Article

Determination of Metallic Contaminants in Human Recombinant Erythropoietin (EPO) Formulations by Adsorptive Cathodic Stripping Voltammetry and UV Photo-Decomposition of Samples

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A contaminação de pacientes com insuficiência renal por espécies metálicas pode estar associada a presença destes na medicação empregada durante o tratamento. Dentre os possíveis contaminantes estão metais nefrotóxicos tais como alumínio, cromo e níquel. No presente trabalho, a presença de Al, Cr e Ni como contaminantes em formulações de eritropoetina (EPO) foi investigada através da otimização de métodos analíticos empregando a voltamétria adsortiva de redissolucao catódica (AdCSV) associada a foto-decomposição de amostras com radiação UV. A irradiação UV de amostras acidificadas de EPO pelo período de 2 a 3 horas na presença de H_2O_2 possibilitou a decomposição eficiente da amostra e a determinação dos contaminantes por AdCSV sem a interferência da matriz. O método otimizado foi aplicado na determinação dos metais em formulações comerciais de EPO utilizadas rotineiramente no tratamento da anemia em pacientes com insuficiência renal crônica. Os níveis de contaminação encontrados nas amostras estudadas variaram de 420,2 a 840,5 ng mL^{-1} (RSD 2-5%) para o Al, de 12,0 a 40,0 ng mL^{-1} (RSD 2-6%) para o Cr e de 4,1 a 8,2 ng mL^{-1} (RSD 3-8%) para o Ni. Os resultados obtidos por AdCSV foram comparáveis aos obtidos por espectrometria de absorção atômica com atomização em forno de grafite (GFAAS). A contaminação de formulações EPO com Al, Cr e Ni mostrou ser uma fonte de contaminação importante para pacientes anêmicos com insuficiencia renal crônica.

The contamination of renal failure patients by metals can be associated to the presence of toxic metals in the medication, since they can be absorbed by the organism. Among the possible contaminants, there are nephrotoxic metals such as aluminum, chromium and nickel. In the present work, the presence of Al, Cr and Ni as contaminants in erythropoietin formulations (EPO) was investigated by optimizing the adsorptive cathodic stripping voltammetric (AdCSV) method and the photo-decomposition of samples by UV irradiation. The UV irradiation of acidified EPO samples from 2 to 3 h in the presence of H_2O_2 allowed the efficient sample decomposition and the determination of the contaminants by AdCSV without matrix interferences. The method was applied for the determination of the metals in commercial EPO formulations used routinely in the treatment of anemia in patients undergoing chronic renal failure. The contamination levels determined in the samples ranged from 420.2 to 840.5 ng mL^{-1} (RSD 2-5%) for Al, 12.0 to 40.0 ng mL^{-1} (RSD 2-6%) for Cr and 4.1 to 8.2 ng mL^{-1} (RSD 3-8%). The results obtained by AdCSV were in good agreement with those obtained by the comparative GFAAS measurements. The contamination of EPO formulations with Al, Cr and Ni was shown to be a serious contamination source for anemic patients undergoing chronic renal failure.

Keywords: adsorptive cathodic stripping voltammetry, UV irradiation, photo-decomposition, erythropoietin

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Introduction

Chronical renal failure causes a wide variety of metabolic disturbances which affect almost every organ in the body system. Despite continuous improvement in dialysis treatment, many organ functions are still impaired. Furthermore, multiple drugs are frequently used for the underlying diseases and uremic complications.[1] The possibility of trace metal contamination in various medications and effects of medications on the metabolism of trace metals have been increasingly studied in the last years. In previous works, we showed that the contamination of hemodialysis patients by toxic metals such as Al, As, Zn, Pb, Cd and Tl can be related to their presence in dialysis concentrates or water used for the preparation of dialysis fluids.[2-9] Besides, Lee and co-workers[1] reported that the contamination of hemodialysis patients could be also related to the medication used in the treatment of anemia, such as the renal antianemic human recombinant erythropoietin (EPO).

