Automated syringe-pump-based flow-batch analysis for spectrophotometric determination of trace hexavalent chromium in water samples

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ABSTRACT

In this work, we established an automated spectrophotometric method for determining hexavalent chromium in water samples using an integrated syringe pump-based environmental water analyzer (*i*SEA) based on the classic 1,5-diphenylcarbazide chemistry. The device was computer-controlled with programs written by LabVIEW. The effects of reagent concentration, sample salinity and foreign ion interferences were investigated. Standard solutions (GBW(E)081584-1) were used to test the accuracy of the method. When equipped with a 5 cm *Z*-shaped flow cell, the detection limit was $0.024\,\mu M$ with sample throughput of $> 30\,h^{-1}$. In order to meet the requirements for trace Cr(VI) determination, the system was modified with a 2.5 m liquid waveguide capillary cell (internal diameter of 0.55 mm). The sample consumption was < 2.1 mL. The detection limit was 0.54 nM with sample throughput of $20\,h^{-1}$ and good linearity with $R^2 = 0.9985$ ($n = 110$, obtained over 14 days). The two methods were successfully applied to determine Cr(VI) level in different water samples including tap water, river water, industrial wastewater and 37 brands of bottled water with spiked recovery in the range of 89.5%–117%.

1. Introduction

Chromium (Cr) is extensively and intensively used in modern industry. Its applications include metal finishing, electroplating, textile dyes, catalyst and wood preservatives. Due to its wide use, large quantities of chromium are released into the environment causing widespread pollution [1]. Chromium typically exists in two oxidation states in surface and ground water - trivalent and hexavalent, which are very different in charge and physicochemical properties [2]. Trivalent chromium [Cr(III)] is considered to be a trace element essential for the proper functioning of living organisms, whereas hexavalent chromium [Cr(VI)] can induce tumors in the alimentary tract of animals via drinking water exposure [3]. Currently, World Health Organization (WHO) has set a $50\,\mu g/L$ ($\sim 0.96\,\mu M$) provisional guideline value for total chromium in drinking water. It should be noted that the guideline value is designated as provisional because of uncertainties in the toxicological database [4]. The Office of Environmental Health Hazard Assessment (OEHHA) of California has published an impressive $0.02\,\mu g/L$ (~ 0.4 nM) Public Health Goal (PHG) for hexavalent chromium in drinking water [5]. Although PHG is based solely on scientific and public health considerations, and not on economic factors and technical feasibility, it underlines the paramount importance of achieving accurate, sensitive and specific detection of Cr(VI) in environmental and potable water for both environmental quality and public health concerns. It should also be noted that OEHHA has initiated the update of the PHG for Cr(VI) in 2016 and is still in process [6].

As a widespread and notorious hazardous material, Cr(VI) has received ongoing attention from both environment researchers and analytical chemists. Instrumental techniques including atomic absorption spectrometry (flame-AAS, graphite-furnace-AAS, electrothermal-AAS) [7–9], inductively coupled plasma optical emission spectrometry (ICP-OES) [10], and inductively coupled plasma mass spectrometry (ICP-MS) [11] etc., have been well established for the analysis for chromium. However, these benchtop instruments typically require trained expertise and time-consuming pretreatments for selective determination of Cr(VI) [12]. Very recently, some novel methods have been reported, such as laser-induced breakdown spectroscopy [13], thermal lens microscopy [14], nanostructured hybrid photoelectrochemical sensor [15] and functionalized fluorescent probes [16,17]. Nevertheless, the requirements of sophisticated instruments and commercially unavailable materials might limit their wide application for routine analysis. Therefore, it remains important to develop novel rapid, accessible and easy-to-use Cr(VI) detection methods.

Spectrophotometric detection based on the classic 1,5-diphenylcarbazide (DPC) chemistry is an attractive approach due to its

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simplicity, good sensitivity and high selectivity for Cr(VI). Both its basic form of colorimetric method and development of ion chromatography-post column reaction method have been accepted as standard methods [18]. Although more than a century has passed since the early establishment of the DPC chemistry [19], the well-known reaction has been continuously coupled to a variety of sample pretreatment processes (solid phase extraction (SPE) [20], solid phase microextraction (SPME) [21], hollow fiber membrane liquid phase microextraction (HF-LPME) [22], dispersive liquid-liquid microextraction (DLLME) [23], etc.) and analytical devices (microfluidic paper-based analytical device (μPAD) [22], 3D printed flow device [24], etc.), thus breathing new life into this chemistry. Among the various modifications and evolutions, flow-based methods [25–28] stand out because of their automation, ease of operation, possible miniaturization, good reproducibility and versatility. For example, Ohira et al. [29] reported an ion transfer device (ITD) to separate oppositely-charged Cr(III) and Cr(VI), and the two different acceptor solutions were subsequently determined by flow injection analysis (FIA), which was easier to operate compared to their previous ICP-MS adaptation of ITD [30].

