ION at 0.097 mM concentration is 2.17 %, of IOP at 0.108 mM concentration is 4.09 % and of DTZA at 0.122 mM concentration is 2.46 %.

Coefficient of variation for the studied X-ray contrast agents (IOP, ION and DTZA) in the other protocols described in the literature ranged from 0.8 to 9.8 %.[26-40] Obtained recovery results of spiked urine samples were given in Table 5.

3.7 Limits of detection and quantification

The limits of detection (LOD) and limits of quantification (LOQ) for determination of analysed drugs in model solutions were calculated on the peak current using following equations: LOD = 3 S.D./a and LOQ = 10 S.D./a, where „S.D." is the standard deviation of the peak currents and „a" is the slope of the related calibration equation.

The limit of detection (LOD) was between 0.010 mM and 0.013 mM for the analysed compounds. LODs for the IOP, ION and DTZA in other articles described in the literature ranged from 0.02 to 1.43 μg L^{-1}.[17-23, 26-40]

The LOD and LOQ values are summarized in Table 4. Both LOD and LOQ values confirmed the sensitivity of the proposed methods.

3.8 Application of the method to urine samples and pharmaceutical formulations

Methods of urine samples for analysis preparation in order to remove matrix effect were elaborated and optimized. Preparation procedure of urine samples containing IOP, ION and DTZA by SPE method gave good results and recoveries of these analytes from urine were found as 94.44-99.27, 94.84-95.13 and 100.82-101.05 %, respectively. Receiving results are average of three measurements parallel prepared samples. The results of these analyses (recoveries, standard deviations, coefficients of variation and confidence intervals) are

summarized in Table 5. Analyte identification was performed according to peak potential by comparison with standard solution, and by the standard addition method.

The developed DPV methods for the IOP, ION and DTZA determination were applied to pharmaceutical formulations (ULTRAVIST™, VISIPAQUE™ and UROGRAFIN™). Conditions of IOP, ION and DTZA determination in pharmaceutical formulations were elaborated. Methods of pharmaceutical formulations for analysis preparation in order to remove matrix effect were elaborated.

The data proved the suitability of only diluting procedure for the determination of investigated compounds from pharmaceutical formulations.

3.9 Specificity

Sometimes voltammetric techniques can pose difficulties in the analysis of biological fluids, which contain reducing or oxidizing substances. Methods of urine samples for analysis preparation in order to remove matrix effect were elaborated and optimized.

The specificity of the method for the analysis of artificial urine samples was evaluated by the determination of selected iodinated X–ray contrast agents in spiked artificial urine with satisfactory results. To the artificial urine samples were added the main components of the real urine. To the artificial urine samples were added: urea, hippuric acid, uric acid and creatinine. It has been shown that urea, creatinine and hippuric acid were non electroactive in over a range of potentials in the data measurement conditions.

Under given measurement conditions only the uric acid underwent oxidation. However, these peaks do not affect the determination of analytes.

The presence of the main components of urine does not interfere in the analysis of ION, IOP and DTZA. No interfering peaks were observed near the peak potentials of examined compounds in artificial urine samples after SPE (Fig. 8).

4 Conclusions

We have developed and validated a new and reliable DPV method for determination of IOP, ION and DTZA and apply the method both to standard solutions and to artificial urine including spiked analysed contrast agents. DPV was used in the voltammetric measurement owing to its good sensitivity and resolving power. It is well known that DPV is suitable for the analysis of the electrochemically active substances. Well-defined oxidation peaks were observed for every of studied X–ray contrasts. The effect of pH at peak potential and peak current were permitted for elaborated the best conditions for determination of compound. The data proved the suitability of SPE procedure for the extraction of investigated compounds from urine samples.

The developed methods showed good recoveries (from 94.4 to 101.1 %) for analysed X-ray contrast agents compared with chromatographic methods. It is necessary to underline fact that it is the first elaborated voltammetric method for the determination of the selected iodinated X–ray contrast agents in standard solutions and in artificial urine samples. In the literature there are no voltammetric methods for the determination of iopromide (IOP), iodixanol (ION) and amidotrizoic acid (DTZA) described. This method may be considered as a suitable alternative to the existing chromatographic methods. Preparation of the sample was easy and the method is not time consuming and cheap.

The disadvantage of voltammetric techniques is smaller selectivity and the fact that the analyzed compounds must be electroactive.

The advantage of the proposed method is relatively simple and inexpensive measuring apparatus compared with chromatographic techniques. One analysis time is very short (two-three minutes) compared with the chromatographic analysis (usually a few minutes).

