Improved voltammetric methodology for chromium redox speciation in estuarine waters

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Abstract

Chromium is a toxic element naturally present in natural waters whose chemical speciation regulates its cycling, mobility and bioavailability. We present here: 1- an improved analytical method for chromium speciation (Cr(VI) vs Cr(III)) in estuarine samples by catalytic adsorptive cathodic stripping voltammetric (cat-AdCSV) and 2- a study highlighting a significant change of redox speciation during summer and winter. Initial measurements first revealed that surface-active substances (SAS) present in estuarine samples strongly influenced the analytical determination of Cr by partially masking the Cr peak through an increase of the background current. We found that the application of a low negative accumulation potential (-1.65 V) resulted in much better voltammograms compared to those obtained using the usual accumulation potential of -1.0 V . Using humic acid (HA) as a model SAS of natural origin, we show that this negative potential clearly prevents adsorption of SAS on the Hg-electrode surface, which in turns benefits the adsorption of the in-situ formed Cr(III)-DTPA complex and the resulting signal. The optimised method was applied to determine chromium redox speciation and distribution along the 23 km long salinity gradient, well oxygenated, Krka River estuary (Croatia). Cr(VI) was found to be the dominant redox species in both summer and winter, with Cr(III) contribution being lower in summer (up to ~30%, average of ~5%) than in winter (up to ~50%, average of ~30%). In summer, lower concentrations of Cr(VI) were found in the freshwater end-member (2.5 nM) than in the seawater end-member (4-5 nM), while the opposite trend was found in winter. Hexavalent chromium exhibited a non-conservative behaviour along the salinity gradient for both seasons. Chromium predominantly exists in dissolved phase, and contribution of particles reactive Cr(III) was minor.

Keywords: chromium redox speciation, estuary, organic matter, surface active substances

1. Introduction

Chromium (Cr) is a redox-sensitive element which, in natural waters, predominantly exists in two stable oxidation states: trivalent {Cr(III)} and hexavalent {Cr(VI)} [1, 2]. The major interest and concern for chromium redox speciation determination in natural waters (including drinking waters) is driven by the fact that in trivalent form (+3) it is an essential element (at trace levels), whereas hexavalent form (+6) is reported to be toxic for both humans and animals (being a possible human carcinogen and mutagen), as well as for other organisms living in natural waters [3, 4]. Natural sources of chromium are varied, from ore mineral, shales, river suspended matter and soils, particularly fine grain size soils. Anthropogenic sources are mainly from metallurgy, electroplating and leather tanning [5]. Cr(VI) is the major oxidation state in oxygenated waters, whereas Cr(III) predominates in anoxic conditions. Under typical conditions in natural waters, Cr(VI) is highly water soluble and mainly exists in forms of stable oxo-compounds CrO_4^{2}-and $Cr_2O_7^{2-}$, and the complexation with organic and inorganic ligands is thought to be insignificant. In freshwaters, predominant inorganic forms of Cr(III) are hydro complexes ($Cr(OH)^{2+}$, $Cr(OH)_3$), while in the presence of chloride (seawater) it forms hexaaquo complex ($[Cr(H_2O)_6]Cl_3$). Unlike the hexavalent form, Cr(III) has affinity to form complexes with natural or anthropogenic organic substances and to adsorb on suspended particulate matter [6, 7]. The oxidation state primarily depends on the aeration status of the water body. In anaerobic conditions, chromium is reduced to Cr(III) by ferrous oxide at pH above 5.5, and by hydrogen sulphide (H_2S) if pH is below that value [8].

The two major issues related to the redox speciation of chromium are identified as environmental [9] and methodological [10, 11]. The ratio of concentrations of Cr redox species in natural waters is highly variable depending on the specific physicochemical conditions of the water column (pH, redox potential, oxygen concentration, presence of appropriate reducers/oxidizers, photochemical redox transformations, mediators acting as ligands or catalysts). However, some experimental evidences show that their actual ratio could deviate from theoretical predictions [12].

Alike other metals, chromium speciation methodology usually involves the following steps: 1. sampling, 2. preservation/storage, 3. species preconcentration/separation, 4. species detection. Each of these steps can modify the natural speciation distribution; the goal is thus to minimize their influences. The first issue of concern for Cr speciation in natural water is sample storage, i.e. preservation of its original concentration and redox speciation. The typical storage conditions for metals are acidification to $pH < 2$ (if only the dissolved concentration has to be determined), or at natural pH (if speciation is of primary interest). However, this storage scheme is not adequate for Cr. On one hand, at natural pH of ~ 8, Cr(VI) is stable (especially under a CO_2 blanket [13]) but Cr(III) is rapidly (minutes to hours) removed from the solution due to adsorption on the container walls. On the other hand, in acidic conditions, Cr(VI) could be reduced to Cr(III) by the oxidation of organic matter. In addition to these storage issues, the relatively low Cr concentration encountered in natural waters (0.1 - 16 nM in seawater, 0.5 - 100 nM in freshwater) is also presenting an analytical challenge.

As a result, there are only scarce studies [3, 14-16] that describe the behaviour and actual distribution of Cr(III) and Cr(VI) in the aquatic environment. Thus, field studies and laboratory model experiments under well-controlled conditions are of importance to help improving our understanding of chromium behaviour and its environmental impact in natural aquatic systems.