EPO is a renal antianemic medicament used in the treatment of renal failure or chronic renal disease caused by diminished EPO production in the kidneys. It is a glycoprotein synthetically produced by the recombinant DNA technology, which contains 165 aminoacids in a sequence identical to the endogen human EPO. Its biological activity is identical to the endogen hormone and it is administered to renal failure anemic patients normally by intravenous injection of a 50 IU/kg dose, three times a week.[10] In the pharmaceutical market, formulations are commercialized as lyophilized powder or intravenous solution in the concentrations 1000 IU, 2000 IU, 3000 IU, 4000 IU or 10000 IU of recombinant EPO. Furthermore, non-active components, such as glycine, human albumin, sodium phosphates and sodium chloride are also present.[11]

The toxicological effects of inorganic contaminants vary among the species. It is well known that the aluminium accumulation in the body is related to neurological disorders and damage of osteous structure of patients.[10,12] Chromium is considered an essential trace element. However, high levels of chromium in the body can lead to increasing vascular damage in hemorragy cases and cerebral trombosis.[12] Nickel, at small concentrations, is considered an important element in the human body as well. However, at elevated levels, it is very toxic and can lead to intestinal irritation, neurological disorders, cardiovascular alterations, and allergic reactions, such as dermatitis, chronic rhinitis and asthma. Furthermore, high levels of nickel in the human body can bring serious consequences leading to necroses and lung cancer.[12]

The contamination of renal failure patients by toxic metals can be strongly related to the presence of elements as contaminants in the medications used in the treatment, since they can be absorbed by the patient organism. The problem is aggravated by the reduced capacity of these patients in eliminating the toxic species from the body. The level of contamination in theses cases depends strongly on the quality of the medication administered during the treatment. Therefore, the existence of analytical methods, which allow the quality control of medicaments used in the treatment of chronic renal failure patients, is relevant to the clinical area and should be systematically studied.

The determination of metals by stripping voltammetry is very sensitive and it is well described in the literature.[13,14] However, in complex matrices, such as EPO formulations, metallic species are normally bound to the organic components of the matrix.[15] The UV irradiation for the photo-decomposition of samples is often employed in combination with the voltammetric determination of metals. Since the method normally uses small amounts of reagents (few µL of acids and H_2O_2) for the photo-decomposition, it eliminates the matrix interference without contaminating the sample before the analysis.[16]

This paper describes the determination of some nephrotoxic metals, such as Al, Cr and Ni, in commercial formulations of EPO by using AdSV methods and UV photo-decomposition of the samples. The methods are based on the AdSV determination of Al in the presence of solochrome violet RS (SVRS), Cr in the presence of diethylenetriaminepentaacetic acid (DTPA) and nickel in the presence of dimethylglyoxime (DMG) as organic ligands. The metals were determined in EPO samples after optimization of a pretreatment step by using UV irradiation in the presence of H_2SO_4 and H_2O_2. The method (UV irradiation + AdCSV determination) allowed the rapid and sensitive determination of Al, Cr and Ni at ng mL^{-1} levels in different EPO formulations used in the treatment of anemic patients undergoing chronic renal failure.

Experimental

Apparatus

Voltammetric measurements were performed using a Metrohm 693 VA Processor in combination with a 694 VA Stand (all from Metrohm, Herisau, Switzerland). The three-electrode configuration was consisted of the hanging mercury drop electrode (HMDE) as working electrode, an Ag/AgCl (3 mol L^{-1} KCl) reference electrode and a platinum wire as auxiliary electrode. The surface area of HMDE was 0.6 mm^2. In differential pulse stripping voltammetric (DPCSV) measurements, scans were performed with a

pulse amplitude of −50 mV, a pulse duration of 40 ms and a scan rate of 20 mV s^{-1}. Scans were also preceded by stirred deposition (2000 rpm) at the HMDE.

Graphite furnace atomic absorption spectrophotometric (GFAAS) comparative measurements of Al, Cr and Ni were performed using an AAS spectrophotometer ZEEnit 600 (Analytik Jena, Jena, Germany) with Zeeman background correction. The experimental conditions for the determinations of Al, Cr and Ni are described in Table 1.

UV photo-decomposition experiments were performed using a Metrohm UV digestor (Model 705, Metrohm, Herisau) with a high-pressure mercury lamp of 500W. Comparative UV photo-decomposition experiments were performed in a home-made UV digestor with a high-pressure mercury lamp of 400 W used for public illumination, as described elsewhere.[15] All UV photo-decomposition experiments were performed at 85 ± 3 °C.