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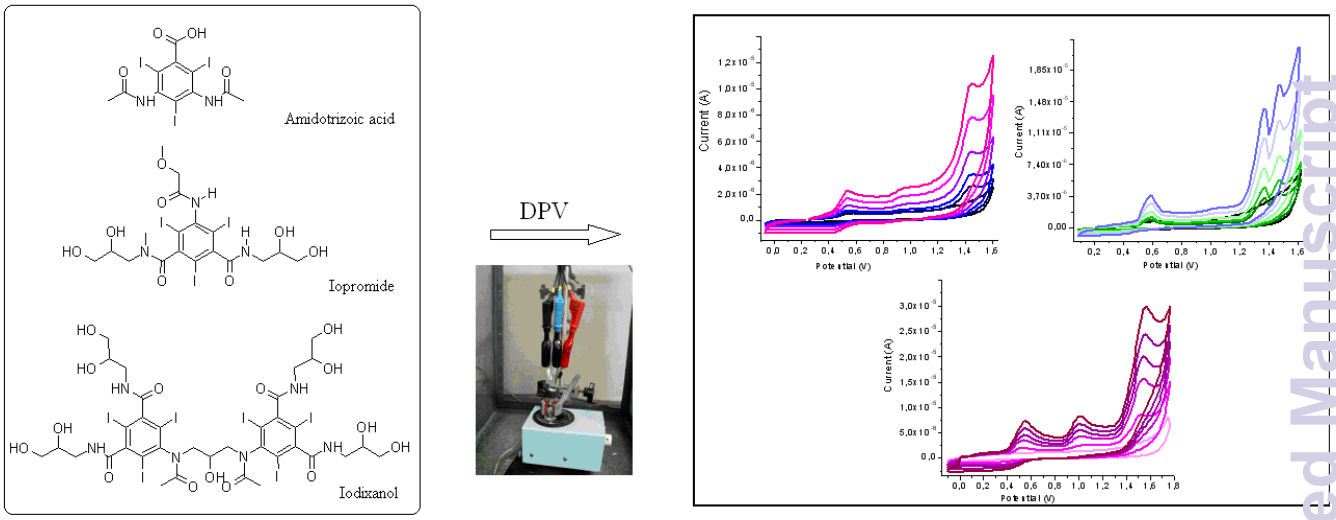
Graphical Abstract



This manuscript shows the ability to quickly detect the concentration of three iodinated X–ray contrast agents (iopromide, iodixanol and amidotrizoic acid) using differential pulse voltammetry method.

Table 1 Molecular structures and chemical names of the ICA under investigation.

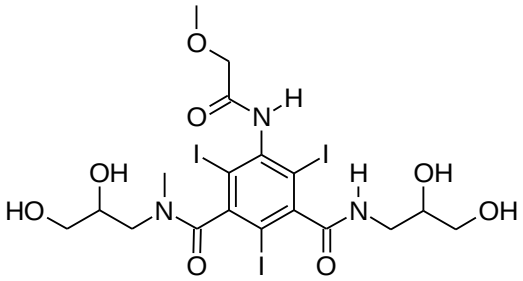
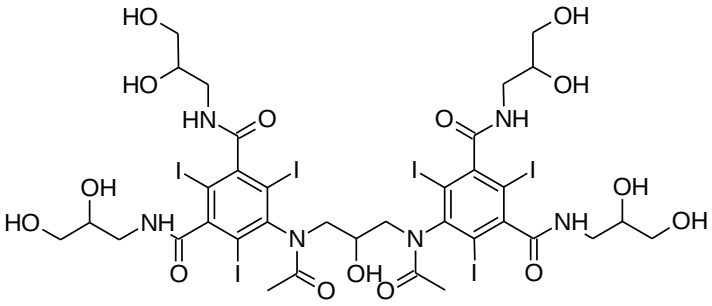
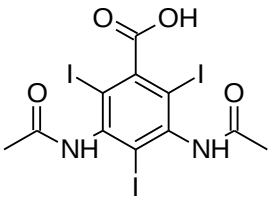
Contrast agents	Molecular structure	IUPAC name
Iopromide (IOP) MW = 791.11		1-*N*,3-*N*-*bis*(2,3-dihydroxypropyl)-2,4,6-triiodo-5-(2-methoxyacetamido)-1-*N*-methylbenzene-1,3-dicarboxamide
Iodixanol (ION) MW = 1550.18		5-{*N*-[3-(*N*-{3,5-*bis*[(2,3-dihydroxypropyl)carbamoyl]-2,4,6-triiodophenyl}acetamido)-2-hydroxypropyl]acetamido}-1-*N*,3-*N*-*bis*(2,3-dihydroxypropyl)-2,4,6-triiodobenzene-1,3-dicarboxamide
Amidotrizoic acid (DTZA) MW = 613.91		3,5-bis(acetylamino)-2,4,6-triiodobenzoic acid

Table 2 Analytical CV parameters for determination of iodinated X–ray contrast agents in model solutions (n = 6).

Contrast agents	Initial Potential [V]	First vertex potential [V]	Second vertex potential [V]	Condition potential [V]	Conditional time [s]	Scan rate [V s^{-1}]	Step potential [V]
ION	0.25	+ 1.60	– 0.05	– 1.20	30.00	0.80	0.015
IOP	0.00	+ 1.75	– 0.10	– 1.50	30.00	0.60	0.015
DTZA	0.20	+ 1.70	+ 0.10	– 1.10	45.00	0.80	0.015

Table 3 Analytical DPV parameters for determination of iodinated X–ray contrast agents in model solutions (n = 6).

Contrast agents	Initial Potential [V]	Start Potential [V]	End Potential [V]	Condition potential [V]	Conditional time [s]	Scan rate [V s^{-1}]
ION	0.25	+ 1.60	– 0.10	– 1.20	75.00	0.80
IOP	0.00	+ 1.75	– 1.00	– 1.50	30.00	0.60
DTZA	0.20	+ 1.80	+ 0.10	– 1.10	60.00	0.80

Table 4 Analytical parameters of calibration curves of all examined compounds in model solutions (n = 6).