From an analytical point of view, the most used techniques for chromium redox speciation measurements in natural waters are high performance liquid chromatography hyphenated to inductively coupled plasma mass spectrometry (HPLC/ICP-MS) [17] and the catalytic adsorptive cathodic stripping voltammetry (Cat-AdCSV) [10, 18-20]. Despite numerous variations of the latter, Cr speciation still remains a challenging task and there is a need for improvement of existing analytical procedures [20-24].

This work is aiming to: (i) develop an improved Cat-AdCSV procedure for Cr determination in samples having a high concentration of organic matter and surface active substances (SAS) and (ii) use this procedure to determine the distribution and behaviour of Cr redox species along the salinity gradient of an estuary (Krka, Croatia).

2. Study site

Krka River and its estuary are part of National Park Krka, which is situated on the eastern coast of Adriatic Sea (Croatia).The river is characterized by numerous lakes formed by tufa barriers, each finishing with waterfalls. Measured flow in Krka River over the last 50 years range from 5-450 m^3s^{-1}, while in the period from 2001 to 2013, the average annual flow spans from 40-60 m^3s^- [25]. This highly stratified estuary is restricted between the last and largest waterfall (Skradinski buk) and Šibenik Channel, measuring in total of ~23 km. The map of the estuary with marked sampling locations is presented in Fig.1. The Krka River estuary is a typical, highly stratified salt-wedge estuary. Its vertical gradient is characterized by three layers: (1) surface fresh/brackish layer (FWL), (2) freshwater-seawater interface (FSI) and (3) seawater layer (SWL). While FWL flows downstream (seaward), the bottom SWL flows in opposite direction, upstream (landward). The halocline is usually positioned between 1.5 and 3 m, and its "thickness" varies between few cm only to 1 m. Due to numerous tufa barriers preceding the estuary and the absence of significant anthropogenic sources, the terrigenous material, nutrients and trace metal river input [26] are very low.

3. Sampling and storage

Sampling was performed using FEP Nalgene bottles which were previously cleaned with 10% HNO_3 (suprapur) and thoroughly rinsed with MQ water (18.2 MΩ, Millipore, USA). Samples were collected using a van Dorn horizontal acrylic sampler or by using grab sampling with 1 L FEP bottle at 16 sites along the whole estuary (Fig. 1). Three sampling campaigns were conducted: summer 2017 and 2018 and winter 2017. For the summer campaigns (summer 2017/2018), both surface (~0.2 m below the surface) and bottom seawater samples were collected, whereas for the winter campaign, due to logistic difficulties, only surface samples were taken. Samples were filtered either immediately onboard or in the laboratory within few hours by using precleaned (MQ +sample) syringe filters 0.22 µm (cellulose-acetate, Minisart, Sartorius). All samples were stored at natural pH at +4°C until analysis in 125 mL FEP bottles which were previously washed using trace metals clean procedure. For total chromium determination, samples were UV-irradiated at 254 nm directly in the FEP bottle for 24h prior to measurement. Concentrations of Cr(VI) in estuarine samples were always determined within two days of sampling. Repeated analyses on the same filtered samples stored for up to 5 days in the dark and at +4 °C did not show any significant differences (within experimental uncertainty, i.e. 10% [12]). This result indicates that adsorption of Cr(III) on the container walls did not occurr, in contrast to previously reported [10], possibly because fluorinated (FEP) bottles were used in this study. Vertical profiles of physico-chemical parameters (S, T, O_2, pH and Chl-a) were recorded using the EXO2 multiparameter CTD probe (YSI).

4. Equipment and chemicals

Voltammetric measurements were performed using a µAutolabIII (EcoChemie) potentiostat coupled with a three-electrode cell (663 VA Stand, Metrohm) with a static mercury drop (SMDE), Ag|AgCl|sat. NaCl and Pt wire as the working, reference and auxiliary electrodes respectively. A home-made sample-changer, five Cavro XE 1000 syringe pumps and home-made software VoltAA were used in conjunction with the potentiostat allowing fully automated measurements to be performed.

Sodium nitrate (3 M stock solution) was prepared by mixing HNO_3 (*suprapur* Merck) and NaOH (*suprapur* Merck), Diethylenetriaminepentaacetic acid (DTPA; 0.25 M stock solution prepared) was purchased from Fluka (analytical grade) and 2-(N-morpholino)ethanesulfonic acid (MES; 1 M stock solution prepared) was purchased from VWR (ultrapure). Stock solutions of Cr(VI) were prepared by appropriate dissolution of K_2CrO_4 (Fluka). Humic acid (HA) was from Sigma-Aldrich. All laboratory solutions were stored in polyethylene bottles, while all the samples were stored in acid-washed FEP (Nalgene) bottles.

5. Basics of Cat-AdCSV method for Cr analysis

The method for chromium determination in freshwater and seawater is based on the *in-situ* formation and adsorption of the Cr(III)-DTPA complex (from Cr(VI) reduction which exists in the solution at potentials more negative than -0.05 V) during the accumulation step. During the stripping step, this complex is further reduced to Cr(II) which is immediately oxidised back to Cr(III) by nitrate, resulting in an enhancement of the signal due to this catalytic effect [10, 18, 19, 27]. Since the original description of this method [28], numerous authors reported on using AdCSV to measure trace levels of chromium in different matrices and using different working electrodes [10, 18, 21, 22, 29-31]. Even though the method was widely used, its analytical application was limited until the mechanism of reaction, formation, adsorption and electrode reaction of Cr(III)-DTPA complex were fully studied [27, 30]. The critical step of the methodology is that the Cr(III) originally present in the solution slowly forms an electro-inactive complex with DTPA. At room temperature, the kinetics of this complexation is believed to take 30 min, allowing then the sole determination of Cr(VI) (increasing the temperature decreases this time [32]).