Table 1. Experimental conditions used in the determination of Al, Cr and Ni by GFAAS

Metal	λ (nm)	Bandwidth (nm)	Current (mA)	Sample volume (μL)
Al	309.3	0.8	6.0	20
Cr	357.9	0.8	4.0	20
Ni	232.0	0.2	5.0	20

Temperature programs

	Drying		Pyrolysis		Atomization	
	Temp. (°C)	time (s)	Temp. (°C)	time (s)	Temp. (°C)	time (s)
Al	90 / 105 / 110	34 / 25 / 12.5	1300	14.8	2450	4.0
Cr	90 / 105 / 110	34 / 25 / 12.5	950	13.4	2450	6.2
Ni	90 / 105 / 110	34 / 25 / 12.5	1100	14.0	2350	6.0

Reagents and solutions

All chemicals were of analytical grade purity. Water was purified by a Milli-Q Ultra Pure Water System (Millipore, Bedford, MA, USA). All chemicals were of analytical-reagent grade. Solochrome violet RS (SVRS) and diethylenetriaminepentaacetic acid (DTPA) were obtained from Acros Organics (Morris Plains, NJ, USA). 30% (m/v) H_2O_2, 37% (m/v) hydrochloric acid, 98% (m/v) sulfuric acid and dimethylglyoxime (DMG) were obtained from Merck (Darmstadt, Germany). All aqueous solutions were prepared with distilled and deionized water, which was further purified by a Milli-Q high purity water device. Metal stock solutions containing 1.00 ± 0.02 g L^{-1} in 0.5 mol L^{-1} HNO$_3$ were prepared from Al(NO$_3$)$_3$·9H$_2$O, Cr(NO$_3$)$_3$·9H$_2$O

and Ni(NO$_3$)$_2$ (Merck, Darmstadt, Germany). The Al, Cr and Ni stock solutions were standardized by complexometric titration with EDTA (Merck, Darmstadt, Germany).[17,18] 1 mg L^{-1} working stock solutions of Ni(II) and Cr(VI) were obtained by suitable dilutions of the 1000 mg L^{-1} stock ones. The 1 mg L^{-1} working stock solution of Al(III) was obtained by the suitable dilution of the 1000 mg L^{-1} stock solution in a 2 mmol L^{-1} solution of SVRS. The pH was adjusted to pH 4.6 by the addition of acetate buffer and the mixture Al-SVRS was heated for 10 min at 40 °C for the completion of the reaction. The acetate buffer pH 4.6 was prepared by a mixture of 5.5 mL of 2 mol L^{-1} acetic acid and 3.7 mL of 1 mol L^{-1} ammonia in a 50 mL volumetric flask. The ammonia buffer pH 9.5 was prepared by a mixture of 10.6 mL of 1 mol L^{-1} hydrochloric acid and 22.5 mL of 2 mol L^{-1} ammonia in a 100 mL volumetric flask. The pH value of all the buffer solutions was adjusted by adding 1 mol L^{-1} NaOH or 1 mol L^{-1} HCl. The DTPA supporting electrolyte was composed of 0.05 mol L^{-1} DTPA, 2.5 mol L^{-1} sodium nitrate and 0.2 mol L^{-1} sodium acetate. A 0.1 mol L^{-1} dimethylglyoxime solution was prepared by dissolution of 0.2900 g in 25 mL of ethanol.

Samples

Commercial EPO formulations used in this work were the following: Eritromax® (Blausiegel, Cotia, SP, Brazil) injectable solution containing 2000, 4000 and 10000 IU of EPO (excipients: sodium chloride, monobasic sodium fosfate and dibasic sodium fosfate); Alfaepoetina® (Blausiegel, Cotia, SP, Brazil) injectable solution containing 4000 IU of EPO (excipients: human albumin, sodium chloride, sodium citrate and citric acid); Hemax-Eritron® (Aché, Guarulhos, SP, Brazil) lyophilized powder containing 4000 IU of EPO (excipients: human albumin, mannitol, sodium chloride, monobasic sodium fosfate and dibasic sodium fosfate).