Contrast agents	Linear range [mM]	a[a]	b[b]	r^{2c}	LOD[d] [mM]	LOQ[e] [mM]
ION	0.032 – 0.258	6.420×10^{-7}	-5.505×10^{-9}	0.998	0.010	0.029
IOP	0.039 – 0.394	4.067×10^{-7}	4.120×10^{-8}	0.999	0.011	0.032
DTZA	0.041 – 0.326	5.906×10^{-7}	9.464×10^{-9}	0.995	0.013	0.040

[a] Slope, [b] intercept, [c] correlation coefficient, [d] limit of detection, [e] limit of quantification.

Table 5 Results of recovery examination for analysed drugs from urine samples by SPE procedure (n=3).

Contrast agents	Concentration added (mM)	Concentration found (mM)	S.D.[a] (mM)	C.V.[b] (%)	L[c] (mM)	Recovery (%)	U[d] (mM)	(%)
ION	0.097	0.092	0.002	2.17	0.092 ± 0.005	94.84	6.13×10^{-3}	6.67
	0.194	0.185	0.003	1.62	0.185 ± 0.007	95.13	10.28×10^{-3}	5.56
IOP	0.108	0.102	0.005	4.90	0.102 ± 0.012	94.44	7.28×10^{-3}	7.14
	0.275	0.273	0.004	1.46	0.273 ± 0.010	99.27	17.06×10^{-3}	6.25
DTZA	0.122	0.123	0.003	2.46	0.123 ± 0.007	100.82	7.23×10^{-3}	5.89
	0.285	0.288	0.006	2.10	0.288 ± 0.015	101.05	13.71×10^{-3}	4.76

[a] Standard deviation of concentrations found, [b] coefficient of variation of concentrations found, [c] confidence interval ($\alpha = 0.05$, $t = 4.303$), [d] expanded uncertainty (for confidence level 95%, coverage factor $k = 2$).[41]

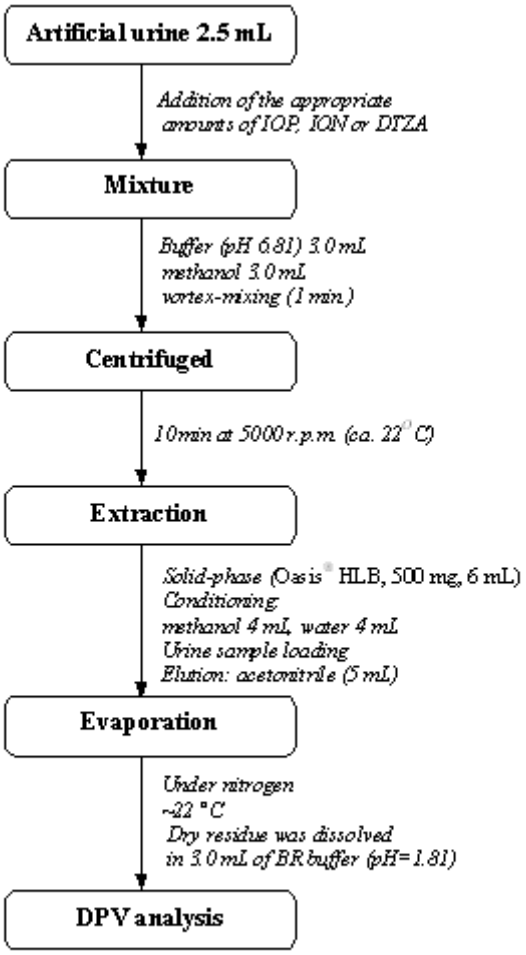


Fig. 1 Procedure of artificial urine samples preparation for determination of the IOP, ION and DTZA.