Among varieties of flow-based methods, flow-batch analysis (FBA) allows implementation of well-established wet chemistries while minimizing operator errors [31]. Combined features of flow-based and batch-wise systems make FBA suitable for routine, field and versatile use [32,33]. Recently, a fully-automated *integrated Syringe-pump-based Environmental-water Analyzer (iSEA)* was developed in our group, which was portable, robust and has been successfully applied to perform high-resolution real-time monitoring of ammonium in fresh water and coastal area [34,35]. The *iSEA* system implemented FBA, but it utilized a programmable syringe pump and only one glass syringe, together with a LED light source and a miniature CCD spectrophotometer. In principle, *iSEA* exploited the concept of "Lab-in-Syringe" (LIS) which was proposed by Maya et al. [36–38]. In LIS format, certain drawbacks involved in FIA and FBA like decreased sensitivity with non-equilibrium detection [39] and volume inaccuracy for long time determination due to roller pulsation of peristaltic pumps [31] are circumvented. To date, the attractive features of LIS have been recognized and the versatility of LIS has been demonstrated by many other applications [28,40–42]. A novel in-syringe magnetic-stirring-assisted dispersive liquid–liquid microextraction for trace determination of Cr(VI) in waters using the DPC chemistry was reported previously [43]. Considering the low concentration of Cr(VI) in typical water samples, especially in drinking water, conventional flow cells with < 5 cm pathlength may not provide sufficient sensitivity. Long pathlength absorbance spectrophotometry, based on Lambert–Beer's law, is a green alternative strategy for trace metal analysis by avoiding laborious pretreatment procedures which commonly involve toxic organic solvents [44]. Liquid waveguide capillary cell (LWCC) has proven its applications in the determination of trace chromium [45–48] and other metals [49–53].

Based on the above background, herein, we present an automated syringe-pump-based flow-batch analysis method using 5 cm *Z*-shaped flow cell and 2.5 m LWCC for measuring Cr(VI) concentrations at different levels. The effects of reagent concentration, sample salinity and interferences on DPC chemistry were evaluated. The proposed method was applied to determine Cr(VI) levels in tap water, river water, industrial wastewater and 37 brands of bottled drinking water with satisfactory recovery.

2. Experimental

2.1. Reagents and solutions

Chemicals used throughout were of analytical grade, and purchased from Sinopharm Chemical Reagent Co., China unless stated otherwise. All chemicals were used as received without further purification. Freshly made ultrapure water (resistivity ≥ 18.2 MΩ cm, Millipore Co.,

USA) was used to prepare solutions. Aged oligotrophic surface seawater collected from South China Sea which contained only nanomolar Cr(VI) was used for studying matrix effects. All containers were sequentially rinsed with ultrapure water, 2 M HCl and ultrapure water prior to use.

Stock solution of Cr(VI) (4.00 mM) was prepared by dissolving solid $K_2Cr_2O_7$, previously dried at 105 °C for 2 h, in ultrapure water. Working standards of Cr(VI) were prepared daily by stepwise dilution of stock solution. DPC solution of 0.5% (*m/V*) was prepared daily by dissolving 0.10 g of 1,5-diphenylcarbazide in 20 mL of high purity acetone (Tedia, HPLC/Spectro). H_2SO_4 solution of 2.5 M was prepared by dilution of concentrated sulfuric acid in ultrapure water (Guaranteed reagent, Xilong Scientific Co., Ltd.).

In interference study, standards of Cu(II), V(V) and Mo(VI) were obtained by dissolving $CuCl_2$ $2H_2O$, NH_4VO_3 (Xilong Scientific Co., Ltd.) and $(NH_4)_6Mo_7O_{24}$ $4H_2O$ (Guangdong Guanghua Sci-Tech Co., Ltd.), respectively, in ultrapure water. Fe(III) stock solution (1000 mg/L) was obtained from CertiPUR® (Merck, Germany). Standard solutions of Cr(VI) (GBW(E)081584-1) used in the accuracy test were purchased from Beijing Haianhongmeng Reference Material Technology Co., Ltd.