UV irradiation of samples analytical procedure

The efficiency of the UV irradiation for the EPO sample decomposition was investigated in acidic medium of H_2SO_4 and in the presence of H_2O_2 for the generation of hydroxyl radicals. The following experimental parameters were investigated in the optimization step using irradiation time (0-4 h) and volume used of 30% (m/v) H_2O_2 solution. All irradiation experiments were carried out using a final sample volume of 10 mL (0.5 mL EPO + 9.5 mL water). The samples were added of H_2SO_4 and H_2O_2 in the beginning of UV irradiation. During the irradiation process, the temperature of the sample solution was maintained

at 85 ± 3 °C. After the irradiation, sample solutions were cooled down in a water bath for the subsequent voltammetric analysis of Al, Cr and Ni by AdCSV.

Voltammetric determination of Al, Cr and Ni in EPO samples

For the Al AdCSV determination, 500 μL of EPO sample was added to 9.5 mL water, followed by the addition of 500 μL acetate buffer (pH 4.6) and 250 μL of 2 mmol L⁻¹ SVRS. Due to the low formation rate of the Al-SVRS, the solution was heated for 10 min at 40 °C in a thermostathized water bath before each AdCSV determination. The Al-SVRS was then deposited at HMDE by applying a potential of −100 mV in the beginning, scaning from −100 to −800 mV with a scan rate of 40 mV s⁻¹. The Al concentration was determined by standard additions ($n = 3$) of Al-SVRS complex 1 mg L⁻¹ stock solution.

For the Cr AdCSV determination, 500 μL of EPO sample was added to 9.5 mL water, followed by the addition of 2.5 mL of a supporting electrolyte composed of 0.05 mol L⁻¹ DTPA, 2.5 mol L⁻¹ sodium nitrate and 0.2 mol L⁻¹ sodium acetate. The final solution pH was adjusted to 6.2 with a 25% (m/v) NaOH solution. The Cr-DTPA complex was then deposited at HMDE by applying a potential of −1000 mV in the beginning, scaning from −1000 to −1450 mV with a scan rate of 20 mV s⁻¹. The Cr concentration was determined by standard additions ($n = 3$) of 1 mg L⁻¹ Cr stock solution.

For the Ni AdCSV determination, 500 μL of EPO sample was added to 9.5 mL water, followed by the addition of 500 μL NH₃/NH₄Cl buffer (1 mol L⁻¹ HCl + 2 mol L⁻¹ NH₃) and 100 μL of 0.1 mol L⁻¹ dimethylglyoxime. The final solution pH was adjusted to 9.5 with a 25% (m/v) NaOH solution. The Ni-DMG complex was then deposited at HMDE by applying a potential of −700 mV for 60 s and the potential was scanned from −700 to −1300 mV. The scan rate was 20 mV s⁻¹. The Ni concentration was determined by standard additions ($n = 3$) of 1 mg L⁻¹ Ni stock solution.

Results and Discussion

Determination of Al, Cr and Ni by AdCSV in EPO samples

The determination of Al, Cr and Ni by AdCSV has already been described in the literature.[13,19-26] It is also well known that AdCSV measurements have seriously drawbacks caused by interferences of organic compounds present in samples of complex matrices. In this work, we observed that the determination of Al, Cr, and Ni by AdCSV directly in diluted (1:10) EPO samples was not possible. Considering that EPO structure contains a sequence of 165 aminoacids,[11] the strong adsorption of organics at HMDE should be firstly eliminated in order to allow the adsoraptive accumulation of metallic complexes. Furthermore, the metallic species should be in a "free" or labile form in order to bind the complexes Al-SVRS, Cr-DTPA and Ni-DMG for the AdCSV measurements. The voltammograms in Figure 1 show the influence of EPO matrix on the AdCSV behavior of Al, Cr and Ni. The absence of voltammetric signals as well as the formation of high amounts of foam in the voltammetric cell during the dearation process of solutions evidenced the necessity of a sample decomposition step prior to AdCSV determinations. Even though a small voltammetric signal could be observed for Ni-DMG complex in diluted (1:10) EPO samples (Figure 1C), it was much lower than that obtained for Ni-DMG in pure supporting electrolyte.