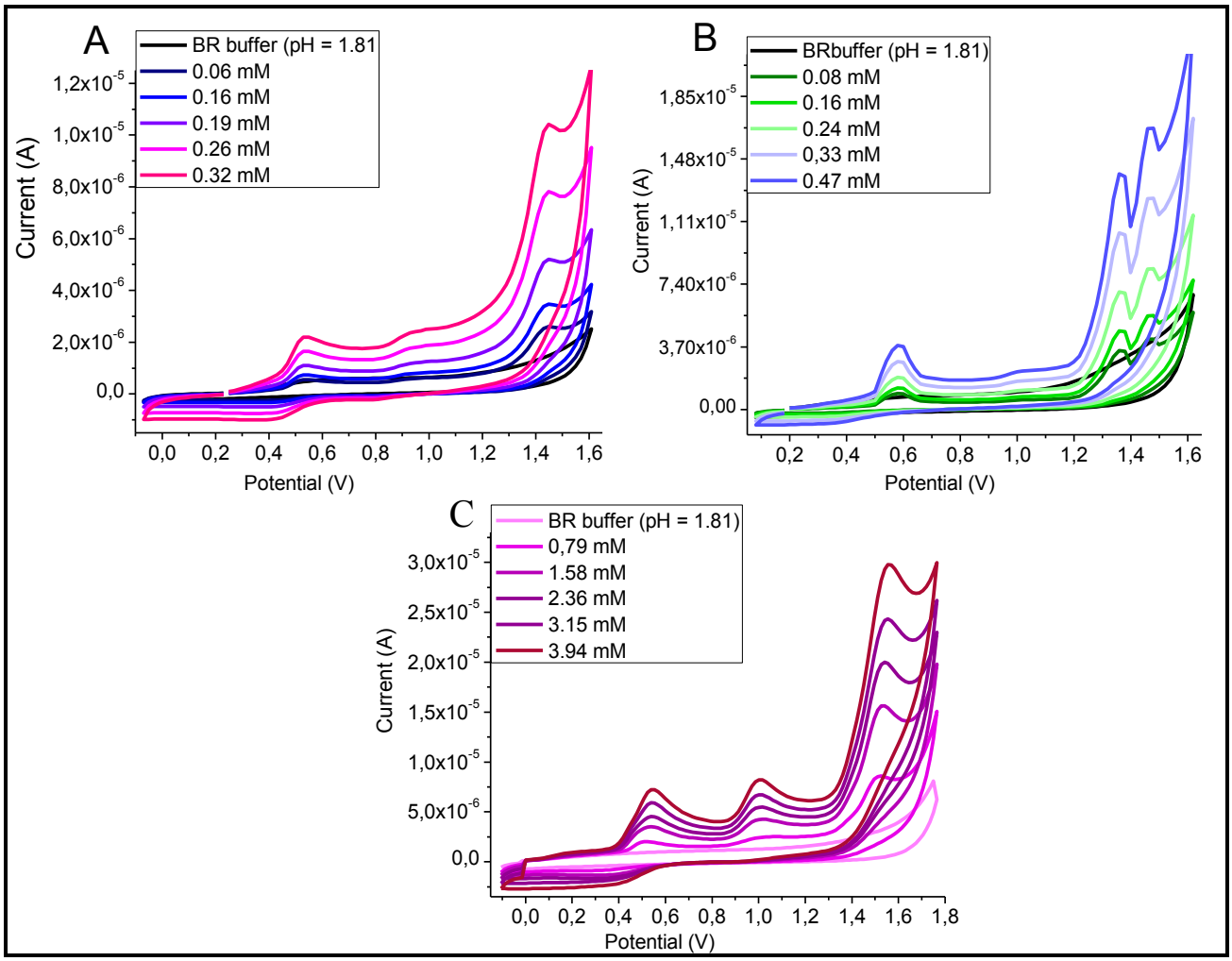


Fig. 2 Cyclic voltammograms recorded for determination of ION (A), DTZA (B) and IOP (C) in mixture of BR buffer at pH 1.81/methanol (9/1, v/v) at GCE (vs. Ag|AgCl||$KCl_{(sat)}$).