2.2. Apparatus and procedures

Schematic diagram of the manifold for Cr(VI) determination is presented in Fig. 1. Detailed description of *iSEA* can be found in our previous work [34]. In brief, the device comprised of a flow module and a detection module. A syringe pump (XCalibur, Tecan, USA) together with a 2.5 mL gastight glass syringe (Hamilton, USA) and a 9-position distribution valve was used to deliver fluids. Polytetrafluoroethylene (PTFE) tubing (0.75 mm i.d.) and 1/4–28 flangeless PEEK fittings (IDEX Health & Science LLC, USA) were used to connect components of the analyzer. Laboratory-programmed software written by LabVIEW (National Instruments, USA) was used to control the system. In the study of reagent concentration, sample salinity, interferences and application to environmental water samples, a 5 cm *Z*-shaped flow cell was used (referred as '*iSEA*-flow cell' Fig. 1(a)). We subsequently demonstrated the feasibility of combining *iSEA* with LWCC (250 cm, 550 μm i.d., World Precision Instrument, USA) (referred as '*iSEA*-LWCC', Fig. 1(b)). A pipette tip of 5 mL was installed as a secondary chamber to enhance the mixing [54]. In both cases, a green LED was utilized as light source, and two optical fibers were used to connect the detector cell and the miniature USB2000 + spectrophotometer (Ocean Optics, USA) (Fig. 1(c)). Data acquisition was performed by Spectrasuite (Ocean Optics, USA). A benchtop UV–Vis spectrophotometer (V1100D, Shanghai Mapada Instruments Co., Ltd) with 5 cm cuvette was used to compare the methods.

The simple operating procedure is described as follows. First, the syringe and the flow cell were cleaned with ultrapure water four times to minimize carryover effect, and the light intensity at 540 nm reaching the detector was set as 100% transmittance. After that, the syringe pump sequentially aspirated 50 μL of 0.5% (*m/V*) DPC, 25 μL 2.5 M H_2SO_4 and 1250 μL sample through Ports 3, 4 and 2, respectively, during which mixing through diffusive process and the chemical reaction occurred concurrently in the syringe. The mixture was then propelled to the flow cell and stopped for 60 s to allow equilibrium of the reaction. The final light intensity was recorded to calculate the absorbance.

When the system was equipped with LWCC, some modifications were made to the procedure. The aspirated volumes of DPC, H_2SO_4 and sample were proportionally increased to 83.3 μL, 41.7 μL and 2083 μL, respectively. The mixture was dispensed to the pipette tip (Port 8) and aspirated back to the syringe (Port 9) twice to mix thoroughly. After propelling the sample-reagent mixture to LWCC, the stopped time was reduced to 40 s. Detailed description of the above procedure is presented in the Supplementary Materials. Before and after use, LWCC was sequentially flushed with ultrapure water, ethanol, 1 M HCl and ultrapure water.
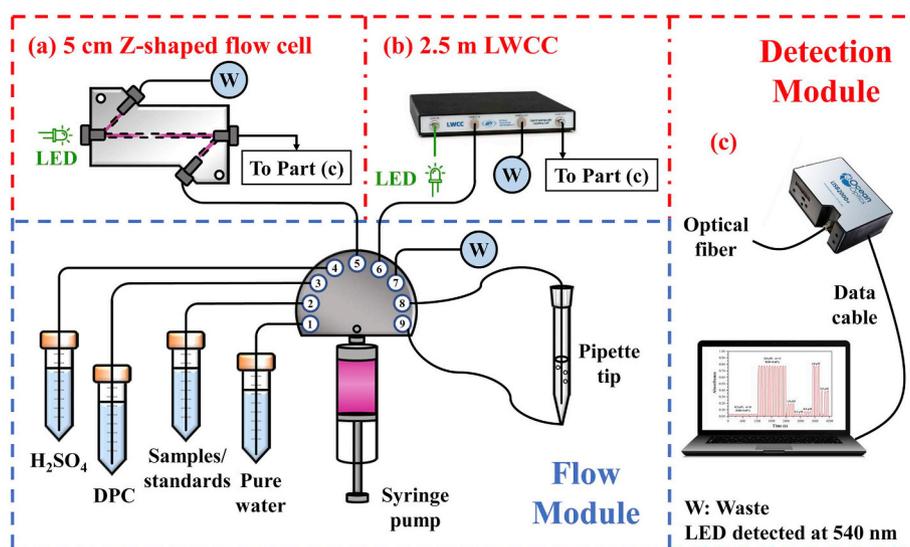
Fig. 1. Schematic diagram of the *i*SEA system for Cr(VI) determination. The detection module consisted of a (a) 5 cm *Z*-shaped flow cell or (b) 2.5 m LWCC and (c) a miniature USB2000+ spectrophotometer and a personal computer.