Considering the interferences observed for EPO samples on the AdCSV determinations showed in Figure 1, we optimized a decomposition step by using UV irradiation for the photo-decomposition of organic compounds in the sample.

Optimization of the UV photo-decomposition of EPO samples

It is well known that the UV photo-decomposition of samples takes place through free radical reactions involving the hydroxyl radicals (•OH).[16,27,28] In order to increase the •OH concentration in solutions, the repetitive addition of H₂O₂ during the irradiation process is normally employed. Furthermore, the photo-decomposition of aqueous samples by action of •OH radicals is accelerated when carried out at about 90 °C, since a higher concentration of •OH is formed in this medium.[16,19,20,27]

Since the complete decomposition of the EPO sample was shown to be necessary (Figure 1), the parameter irradiation time and H₂O₂ concentration were firstly optimized for 1:10 diluted EPO samples acidified with 20 μL of concentrated H₂SO₄. Firstly, it could be observed that the irradiation of spiked EPO samples from 0.5 to 6 h without any addition of H₂O₂ was not sufficient for the decomposition of samples prior to AdCSV determinations. Thus, we decided to add 30% (m/v) H₂O₂ to all the samples in the beginning of the irradiation processes, which ranged from 0.5 to 6 h. It was observed that the addition of H₂O₂ increased significantly the decomposition of EPO samples. Besides, the form of the voltammograms obtained for the Al-SVRS, Cr-DTPA and Ni-DMG complexes changed, and recovery values for Al, Cr and Ni added to EPO samples increased as well. The best recovery values (92-103%) in spiked EPO samples were obtained by using the conditions
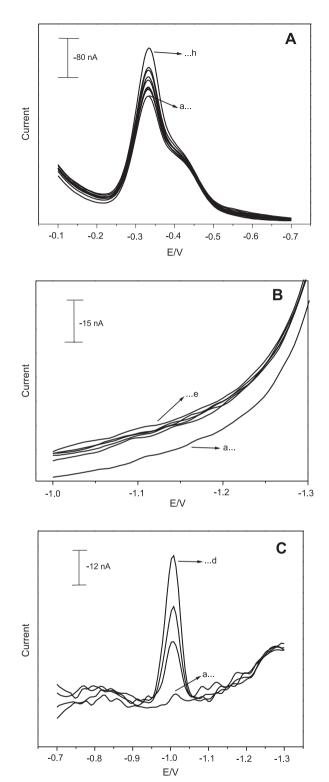
Figure 1. AdCSV behavior of (A) Al, (B) Cr and (C) Ni in (a) EPO samples without UV photo-decomposition. (A): standard additions of Al from (b) 10 μg L^{-1} to (h) 80 μg L^{-1}; sample solution: 1 mL EPO in 10 mL of supporting electrolyte containing 0.05 mmol L-1 SVRS and 0.1 mol L^{-1} acetate buffer (pH 4.6). (B): standard additions of Cr from (b) 1 μg L^{-1} to (e) 5 μg L^{-1}; sample solution: 1 mL EPO in 10 mL of supporting electrolyte containing 0.01 mol L^{-1} DTPA, 0.5 mol L^{-1} NaNO$_3$ and 0.04 mol L^{-1} sodium acetate (pH 6.2). (C): standard additions of Ni from (b) 1 μg L^{-1} to (d) 3 μg L^{-1}; sample solution: 1 mL EPO in 10 mL of supporting electrolyte containing 1 mmol L^{-1} DMG and 0.1 mol L^{-1} ammonium buffer (pH 9.5).

described in Table 2. The results obtained in the recovey experiments for Al, Cr and Ni under optimized conditions are also summarized in Table 2. The voltammograms in Figure 2 show the AdCSV peaks obtained for Al, Cr and Ni in spiked EPO samples submitted to optimized UV irradiation conditions described in Table 2.