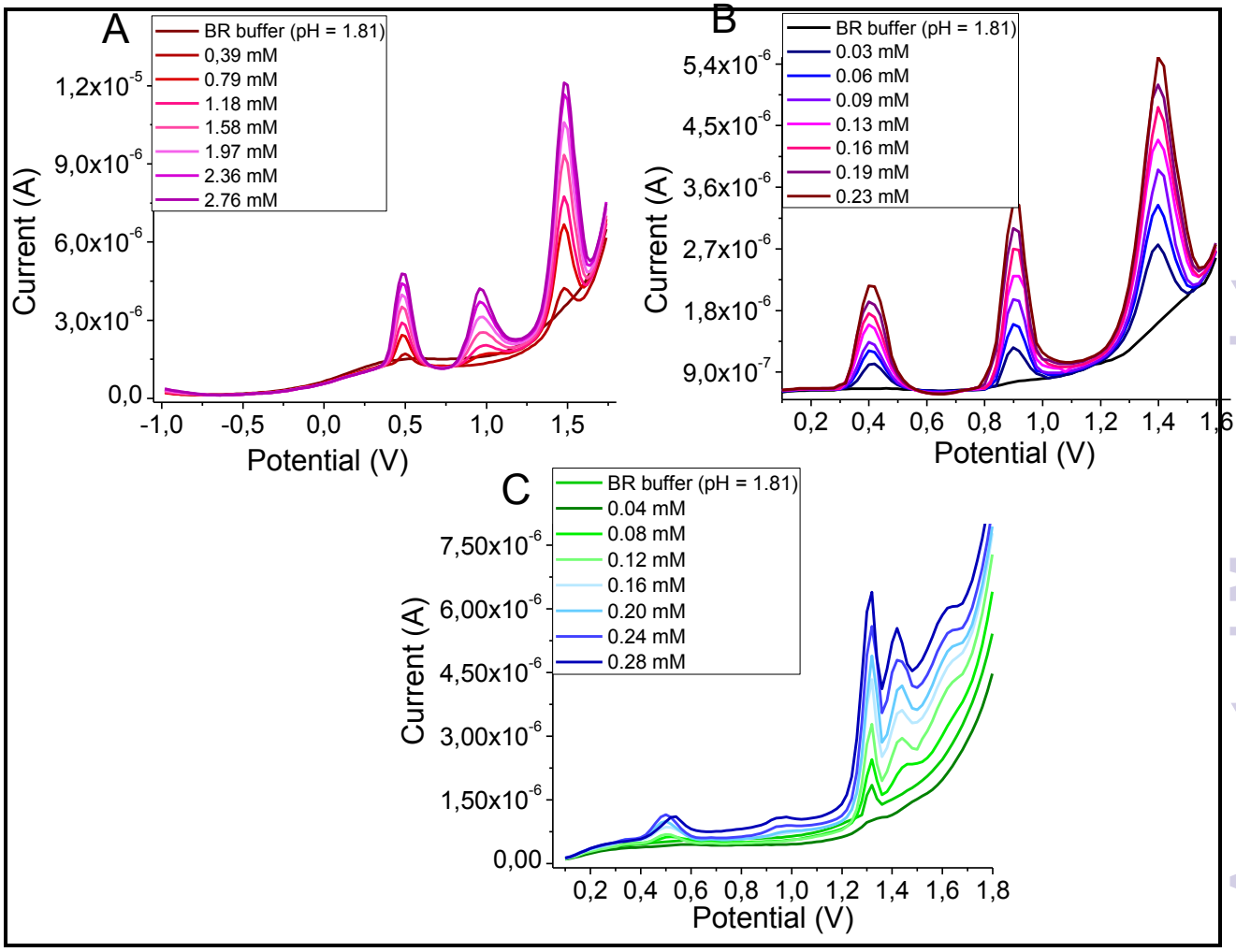


Fig. 3 DPV voltammograms recorded for determination of ION (A), IOP (B) and DTZA (C) in mixture of BR buffer at pH 1.81/methanol (9/1, v/v) at GCE (vs. Ag|AgCl||$KCl_{(sat)}$).

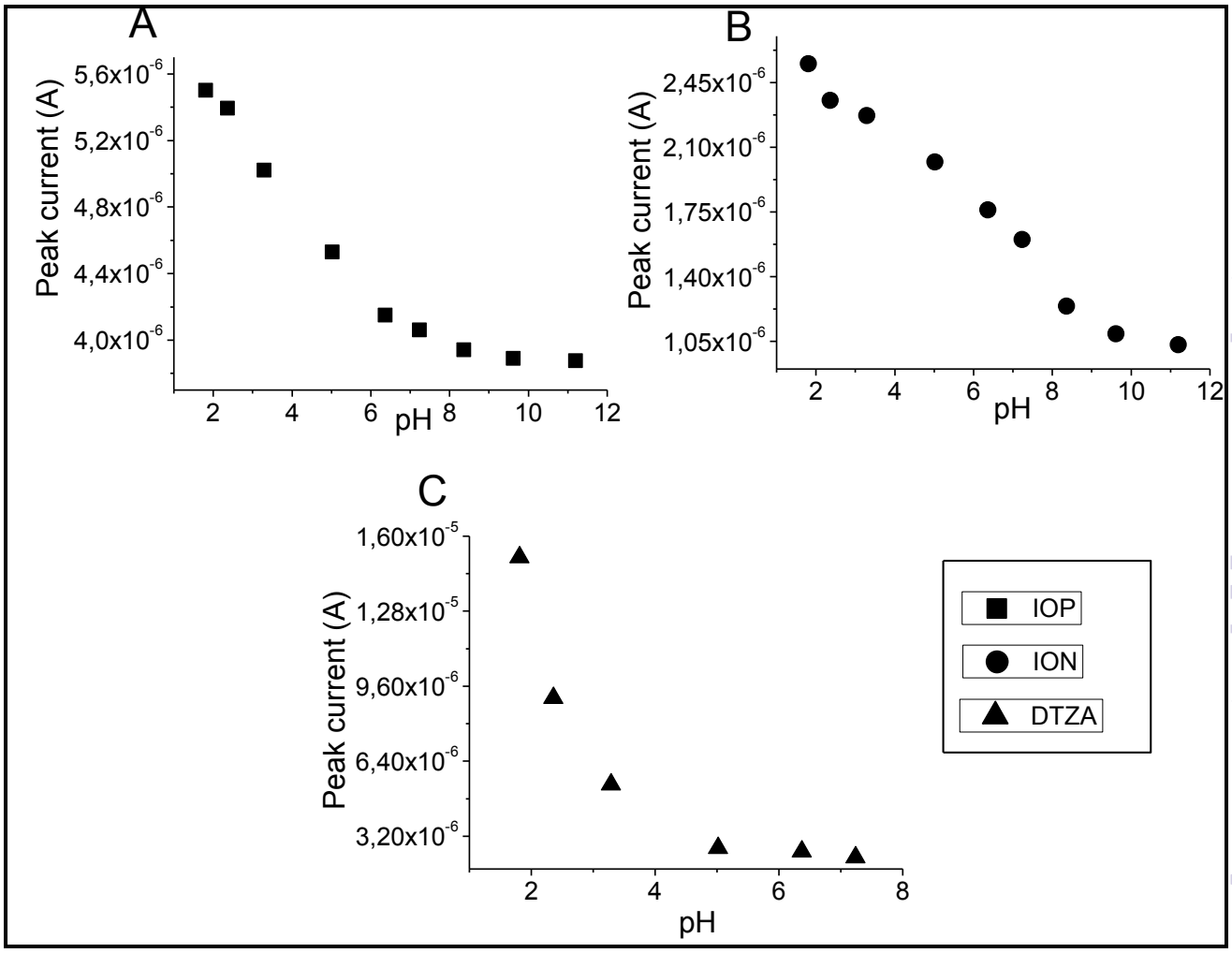


Fig. 4 Plot of oxidative peak current response versus pH of BR buffer containing 3.9 x 10^{-5} M IOP (A), 6.0 x 10^{-6} M of ION (B), 4.0 x 10^{-6} M of DTZA (C) at GCE (vs. Ag|AgCl||$KCl_{(sat)}$).