In this work, the determination of Cr concentrations was performed in buffered samples (MES, pH 5.5) using the following fully automated procedure: rinsing of the cell between samples by acidified MQ (pH 2, 10 mM HCl), sample exchange, addition of reagents (DTPA, $NaNO_3$) and Cr(VI) standard using syringe burettes. Adequate volume of Cr standard was determined by using predefined sensitivity. Prepared samples were measured ~ 1 h after the addition of reagents when all originally present Cr(III) was transferred into an electroinactive complex. Total Cr is determined after 24h UV-irradiation of the sample at neutral or slightly acidic pH (to convert the existing Cr(III) to Cr(VI)). Cr(III) concentration is calculated as the difference between total Cr and Cr(VI). Typical voltammetric conditions were: 3 min initial purging, accumulation at -1.65 V for 60 s, stripping from -0.95 to -1.35 V using differential pulse mode (2 mV potential step, 0.1 s interval time, 0.040 s pulse time, 10 or 40 mV amplitude).

6. Results and discussion

6.1. Optimization of the analytical procedure

Analytical parameters used for Cr voltammetric determination by various authors [10, 21, 30, 31] are shown in Table 1. Except for the work of Korolczuk [21] who used a very negative deposition potential (-1.7 V) in conjunction with a matrix exchange procedure, all other work applied the usual deposition potential of -1.0 V. Solutions were always buffered at pH ranging from 5 to 6.5, depending on if freshwater or seawater is analysed. We found here that the addition of 5 mM MES buffer at pH 5.5 resulted in a similar sensitivity at all salinities (0 – 38). While this is contrast to Li and Xue [30] who reported a low sensitivity when using MES as a buffer, it agrees with the approach proposed by Korolczuk [33]. Previous studies showed that deviations from the optimized pH may cause decrease in sensitivity [10, 30], but the optimized pH varies from study to study, ranging from 5 to 6.5 (Table 1). In this study pH = 5.5 was used and the sensitivity were found to be adequate for the field study across the salinity range (Fig. S1), so no further tests on the pH was performed.

Initial tests in estuarine samples revealed that the sensitivity and the shape of voltammograms were changing from sample to sample when using a deposition potential of -1.0 V. In contrast, we obtained much better stability and better-shaped peaks when using a more negative deposition potential (e.g. -1.65 V). This is also corroborated by previous studies: a low deposition potential was found optimal by Korolczuk *et. al* [21, 22] when used with a medium exchange procedure and an increase of the Cr peak was also reported at low deposition potentials (-1.8 V) at a vibrating silver amalgam microwire electrode [20], although that potential was not suggested as the optimum one. The same study reported that Cr(VI) determination in samples without UV-irradiation step was not possible due to interference by dissolved organic matter (DOM), although no further study was performed by the authors. Surface active substances (SAS), naturally present in aqueous samples, were identified to have interference in the determination of chromium by AdCSV [23] and were removed using a fumed silica column. It is thus likely that the application of a low deposition potential minimise the interference from SAS, similar to what was observed for Cu complexation studies [34] or for the determination of platinum [35].

Below, we show that the use of a low deposition potential removes SAS interferences from our estuarine samples, without the need of a medium exchange procedure such as that used by Korolczuk *et. al* [21]. Humic acid (HA) was used as a model of natural organic substance, which is common in coastal environment [36]. The concentration of HA was increased up to 1 mg/L, which is equivalent to ~0.5 mg/L dissolved organic carbon (DOC). This DOC concentration is lower than that previously reported for the Krka River estuary (0.8-1.5 mg/L) [25], but humic substances (HS) are not the sole contributor to DOC. Voltammograms were recorded in UV-irradiated seawater spiked with 6 nM Cr(VI) at deposition potentials of -1.0 and -1.65 V (Fig. 2). When using the former, HA visibly interferes: the baseline current is strongly increased at more negative potentials, while the Cr peak gradually diminished and practically disappeared at HA concentration of 1 mg/L. At HA concentrations above 0.5 mg/L, the chromium peak is poorly expressed, even at such high Cr concentrations. When using a more negative accumulation potential of -1.65 V (Fig 2., inset), the Cr peak and background currents are clearly much less affected by addition of HA. A very small increase of the baseline current is still observed at more negative potentials (note the difference in the range of Y-axis for two plots), but the Cr peak remains well shaped, despite decreasing down to around 30% of the initial value (without HA addition). This decrease suggests that the interferences from SAS is not entirely removed or that Cr(VI) is complexed by HA.

To identify if the decrease of the signal is due to SAS interference on the voltammetric signal or is due to complexation by HA, we carried out analytical determinations of Cr by the method of standard addition at both potentials (-1.0 and -1.65 V) at each HA concentration (calibrations not shown). At low HA concentrations, below 0.7 mg/L, despite a strong decrease of signal intensity, the accuracy of Cr determination is not significantly impacted, at both potentials. At higher HA concentrations, analysis of Cr using -1.0 V deposition potential was not possible, whereas using deposition at -1.65 V a reproducible and accurate determination of Cr was always obtained (recovery ~100%), suggesting that complexation of Cr(VI) with HA did not occur within the time frame of the experiment. The only problem that was sometimes observed when using an accumulation potential of -1.65 V was the dislodgement of the Hg-drop. However, as the accumulation time for most of the samples measured in natural water is only 60 s, this problem did not impact Cr determination because duplicate or triplicate measurements were always performed (for the sample and for each addition of Cr standard).