Table 2. Recovery experiments (*n* = 3) for Al, Cr and Ni added to EPO samples (1:10 diluted) submitted to UV irradiation (85 ± 3°C)

Metal	Metal added to EPO sample (ng mL^{-1})	Metal recovery (%)	UV irradiation conditions
Al	100	98 ± 3	2 h irradiation under addition of 50 μL H$_2$O$_2$ 30% and 20 μL H$_2$SO$_4$ 98%
	200	100 ± 2	
	300	95 ± 3	
Cr	20	102 ± 2	3 h irradiation under addition of 50 μL H$_2$O$_2$ 30% and 20 μL H$_2$SO$_4$ 98% (repeated addition after 1.5 h irradiation)
	40	103 ± 3	
	80	98 ± 3	
Ni	20	93 ± 2	2 h irradiation under addition of 50 μL H$_2$O$_2$ 30% and 20 μL H$_2$SO$_4$ 98%
	40	92 ± 3	
	80	95 ± 3	

The power of UV radiation source has also been shown to have a strong influence on the decomposition efficiency of samples with high organic content.[16] In order to investigate the influence of this parameter, we submitted the spiked EPO samples to the UV irradiation process in a home-made UV digestor, which contained a high-pressure mercuy lamp of 400 W routinely used for public illumination.[15] As it can be seen in Table 3, lower recovey values (85-96%) were obtained for Al, Cr and Ni in spiked EPO samples irradiated under the optimized conditions (Table 2) by using a 400 W radiation source. The results in Table 3 show that the Hg lamp power influences the decomposition efficiency of diluted EPO samples. However, it is possible to use a non-commercial device for decomposition of the samples if the irradiation time is increased to 4 h, where recovery values ranging from 93 to 99% were obtained for Al, Cr and Ni under the same conditions described in Table 2. Similar results have already been obtained in the photo-decomposition of undiluted urine samples by using the same home-made UV irradiation system described elsewhere.[15]

Interferences on the determination of Al, Cr and Ni by AdCSV in decomposed EPO samples

The influence of some metallic ions on the AdCSV determination of Al, Cr and Ni after UV photo-decomposition was studied in a subsequent step. Possible

interferences included other electroactive elements with a reduction potential in the region where the complexes Al-SVRS, Cr-DTPA and Ni-DMG are deposited and stripped
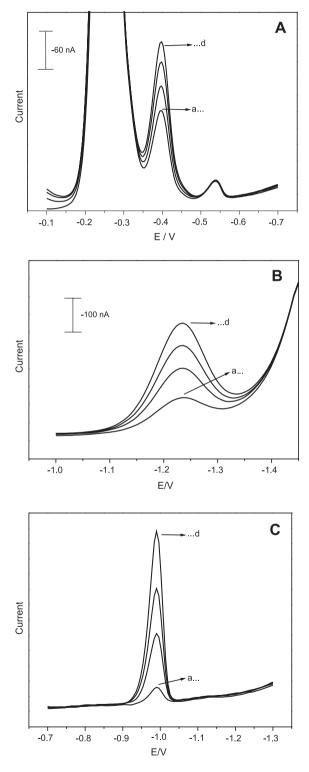

Figure 2. AdCSV behavior of (A) Al (B) Cr and **(C)** Ni in EPO samples after the optimized UV photo-decomposition step. (a) sample, (b) sample + 10 µg L^{-1} Al / 1 µg L^{-1} Cr / 1 µg L^{-1} Ni, (c) sample + 20 µg L^{-1} Al / 2 µg L^{-1} Cr / 2 µg L^{-1} Ni and (d) sample + 30 µg L^{-1} Al / 3 µg L^{-1} Cr / 3 µg L^{-1} Ni; other experimental conditions as described in Figure 1 and Table 2.

Table 3. Recovery experiments (*n* = 3) of Al, Cr and Ni after 3 h UV irradiation (85 ±3°C) for the comparative study of sample digestion efficiency using different UV irradiation systems; UV irradiation conditions as described in Table 2

Metal	Metal added to EPO sample (ng mL^{-1})	Recovered (%)	
		UV digestor 1[a]	UV digestor 2[b]
Al	100	92 ± 3	88 ± 3
	200	93 ± 2	85 ± 3
Cr	20	94 ± 5	96 ± 4
	40	93 ± 5	87 ± 3
Ni	20	92 ± 3	86 ± 3
	40	93 ± 3	91 ± 5