2.3. Water samples

Tap water was collected in centrifugal tubes from our lab in Xiang'an campus of Xiamen University, China. After collection, the sample was immediately filtered through 0.45 μm syringe filters and analyzed. River water was collected from Jiulong River, Fujian, China, filtered and analyzed as soon as possible. Industrial wastewater samples were collected using low density polyethylene bottles from an anonymous chromium electroplating steelworks in Zhangzhou, Fujian, China. Samples were transported to the lab, filtered and analyzed within 24 h.

For trace Cr(VI) determination, 37 brands of bottled water samples were purchased from local markets and online store in duplicate (except that 7 brands had only one sample) and kept sealed until analysis. The water samples were divided into three categories in terms of the water type: (1) purified or pure drinking water (8 brands), (2) natural mineral/spring/lake water (25 brands), and (3) drinking water with artificial additives (oxygen or minerals like KCl or MgSO$_4$, 4 brands).

3. Results and discussion

3.1. Optimization of H$_2$SO$_4$ and DPC concentrations

As described above, the *i*SEA system implements combined flow-batch analysis and stopped-flow analysis. Additionally, with the aid of programmable syringe pump, it is convenient to carry out reagent concentration optimization and investigate the kinetics of the reaction without changing any hardware. To this end, ultrapure water was spiked with 4.0 μM and 0.5 μM of Cr(VI) standards and then reacted with varying volumes of H$_2$SO$_4$ (final concentration 0.014 M–0.136 M) and DPC solution (final concentration 0.005%–0.036%). Absorbance was recorded for 5 min. Reaction rate, sensitivity and repeatability were taken into overall consideration.

As shown in Fig. 2, the reaction rate saw an upward trend with increasing concentrations of H$_2$SO$_4$ and DPC in the cases of both high and low Cr(VI) concentrations. With H$_2$SO$_4$ concentration ≥ 0.048 M and DPC concentration ≥ 0.019%, the reaction reached equilibrium in approximately 60 s. However, with further increase in H$_2$SO$_4$ concentration, irregular signals occurred and repeatability became worse. This was because high concentration of H$_2$SO$_4$ resulted in incomplete mixing in the syringe. Therefore, 0.048 M H$_2$SO$_4$ and 0.019% DPC were selected for further experiments and the corresponding aspirated

volume ratio of H$_2$SO$_4$, DPC and sample was 1:2:50. Stopped time was set at 60 s, at the end of which the absorbance was about 90% of that at equilibrium. It is noteworthy that the aspirated volume and the stopped time can be changed as needed (e.g. to achieve higher throughput or reduce interferences) in practical determination.

3.2. Effect of salinity

The coastal areas are often polluted by wastewater, and thus, it is important to measure the heavy metal concentrations in seawater. To study the effect of sample salinity on Cr(VI) detection, seawater was diluted by ultrapure water to obtain matrices with salinity ranging from 0 to 35. The Cr(VI) standard solutions were 0, 0.2, 0.5, 0.8, 1.0 and 2.0 μM. The slopes and intercepts of 11 calibration curves were compared, as presented in Fig. 3.

There was no significant difference between 11 slopes and intercepts, indicating that salinity had negligible effects on the Cr(VI)-DPC reaction, which was similar to the results obtained by Yao and Byrne [45]. In flow analysis, high ionic strength may affect the reaction rate and end point [55]. If non-equilibrium detection is employed, as is often the case in FIA, the sensitivity may vary. The difference in refraction index (RI) values between salinity samples and carrier (Schlieren effects) can also be troublesome [56]. These problems can be partially addressed by thoroughly mixing and stopped-flow analysis. However, further careful investigations are needed for the specific chemistry and experimental design.

3.3. Interference of foreign ions

The reaction of Cr(VI) with 1,5-diphenylcarbazide has good specificity [18]. However, potential interferences caused by some metal ions such as Fe(III), V(V), Mo(VI), Cu(II) and Hg(II) have been documented [29,45,57,58]. In this study, interference test was carried out by increasing the concentrations of Fe(III), V(V), Mo(VI) and Cu(II) while keeping the Cr(VI) concentration constant at 1.0 μM. The concentration of foreign ions causing no more than ± 5% variation in recovery was defined as the tolerance limit.