Figure 3 shows the variation of the peak intensity versus accumulation potential (so-called "pseudopolarograms") at different HA concentrations in UV digested seawater. An initial increase of peak intensities with decreasing accumulation potentials was first observed, reaching a maximum at -1.05 V, followed by a strong decrease to an almost complete loss of the signal (-1.2 to -1.5 V) before finally increasing again up to the lowest deposition potential tested here (-1.7 V). The maxima at -1.05 V disappears at the highest HA concentration of 1 mg/L while the signal at -1.7 V is much less affected. Very similar U-shaped relationships was also found in our estuarine samples of different salinities (Fig. S2, SI), similar to previously reported at a vibrating amalgam micro-wire electrode [20]. In seawater, the loss of the Hg drop was observed at accumulation potentials more negative than -1.7 V, possibly due to partial reduction of major cations. In freshwater, that negative accumulation potential limit was shifted far more negative (down to -2.4 V) (Fig. S2) with a much-improved sensitivity. For instance, the maximum peak height obtained at -2.2 V was ~4× higher than the one obtained at -1.0 V.

The Cr redox mechanism occurring at accumulation potentials more negative than -1.5 V has already been described [18, 22]: Cr(VI) is reduced to its metallic state Cr(0) and accumulated at the Hg surface. At the start of the stripping (-0.95 V), Cr(0) is oxidised to Cr(III) that immediately forms a complex with DTPA and the stripping is occurring along the catalytic pathway described above (section 4).

6.2. Voltammogram shape and baseline elimination

The most common way of expressing sensitivity is in terms of nA/nM. However, in cases where the signal is positioned at the steep part of the baseline (as for Cr), the signal to baseline shape is much more important than just pure (and high) sensitivity expressed in nA/nM. A typical example is given in Fig. 4: while the peaks obtained at -1.0 and -1.65 V are of the same absolute intensity (~4.5 nA; determined using curvature baseline), the shape of the latter peak is much better resolved than the former (see also voltammograms in Fig. 2). Thus, when optimising any voltammetric procedures, both the absolute intensity and the shape of the voltammogram should be improved. This is especially important at low signal amplitude, where the steep baseline could mask the analyte signal. In such cases, the application of derivative transformations is beneficial: it eliminates the curvature of the baseline (see inset of Fig. 4) and consequently lower detection limits [35, 37]. We used here the peak height of the 2nd derivative transformation for quantification of Cr concentration.

6.3. Hydrography of the estuary

Physico-chemical parameters for the sampling periods are presented in Fig. S3. The vertical and horizontal profiles of salinity are typical of two contrasting sampling periods (summer and winter): the halocline is deeper and the low salinity brackish layer extends more within the estuary in winter than in summer. The pH of samples (not shown) was between 8.4 (at the freshwater part) and 8.2 (at the seawater). Higher pH in freshwater part is related to CO_2 removal at waterfalls which precede the estuarine transect [25]. Temperature profiles follow a similar trend as those of salinity for both periods, whereas oxygen profiles were the most variable. A clear increase of oxygen levels below the halocline (reaching value up to 140 % of oxygen saturation) between 3rd and 20th kilometre were observed in summer, due to high productivity occurring in this lacustrine part of the estuary (Prokljan lake) [38]. On the other hand, hypoxic conditions were found in the deeper regions of the upstream part of the estuary during winter. This is due to progressive degradation of organic matter produced during summer period, associated with the high residence time of the seawater layer in that upper part of the estuary. Fig. S4 presents typical profiles of dissolved organic carbon (DOC) for the winter and summer periods. DOC concentrations were higher in summer (up to ~150 μM) than in winter (up to ~80 μM). Typically, DOC was lower (~50 μM) in winter in the freshwater end-member compared to the seawater end-member, whereas for the summer period it could be the opposite, due to developed biological productivity in the freshwater Visovac Lake, that is located before the waterfalls and the estuary [39].

6.4. Chromium distribution along the Krka River estuary

The distributions of dissolved Cr(VI) and Cr(III) along the estuarine transect are presented in Fig. 5 as a function of salinity. Due to the fully oxygenated samples, Cr(VI) predominates both in summer and winter. Although the suspended particulate matter is generally low in the Krka River estuary (< 5 mg/L) [25], some portion of Cr(III), which is particle-reactive in contract to Cr(VI), could be adsorbed on the particulate matter. Thus, higher concentrations of Cr(III) in non-filtered samples were expected but these were not found. In unfiltered samples, slightly higher (<5 %) concentration of Cr(III) were found compared to the dissolved ones

(Fig. S5). One of the known challenge in Cr determination is that Cr(III) is strongly adsorbed on the bottle walls [10]. In cases when UV-irradiation of sample to remove organic matter is performed in separate, usually quartz tubes [20], the adsorbed Cr(III) is not recoverable because the sample is transferred from storage bottle to the UV-digestion vessel. In our work, Teflon (FEP) bottles were used to store the samples and UV-irradiation was performed directly in these bottles (FEP is UV transparent), with adaptation of samples pH to around 5. In this way, any adsorbed Cr(III) is expected to be recovered and reliably quantified.

For both summer campaigns, very similar transects of both Cr redox species were obtained. Concentration of dissolved Cr(VI) slightly increased with the salinity (or distance), with a profile that could be characterised as non-conservative. The concentration level of Cr(VI) in the Krka River end-member was around 2.5 nM, while at the seawater end-member, it was between 4 and 5 nM (Fig. 5). In contrast, an opposite trend was found in winter with higher Cr(VI) found at the lower salinities. In winter, higher concentrations in the freshwater end-member can be explained by the higher river flow, leading to significant weathering processes while no significant differences were found in the seawater end member between winter and summer.