[a] Mercury high-pressure lamp with 500 W power (Metrohm UV Digestor);
[b] Mercury high-pressure lamp with 400 W power (Home-made UV Digestor).[15]

out as well as metallic ions that can also form complexes with SVRS, DTPA and DMG. Among the interferences, we tested the influence of the metallic ions Pb^{II}, Cd^{II}, Cu^{II}, Zn^{II} and Fe^{III}, since they were also shown to be present as contaminants in EPO formulations. Furthermore, Fe^{III} ions bound the complex Fe-SVRS under the same conditions described for Al^{III}, having a peak potential close to −0.55 V (Figure 2A). Zn^{II} ions were determined in this work by ASV using acetate buffer as supporting electrolyte[13,29] and their concentrations in decomposed EPO samples were found to be between 1.60 and 5.50 µg mL^{-1}. Thus, Zn^{II} was considered the main inorganic contaminant of EPO samples and a possible interfering species. Pb^{II}, Cd^{II} and Cu^{II} ions were also found to be present as contaminants in EPO samples at concentrations ranging from 0.08 to 8.8 ng mL^{-1} and, therefore, they were also considered possible interferents on the AdCSV. The metallic ions Co^{II}, Tl^{I}, Mn^{II} and Sb^{III} as well as the anions CrO$_4^{2-}$, SeO$_3^{2-}$, and AsO$_3^{2-}$ are electroactive species that were also investigated. However, they had no interference on the AdCSV for Al, Cr and Ni in decomposed EPO samples.

Among the possible contaminants of EPO samples, it can be seen in Figure 3 that only Pb^{II} is considered an interferent on the determination of Cr by AdCSV. A decrease of *ca.* 45% in the Cr-DTPA peak was observed for a small excess of Pb^{II} ions in relation to Cr^{III} ions in the voltammetric cell containing UV decomposed EPO samples. However, the determination of Cr-DTPA in the presence of Pb^{II} ions can be carried out by the standard addition method, which eliminates the Pb^{II} interference. The interference of Zn^{II} ions on the Al determination by AdCSV was shown to be serious only if the determination is performed by the AdCSV method using 1,2-dihydroxyanthraquinone-3-sulfonicacid (DASA) as complexing agent, as already observed in previous works.[7,30]
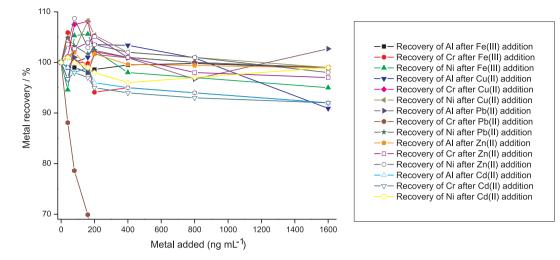
Figure 3. Recovery experiments for Al (40 µg L^{-1}), Cr (3 µg L^{-1}) and Ni (3 µg L^{-1}) in the presence of some metallic ions as interfering species in irradiated EPO samples (n = 3); UV irradiation conditions as described in Table 2.

Figures of merit

Linear ranges (r = 0.999) for the determination of metals by AdCSV in the voltammetric cell containing 10 mL of 1:10 diluted decomposed EPO samples ranged from 0 to 200 µg L^{-1} for Al (r = 0.999), 0 to 20 µg L^{-1} for Cr (r = 0.997) and 0 to 15 µg L^{-1} for Ni (r = 0.998) using the HMDE as working electrode and a deposition time of 60 s for Ni. The linear range can be expanded up to 100 µg L^{-1} for Cr (r = 0.995) if the static mercury drop electrode (SMDE) is used as working electrode.

The following limits of detection for the metals in EPO samples decomposed by UV irradiation were calculated from the (3σ) standard deviation associated with the mean of 10 measurements of the background current divided by the slope of the respective calibration function:[31] 19.0 ng mL^{-1} for Al, 2.0 ng mL^{-1} for Cr and 1.2 ng mL^{-1} for Ni (deposition time of 60 s at the HMDE). The following limits of quantification for the metals in EPO samples decomposed by UV irradiation were calculated from the (10σ) standard deviation associated with the mean of 10 measurements of the background current divided by the slope of the respective calibration function:[31] 63.0 ng mL^{-1} for Al, 7.0 ng mL^{-1} for Cr and 4.0 ng mL^{-1} for Ni (deposition time of 60 s at the HMDE).