As illustrated in Fig. 4, the effects of Fe(III) and V(V) showed similar patterns. Increased concentrations of Fe(III) led to elevated recovery. It was observed that Fe(III) at high concentrations formed a yellow product with DPC. In the present experimental design, the tolerance limit for Fe(III) was 50 μM (2.80 mg/L), which should not be a problem in the
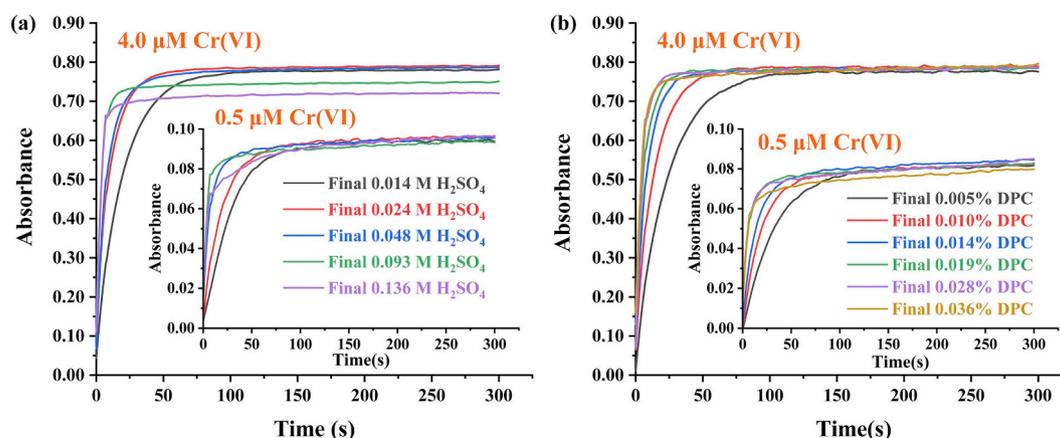
Fig. 2. Effects of reagent concentration. (a) H₂SO₄ concentration (final DPC concentration at ~0.019%), (b) DPC concentration (final H₂SO₄ concentration at ~0.047 M).
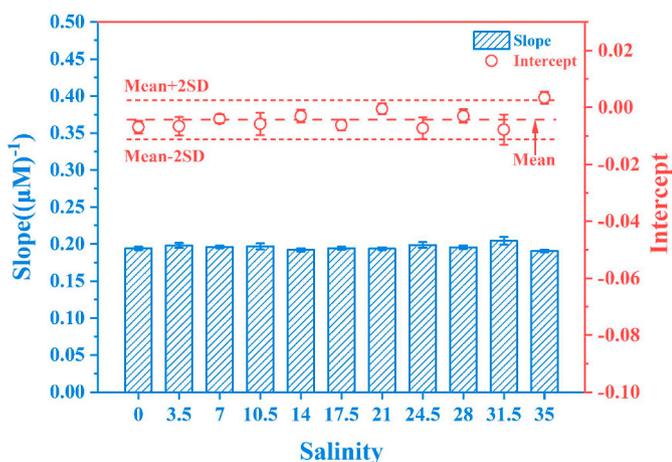
Fig. 3. Effects of salinity on slopes and intercepts of calibration curves. Error bar denotes slope/intercept ± standard error (*n* = 3).

determination of environmental waters. > 5 μM (0.26 mg/L) of V(V) can cause positive interference. However, it was found that the interference of 10 μM V(V) decreased from +12.0% to +5.9% after 10 min of reaction, as shown in Fig. S1. This self-elimination of interference has

been previously reported [29]. If V(V) is an issue in practical determination, then prolonging the reaction time (for example, from 60 s to 10 min) can be considered as a simple method to overcome the interference. On the other hand, Cu(II) and Mo(VI) exerted negative interferences, but their tolerance limits were as high as 700 μM (48.8 mg/L) and 300 μM (28.80 mg/L), respectively.

3.4. Adapting the configuration for LWCC

In the above sections, we have preliminarily demonstrated the feasibility and applicability of utilizing *i*SEA for Cr(VI) determination. Further validation and application will be presented in Sections 3.5 and 3.6. In the following section, we will describe the adaptation and modification of the manifold to couple with LWCC.