As for Cr(VI), no clear difference was found between dissolved and unfiltered Cr(III) concentrations, indicating that the fraction of Cr(III) associated to particles was minor. Hexavalent chromium can be photo-reduced to its trivalent state that can be then rapidly complexed with naturally occurring dissolved organic matter [40, 41]. However, according to our results, this process does not seem to occur, even in summer. In the work of Achterberg and van den Berg [41] in which Cr distribution in Mediterranean waters was studied, Cr(III) was found higher in surface layers for both winter and summer periods. It was suggested that this is possibly caused by photochemical conversion of Cr(VI) to Cr(III) during summer periods and by atmospheric inputs during winter periods. Atmospheric input of Cr(III) could be envisaged since it is abundant in atmospheric particulate matter [42]. On the other hand, in summer period, Cr(III) could be photo-oxidized to Cr(VI) while in the winter time, such conversion is limited due to lower solar irradiance. Additional seasonal sampling campaigns would be required to tests the hypothesis that atmospheric/aerosol inputs of Cr(III) is regulating its concentration in the surface layer of the estuary and that Cr(III) is photo-oxidised to Cr(VI).

The transect of chromium in the bottom water is unusual. While an upstream increase of trace metals was recorded for many metals in the bottom layer [25], Cr concentrations here follow an opposite trend, similar to that observed in surface water. A lower concentration is detected on the freshwater side compared to those measured lower in the estuary (see Fig. S6), reaching a stable value of c.a. 4.4 nM from 6 km downstream. This unusual behaviour can be explained by a reduction of Cr(VI) to Cr(III) in the upstream part, followed by removal of Cr(III) through adsorption onto sinking particulate matter, which is known to be increased in that part of the estuary [25]. Reduction of Cr(VI) to Cr(III) can be favoured by the hypoxic conditions in that section of the estuary (Fig. S3) and/or through reduction by low molecular weight organic matter, as previously suggested [43].

7. Conclusions

We have developed here an optimised voltammetric procedure to measure Cr in presence of SAS, substances that are ubiquitous in natural waters. By applying a lower negative accumulation potential (-1.65 V) than the usual one (-1.0 V), the interfering effect of SAS adsorption on the mercury drop electrode is strongly minimised. Experiments performed in UV-digested seawater with addition of humic acid (HA) showed clear evidence of the benefits of using such low deposition potential. This optimised voltammetric procedure was successfully applied for the Cr speciation along the Krka River estuary in winter and summer. Cr(VI) was found to be the predominant redox species in all samples and higher Cr(III) concentrations were found in winter. While Cr concentration for summer samples increased towards the open sea, an opposite trend was found for winter campaign, probably related to weathering processes and higher Krka River flow, which increased Cr concentration in the freshwater part.

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Table 1. Parameters of the method for cat-AdCSV measurement of trace levels of chromium using DTPA.

	Boussemart [10]	Li and Xue [30]	deSouza [31]	Korolczuk [33]	This study
Deposition potential /V	-1.0	-1.0	-1.0	-1.7*	-1.65
Deposition time /s	60	60	60	60	60
DTPA / c(mM)	2.5	5	1.25	10	1.25
NO_3^- / c(mM)	500	500	1500	500	500
MES / c(mM)	-	-	-	10	5
pH seawater	5.0	5.7	5.0	-	5.5
pH freshwater	6.4	5.7	-	6.1	5.5

* - with matrix exchange

Figure Captions

Figure 1. Left: Map of the Krka River estuary with indicated locations of sampling sites (open diamonds). Right: horizontal bottom depth profile with positions of sampling sites and specific regions along the estuary.

Figure 2. Voltammograms recorded in UV-irradiated seawater spiked with 6 nM Cr(VI) at varying concentrations of HA using -1.0 V (main plot) and -1.65 V (inset) as accumulation potentials.

Figure 3. Variation of peak area on the accumulation potential without and with increasing concentration of HA in UV-irradiated seawater (UVSW) with total Cr concentration of 6 nM.

Figure 4. Voltammograms obtained at -1.0 V and -1.65 V accumulation potential in seawater sample ([Cr] = 4.5 nM). Inset: 2nd derivative vs potentials.

Figure 5. Distribution of dissolved chromium along the salinity gradient of the Krka River estuary in surface layer. Blue dotted line corresponds to conservative mixing line.