Relative standard deviations calculated for five measurements of the metals in the voltammetric cell containing 10 mL of 1:10 diluted EPO samples after the UV irradiation step were 2.5% for 25.0 µg L^{-1} Al, 3.5% for 5.0 µg L^{-1} Cr and 3.0% for 5.0 µg L^{-1} Ni.

Analytical application

The determination of nephrotoxic metals in fluids and medicaments used in the treatment of chronic renal failure is very important, since they are common contaminats of pharmaceutical formulations and inorganic salts added as excipients. The method described above, which combines the decomposition of EPO samples by UV irradiation and the sensitive AdCSV determination of metals, was applied to the determination of Al, Cr and Ni in three different formulations of recombinant human EPO. Twelve different samples of Eritromax® injectable solution, Alfaepoetina® injectable solution and Hemax-Eritron® lyophilized powder were submitted to the UV irradiation step and the metal content was determined by the respective AdCSV method. Table 4 shows the results obtained with three replicate determinations using the standard addition method. According to these results, Al is present in all the commercial EPO formulations in concentrations ranging from 420.2 to 840.5 ng mL^{-1} (RSD 2-5%), which represents a serious contamination source for patients undergoing renal failure. Cr is also present as a contaminant in all the EPO formulations in concentrations ranging from 12.0 to 40.0 ng mL^{-1} (RSD 2-6%). Among the toxic metals studied here, Ni was found to be the minor contaminant in all the EPO formulations, being its concentration ranging from 4.1 to 8.2 ng mL^{-1} (RSD 3-8%). Table 4 also compares the results obtained in all the EPO formulations analyzed by the proposed AdCSV method and the comparative GFAAS method. As can be seen, the results obtained by AdCSV are in good agreement with those obtained by the comparative GFAAS method for Al, Cr and Ni in some analyzed samples.

Conclusions

The proposed method provided a sensitive voltammetric determination of Al, Cr and Ni as contaminants of EPO samples used in the treatment of chronic renal failure. The

Table 4. Determination of Al, Cr, and Ni as contaminants in EPO samples by AdCSV and GFAAS after UV irradiation ($85 \pm 3 °C$); UV irradiation conditions as described in Table 2

Sample	Metal concentration (ng mL^{-1})					
	Al		Cr		Ni	
	AdCSV[a]	GFAAS[b]	AdCSV[c]	GFAAS[d]	AdCSV[e]	GFAAS
1	840.0	880.1	12.0	10.5	6.0	< 10.0
2	610.5		40.0	42.8	5.2	< 10.0
3	571.0	548.8	19.0	18.3	4.3	< 10.0
4	662.3	630.5	18.2	17.5	4.1	< 10.0
5	840.5	890.2	21.0		4.1	< 10.0
6	620.2		25.2	27.1	6.3	< 10.0
7	792.5	778.4	23.1		8.0	< 10.0
8	652.4		22.2	20.4	7.2	< 10.0
9	532.0	560.1	27.5		8.2	< 10.0
10	450.3		17.2		7.2	<10.0
11	420.2		15.5	17.2	4.2	< 10.0
12	470.2	450.0	22.3	24.1	5.5	< 10.0

[a] RSD (n = 3): 2-5% ; [b] RSD (n = 3): 3-8% ; [c] RSD (n = 3): 2-6% ; [d] RSD (n = 3): 4-9% ; [e] RSD (n = 3): 3-8%.

developed decomposition step for EPO samples using UV irradiation was shown to be efficient for eliminating the matrix interferences observed on the AdCSV determination. The irradiation of acidified EPO samples from 2 to 3 h in the presence of H_2O_2 allowed the efficient decomposition and the determination of contaminants without matrix interferences. The method was shown to be free of interferences from other metallic species, which can be present as contaminants in the samples. Contamination levels observed for Al, Cr and Ni differed significantly, being Al the main contaminant among the studied toxic metals. The studied nephrotoxic metals were found to be present in all the EPO formulations in concentrations from 4.1 to 840.5 ng mL^{-1}, representing a serious contamination source for anemic patients undergoing chronic renal failure.

Acknowledgments

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