To ensure that the capillary cell was completely filled with mixture, the aspirated volumes of DPC, H₂SO₄ and sample were proportionally increased to 83.3 μL, 41.7 μL and 2083 μL, respectively. This helped to eliminate dilution by dead volume and RI effects [59]. For *i*SEA, the syringe itself served as an efficient primary mixing chamber, based on the principle of optimizing the aspirating and dispensing rates to maximize the dispersion process [34]. However, when the mixture was propelled to LWCC, irregular signals and unsatisfactory repeatability were observed. LWCC has the characteristics of small diameter, long path length and high sensitivity. Therefore, the dispersion phenomena
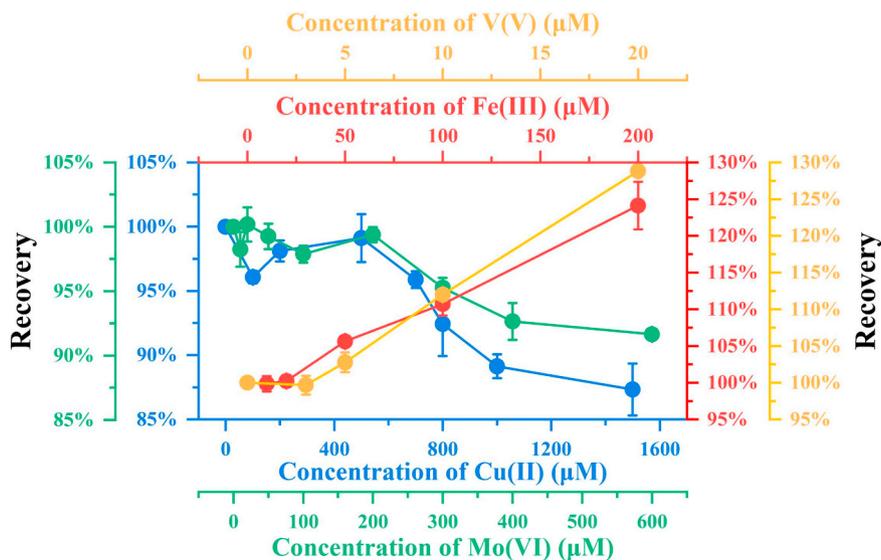
Fig. 4. Interference from Fe(III), V(V), Mo(VI) and Cu(II) ions with different concentrations on the determination of 1.0 μM Cr(VI).

Table 1
Summary of analytical performance of the proposed method.

Characteristics	*i*SEA-flow cell	*i*SEA-LWCC
Slope	0.1909 ± 0.0004	$0.0091 \pm 3.4E\text{-}5$
Intercept	-0.0031 ± 0.0006	0.0063 ± 0.0019
Linearity	$R^2 = 0.9992$, $n = 144$, 14 days	$R^2 = 0.9985$, $n = 110$, 14 days
MDL	0.024 μM	0.54 nM
RSD	2.65% for 0.2 μM ($n = 11$)	4.04% for 10 nM ($n = 10$)
	0.85% for 1.0 μM ($n = 11$)	0.47% for 20 nM ($n = 10$)
	0.40% for 4.0 μM ($n = 11$)	0.47% for 40 nM ($n = 10$)
Throughput	$> 30\,h^{-1}$	$20\,h^{-1}$

may differ from U or Z shaped flow cells [60], and might require better mixing conditions. It was assumed that for trace Cr(VI) determination with LWCC, insufficient mixing in the syringe resulted in differences in mixture homogeneity and reaction rate. To test the effect of mixing further, a series of mixed reagents (MR) was prepared by mixing 0.5% (*m/V*) DPC and H_2SO_4 solutions with concentrations ranging from 0.5 M to 2.5 M at volume ratio of 2:1. It was found that the signal and repeatability were improved by mixing MR with sample and propelling the mixture to the LWCC. However, the stability of MR was not satisfactory and all MR solutions developed appreciable color within one day (Fig. S2). It was found that, the higher the H_2SO_4 concentration, the faster was the color development. Deteriorated colorimetric reagents cause increase in absorbance, especially for blank solution, and the rate of deterioration may be related to environmental conditions such as temperature and humidity. The single DPC solution can be kept at room temperature for > 30 days, during which no significant loss of sensitivity was observed for the determination of 0–4.0 μM Cr(VI) standards with 5 cm flow cell (Fig. S3). The stability of MR might be improved if the concentrations of DPC and H_2SO_4 were much lowered. However, as shown in Fig. 2, the reaction time would be consequently prolonged.

As recommended by Amornthammarong et al. [54], a 5 mL pipette tip was utilized as a secondary mixing chamber. The dispersion and aspiration of the mixture took turns between the tip and the syringe. Complete mixing was achieved as evidenced by the good similarity in the results of the determination of mineral water samples by *i*SEA-LWCC and by manually mixing reagents and samples and then injecting for LWCC detection (see Fig. 6, red circles).