Figure 1

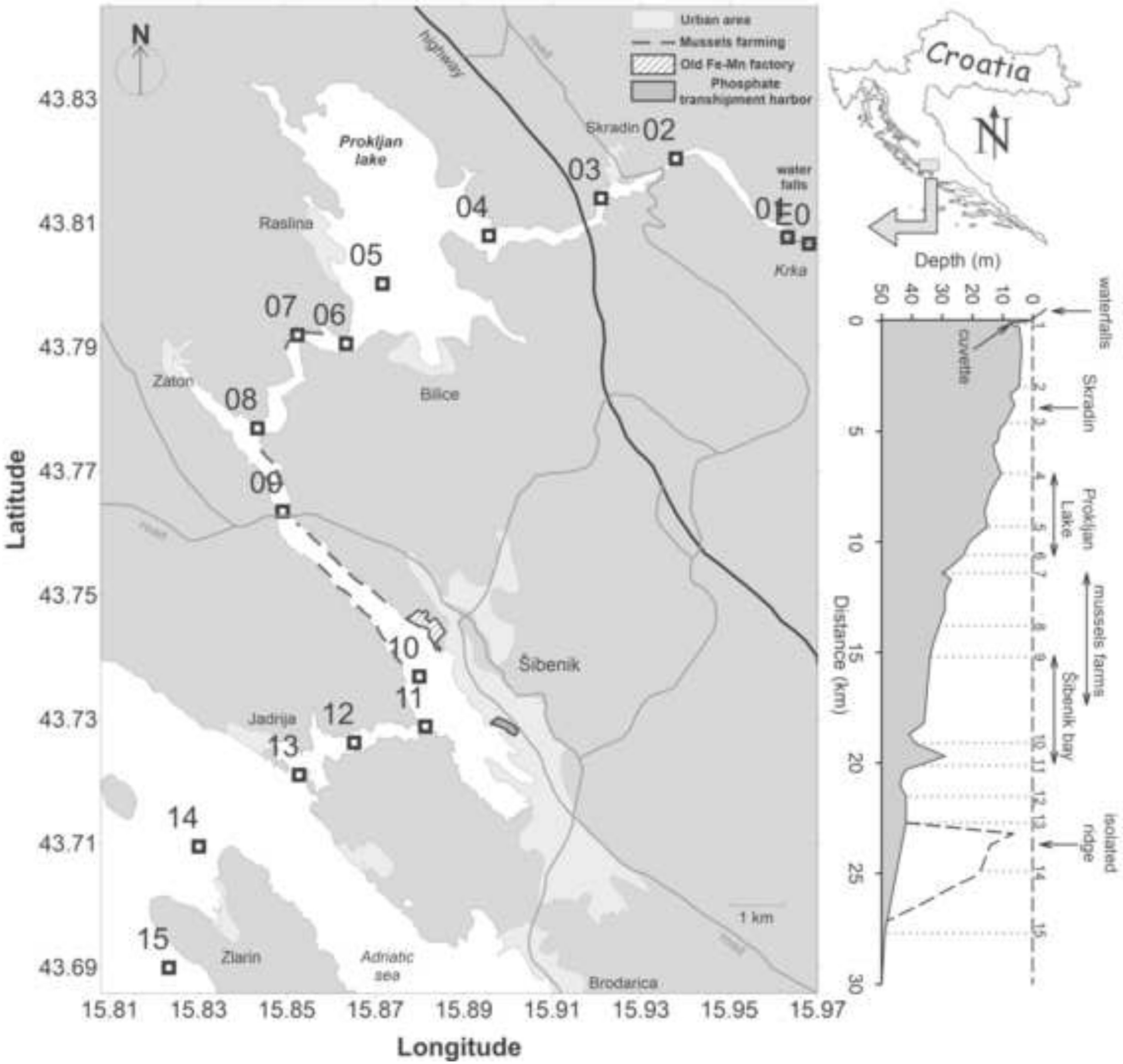

N
Urban area
Mussels farming
Old Fe-Mn factory
Phosphate transhipment harbor
Croatia
N
highway
Skradin
02
03
04
01
E0
water falls
Krka
Prokljan lake
Raslina
05
07
06
Zaton
08
Bilice
09
10
Šibenik
11
12
Jadrija
13
14
15
Zlarin
Adriatic sea
Brodarica
1 km
43.83
43.81
43.79
43.77
43.75
43.73
43.71
43.69
Latitude
15.81
15.83
15.85
15.87
15.89
15.91
15.93
15.95
15.97
Longitude
Depth (m)
50
40
30
20
10
0
0
5
10
15
20
25
30
Distance (km)
waterfalls
curvette
Skradin
Prokljan Lake
mussels farms
Šibenik bay
isolated ridge
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