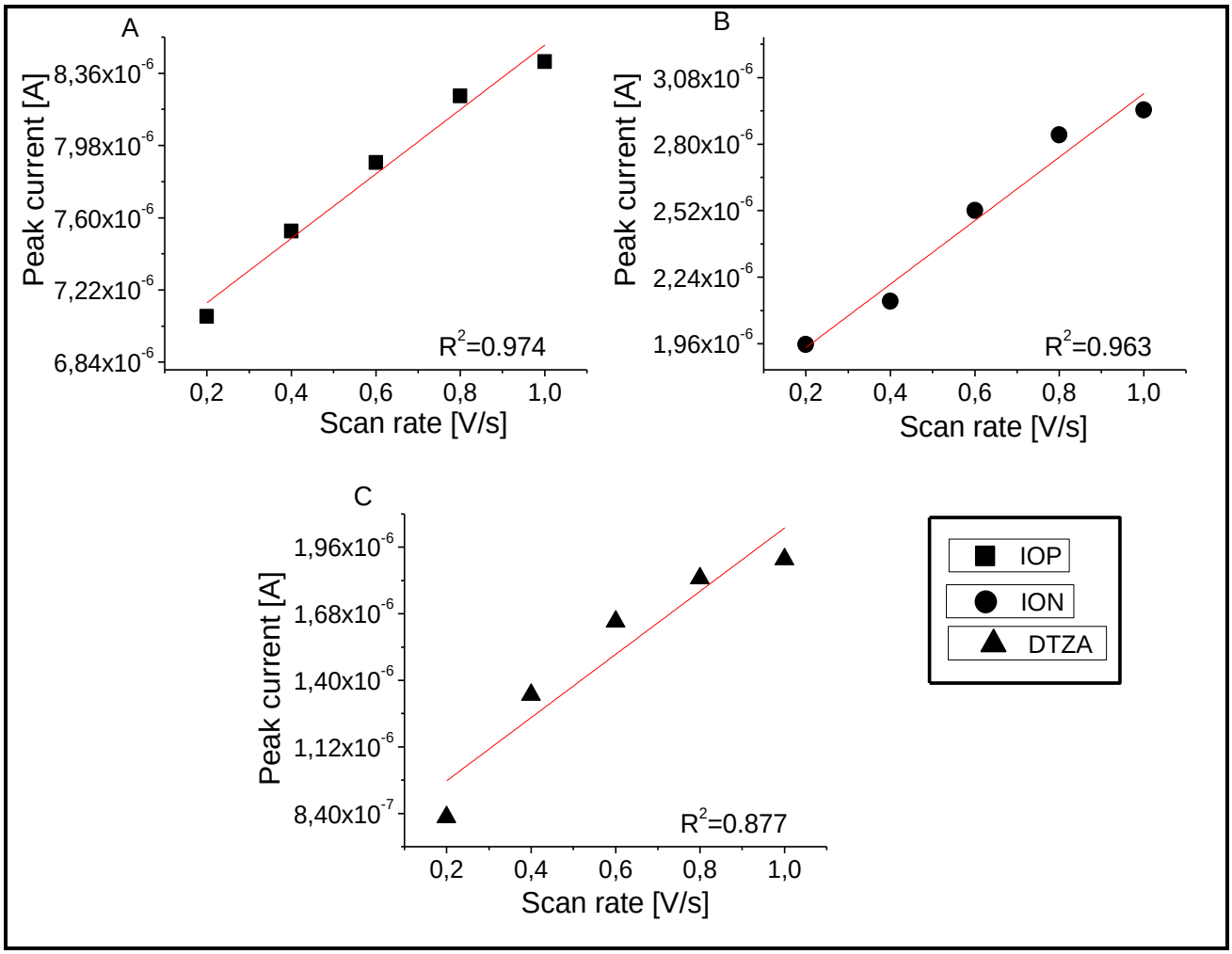


Fig. 5 Plot of oxidative peak current versus potential scan rate of 1.18 x 10^{-4} M of IOP (A), 0.6 x 10^{-4} M of ION (B) and 0.8 x 10^{-5} M of DTZA (C) in BR buffer (pH = 1.81) at GCE (vs. Ag|AgCl||$KCl_{(sat)}$).