3.5. Analytical performance and validation

Under the optimized conditions, the analytical figures of merit of the proposed analyzer are summarized in Table 1. Calibration curves were constructed using the data obtained during 14 days, indicating good reproducibility of the method. Fig. 5(a) shows the calibration curve of *i*SEA-LWCC for Cr(VI) detection and the inset illustrates the
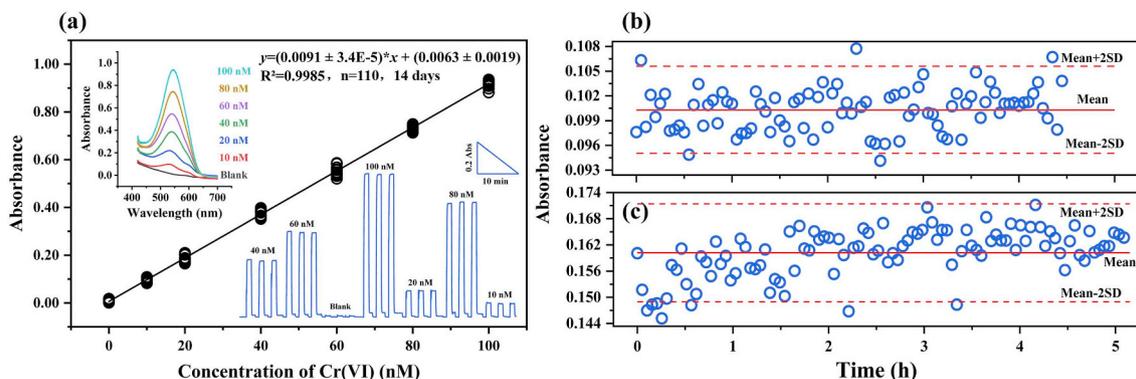
corresponding spectrum and typical signal output. For *i*SEA-flow cell, the calibration curve and typical signal output are presented in Fig. S4. Method detection limit (MDL) was calculated by three times the standard deviation (SD) of the measurement blanks ($n = 11$) divided by the slope of the calibration curve, and estimated to be 0.024 μM and 0.54 nM for 5 cm flow cell and 2.5 m LWCC, respectively. Repeatability of the analytical results was characterized by relative standard deviations (RSDs) of consecutive determinations, and the RSD values were within 5% for all concentrations (see Table 1), indicating high precision of the results. To further evaluate the reliability of *i*SEA-LWCC, continuous determination of 10 nM Cr(VI) standards ($n = 90$) and mineral water samples (~14 nM, $n = 100$) was performed (Fig. 5(b) & (c)). The corresponding RSDs were 2.63% and 3.50%, respectively, suggesting good working conditions for long-time continuous determination. Standard solutions (GBW(E)081584-1) were used to test the accuracy of the method. Results in Fig. 6 (blue triangles) indicated that concentration measured by *i*SEA agreed well with the certified values, showing good accuracy of the method. High throughput rates of > 30
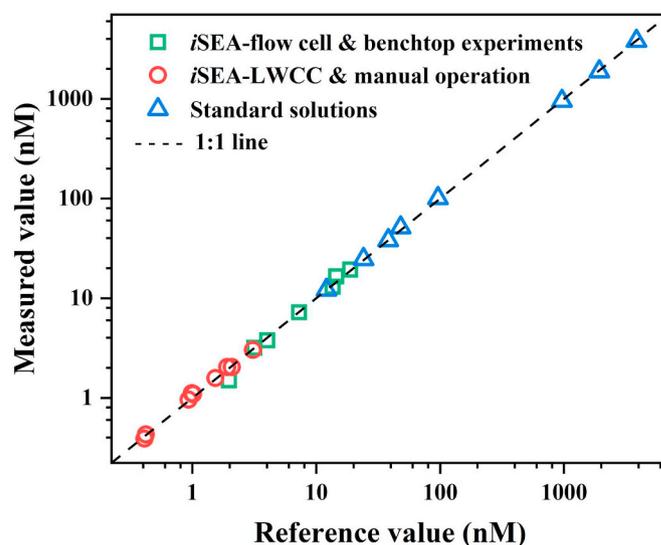
Fig. 6. Comparison of determination results of the proposed method with standards. Red circles denote results obtained by *i*SEA-LWCC and by manually mixing reagents and samples, followed by LWCC detection ($n = 7$). Green squares denote results obtained by *i*SEA-flow cell and by benchtop spectrophotometer with manual operation ($n = 12$). Blue triangles denote determination of standard solutions (analyzed after 500–160,000-fold dilution, respectively). Note the log scale of axes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 5. (a) Calibration curve for *i*SEA-LWCC Cr(VI) detection with concentration varying from 0 to 100 nM. Inset illustrates corresponding spectrum and typical signal output. (b) *i*SEA-LWCC continuous determination of 10 nM Cr(VI) standards ($n = 90$, RSD = 2.63%) and (c) mineral water sample (~14 nM, $n = 100$, RSD = 3.50%).