Figure 2

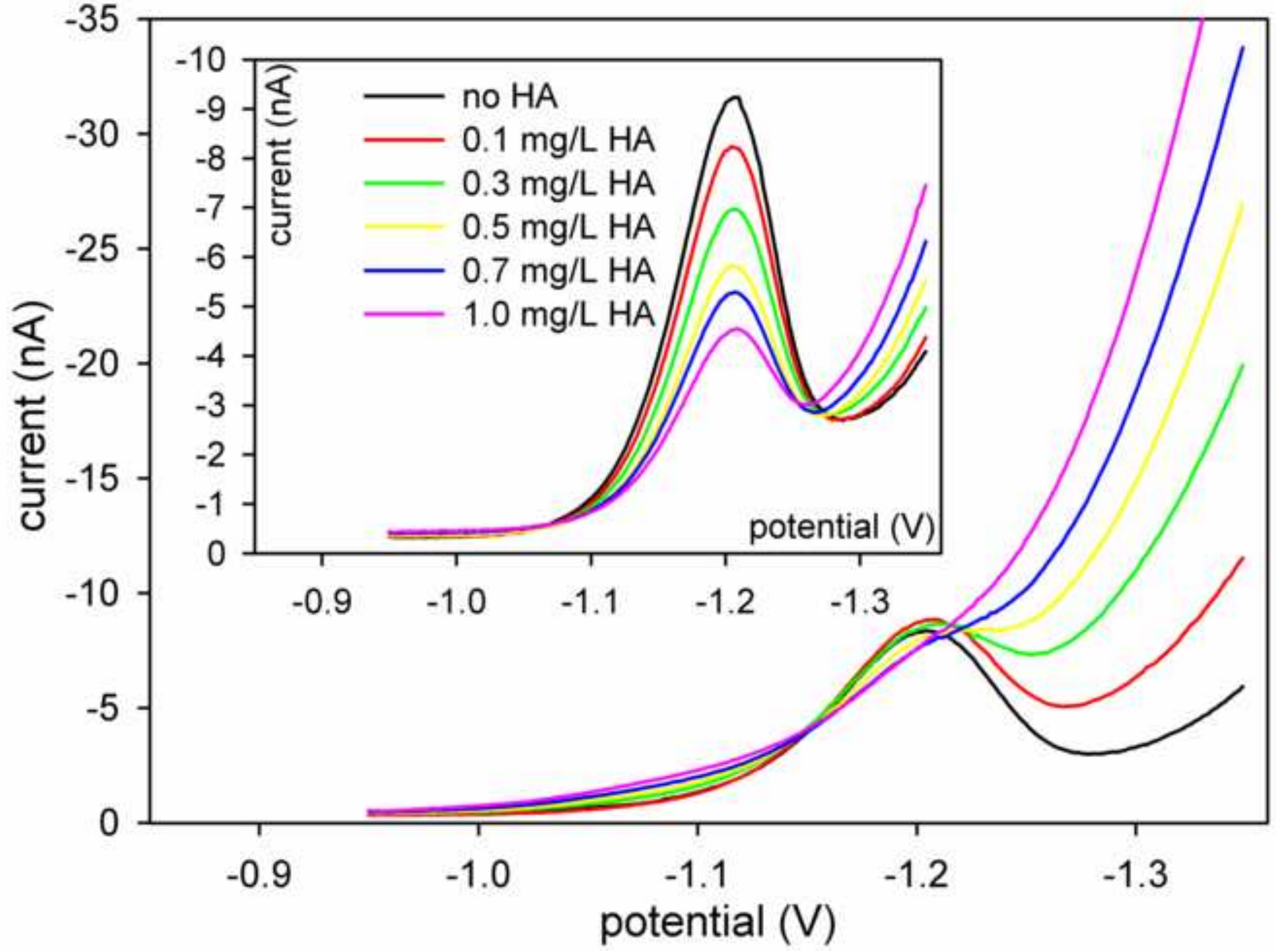

-35
-30
-25
-20
-15
-10
-5
0
current (nA)
-0.9
-1.0
-1.1
-1.2
-1.3
potential (V)
-10
-9
-8
-7
-6
-5
-4
-3
-2
-1
0
current (nA)
no HA
0.1 mg/L HA
0.3 mg/L HA
0.5 mg/L HA
0.7 mg/L HA
1.0 mg/L HA
potential (V)
-0.9
-1.0
-1.1
-1.2
-1.3