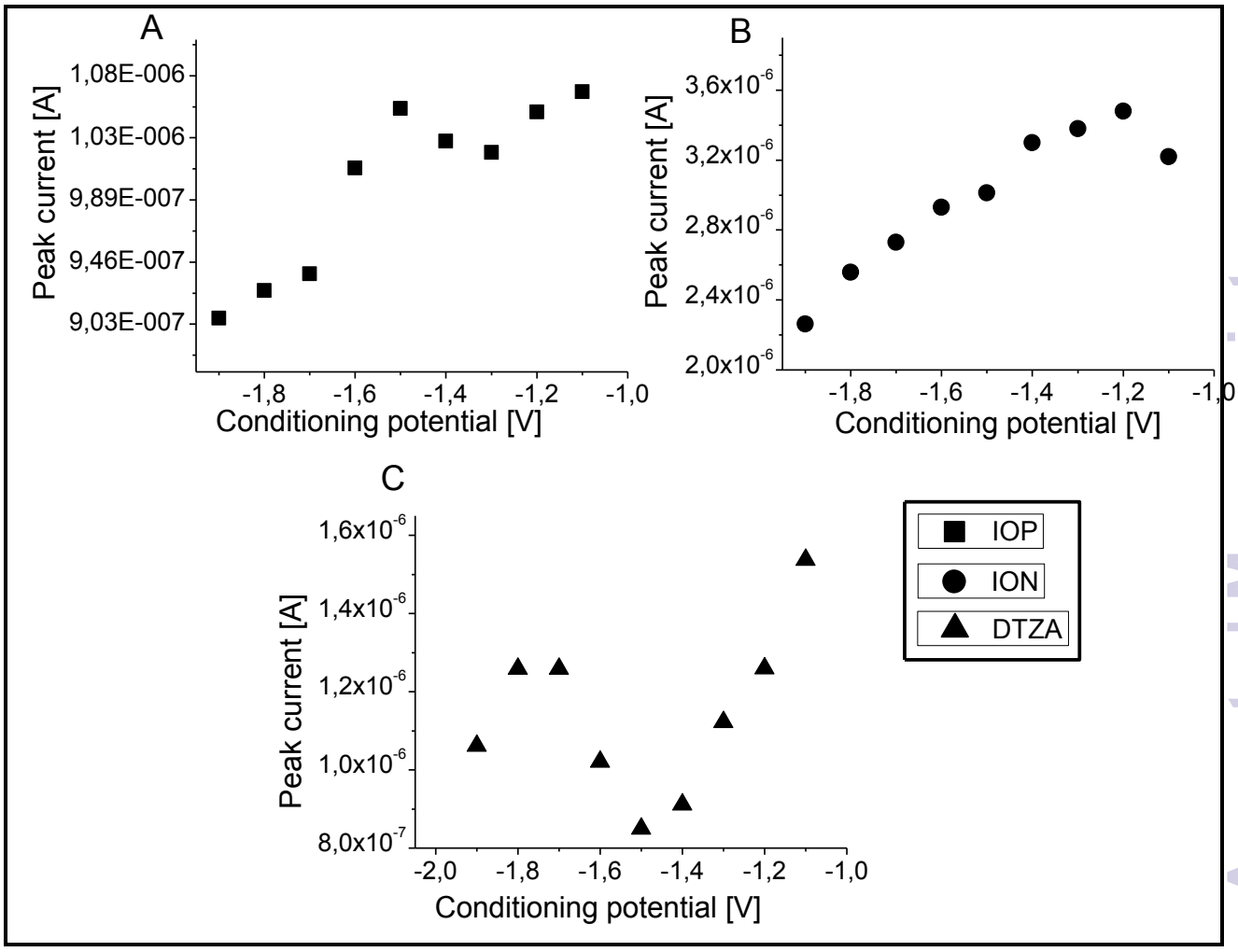


Fig. 6 Plot of oxidative peak current response versus conditioning potential of 3.9 x 10^{-5} M IOP (A), 6.0 x 10^{-6} M of ION (B), 4.0 x 10^{-6} M of DTZA (C)vat GCE (vs. Ag|AgCl||$KCl_{(sat)}$).