Figure 3

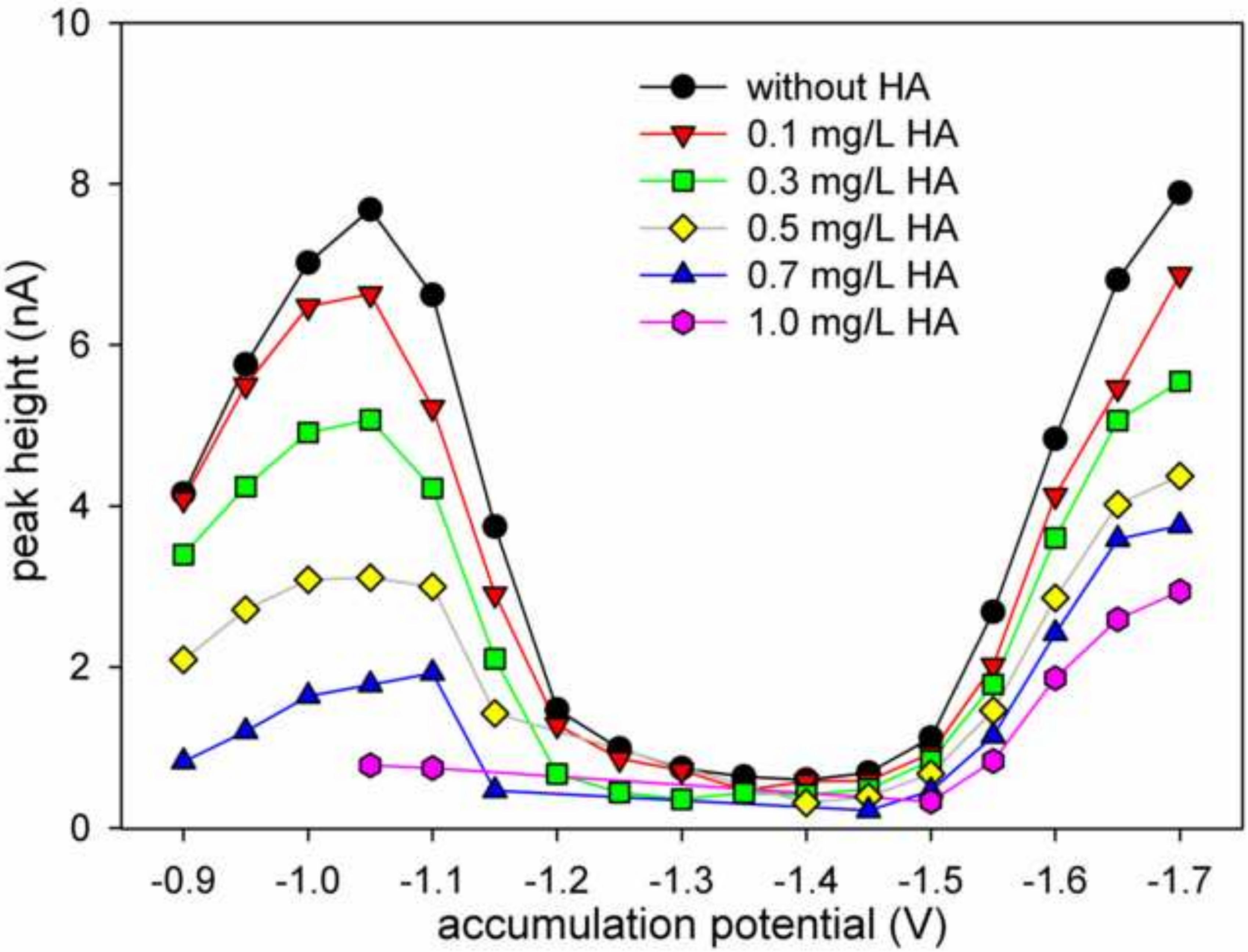
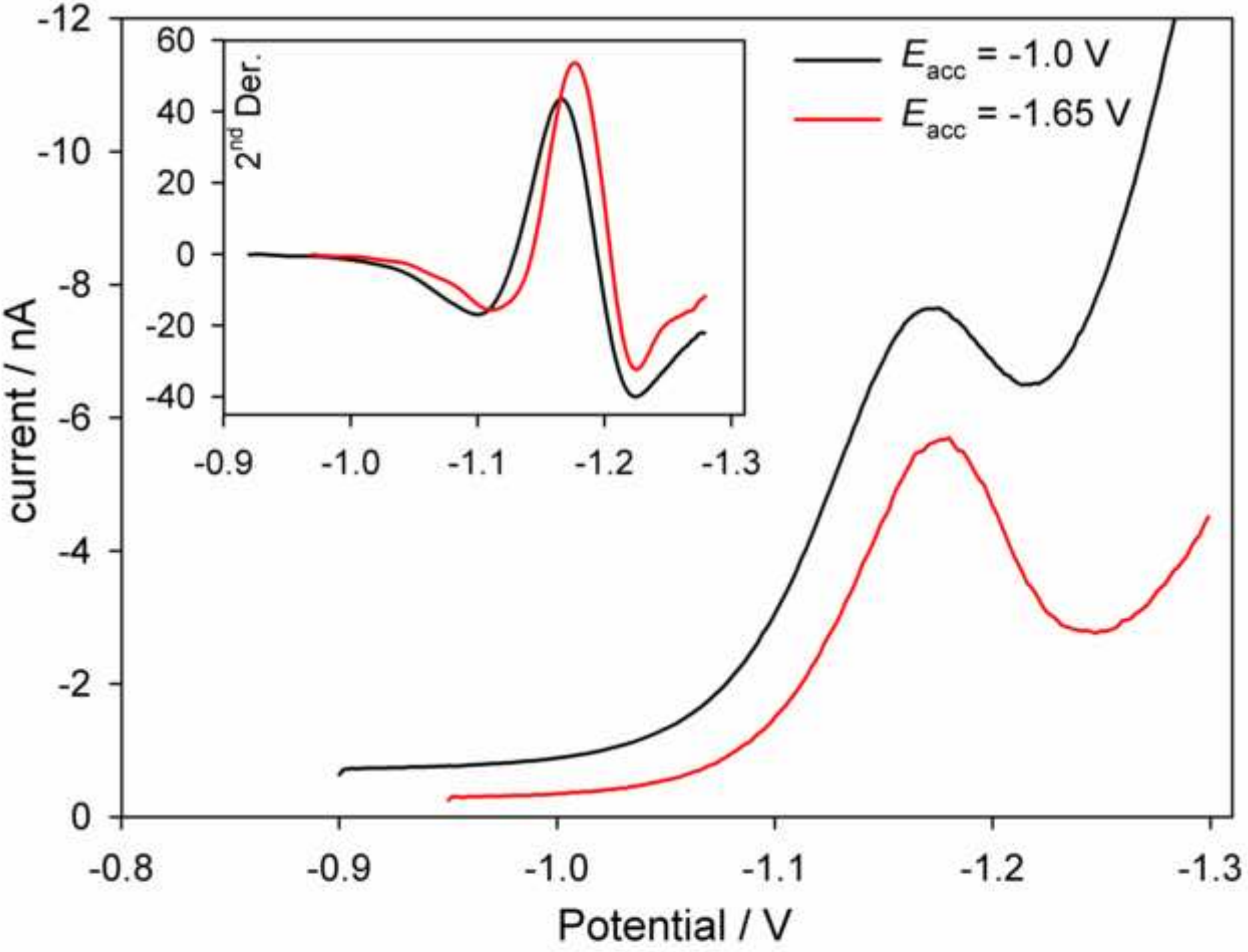

peak height (nA)
0
2
4
6
8
10
accumulation potential (V)
-0.9
-1.0
-1.1
-1.2
-1.3
-1.4
-1.5
-1.6
-1.7
without HA
0.1 mg/L HA
0.3 mg/L HA
0.5 mg/L HA
0.7 mg/L HA
1.0 mg/L HA

Figure 4



E_{acc} = -1.0 V
E_{acc} = -1.65 V
current / nA
-12
-10
-8
-6
-4
-2
0
-0.8
-0.9
-1.0
-1.1
-1.2
-1.3
Potential / V
2nd Der.
60
40
20
0
-20
-40
-0.9
-1.0
-1.1
-1.2
-1.3

Figure 5

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