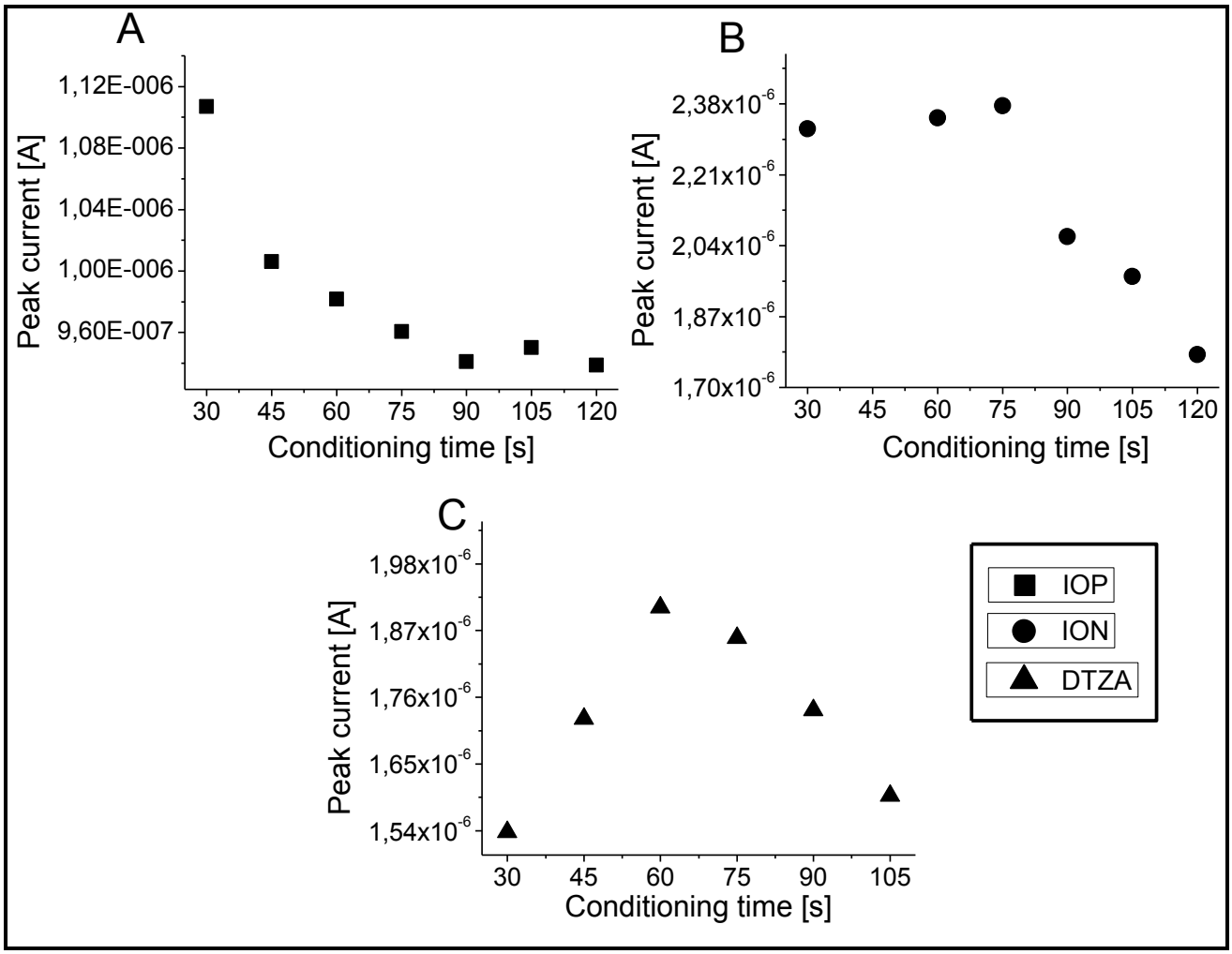


Fig. 7 Plot of oxidative peak current response versus conditioning time of 3.9 x 10^{-5} M IOP (A), 6.0 x 10^{-6} M of ION (B), 4.0 x 10^{-6} M of DTZA (C) in BR buffer (pH = 1.81) at GCE (vs. Ag|AgCl||$KCl_{(sat)}$).

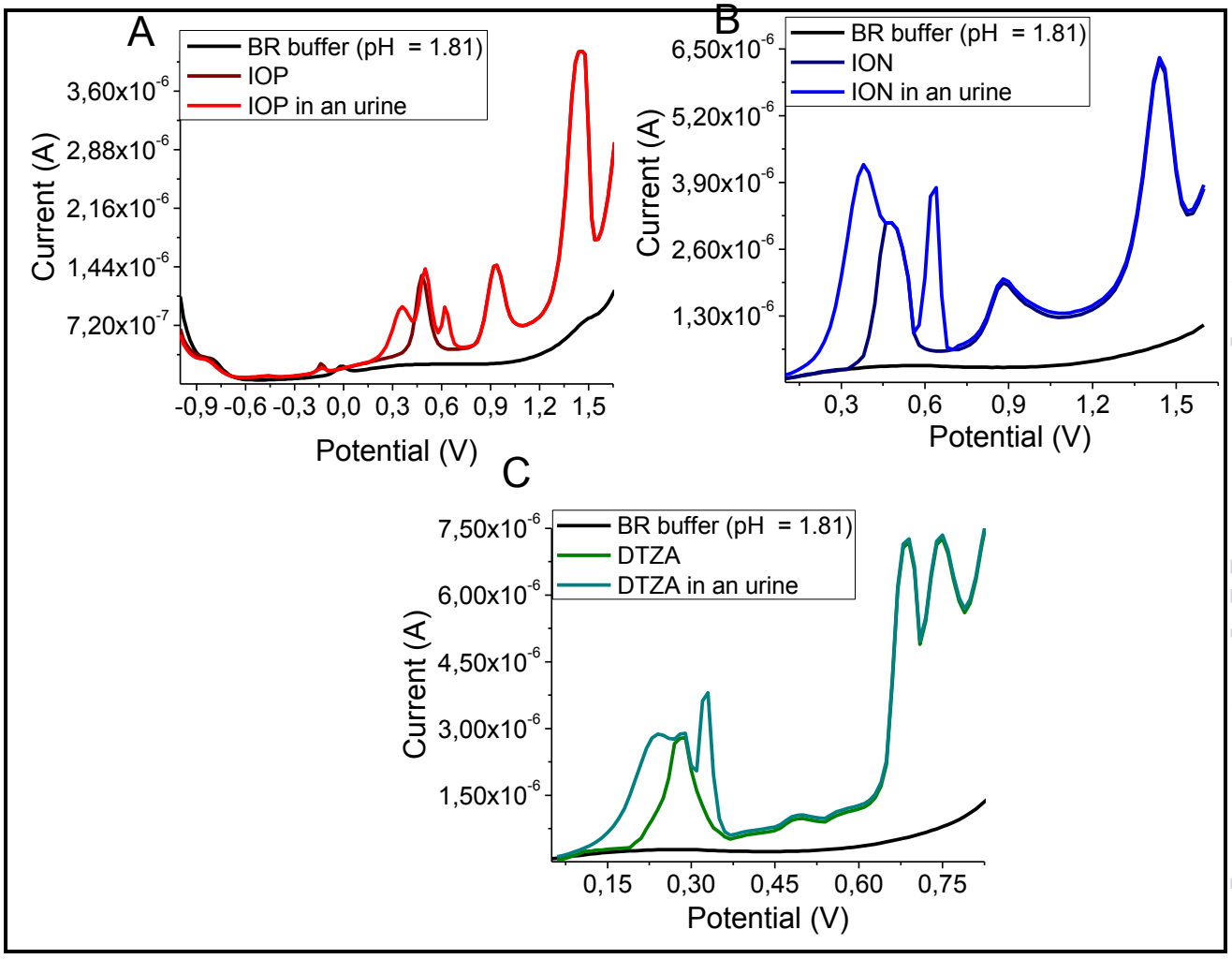


Fig. 8 DPV voltamogramms of ION (A), IOP (B) and DTZA (C) in BR buffer (pH = 1.81) of artificial urine samples after SPE procedure at GCE (vs. Ag|AgCl||$KCl_{(sat)}$).