Table 2
Determination and spiked test of real samples by *i*SEA-flow cell.

Sample	Spiked (μM)	Found (μM)[a]	Recovery (%)
Tap water	0.0	ND[b]	–
	0.4	0.41 ± 0.01	102.3 ± 2.1
	1.0	1.01 ± 0.01	101.0 ± 1.1
River water	0.0	ND	–
	0.4	0.42 ± 0.01	105.5 ± 2.4
	1.0	0.99 ± 0.01	98.6 ± 1.3
Industrial wastewater 1[c]	0.0	0.93 ± 0.01	–
	1.0	1.91 ± 0.02	98.0 ± 1.6
	2.0	3.08 ± 0.03	107.2 ± 1.3
Industrial wastewater 2[c]	0.0	1.01 ± 0.00	–
	0.5	1.53 ± 0.02	103.9 ± 3.5
	1.0	2.08 ± 0.02	107.4 ± 2.1

[a] Mean ± standard deviation of three determinations.
[b] Not detected.
[c] Samples analyzed after 10,000-fold dilution with ultrapure water.

Table 3
Determined Cr(VI) level and recoveries of bottled water samples.

Water samples	Concentration (nM)	Spiked (nM)	Recovery (%)
Purified or pure drinking water	ND-2.6	10 or 20	90.2–100.7
Sample 1 (treated with two-pass reverse osmosis)	ND	10	97.6 ± 2.8
	ND	10	100.2 ± 1.2
Sample 2 (treated with nano-filtration)	ND	20	96.3 ± 0.5
	ND	20	99.2 ± 0.9
Natural mineral/spring/lake water	1.2–27.6	10, 20 or 40	90.6–116.6
Sample 1 (subject to deep filtration in Karst)	3.6 ± 0.1	10	93.7 ± 3.5
	3.9 ± 0.3	10	93.8 ± 2.2
Sample 2 (cold spring from effusive rocks)	14.5 ± 0.1	10	91.3 ± 8.3
	13.8 ± 0.3	10	112.6 ± 4.2
Drinking water with artificial additives	ND-1.5	10	89.5–99.2
Sample 1 (oxygenated drinking water)	ND	10	90.2 ± 0.7
	ND	10	95.7 ± 1.0
Sample 2 (KCl, MgSO₄ added, treated with two-pass reverse osmosis)	1.4 ± 0.1	10	95.4 ± 1.2
	1.3 ± 0.0	10	97.6 ± 0.5

h^{-1} and $20\,h^{-1}$ were obtained for *i*SEA-flow cell and *i*SEA-LWCC, respectively, which were adequate for routine analysis.

3.6. Application to water samples

The *i*SEA with 5 cm flow cell was successfully applied to determine Cr(VI) concentrations in tap water, river water and industrial wastewater. As can be seen in Table 2, concentrations in the tap water and river water samples were below the LOD. Two industrial wastewater samples contained very high levels of Cr(VI) so that they have to be diluted 10,000 times before analysis. Concentrations of the original samples were calculated to be 9.3 mM and 10.1 mM, respectively, and specific wastewater treatment should be carried out before discharge. Spiked recoveries at different levels in different matrices were in the range of 98.0%–107%, indicating no significant matrix interferences. However, analysis of other industrial wastewater may be performed with extra pretreatment efforts, such as extraction or masking of other metal ions, since components of wastewater of different origin can vary greatly. In addition, the determination and spiked test were performed on a commercial benchtop spectrophotometer with manual operation (see Fig. 6, green squares). Two sets of data passed paired *t*-test, with calculated *t* value (1.925) less than the critical value (2.228), showing no significant difference at 95% confidence level.

Ma et al. [48] previously found that major components in natural mineral waters such as Na^+, Cl^-, Mg^{2+}, SO_4^{2-}, HCO_3^-, Ca^{2+},

$HSiO_3^-$, K^+, Li^+ and Sr^{2+} (mg/L to g/L level) caused negligible interference in the determination of 10 nM Cr(VI). The *i*SEA with LWCC was utilized to determine Cr(VI) in 37 brands of commercially available bottled water samples. The samples were spiked with Cr(VI) standards of 10 nM, 20 nM or 40 nM concentrations to examine the measurement accuracy. Determined Cr(VI) levels and recoveries of the three categories of water are listed in Table 3. For each category, two typical samples were represented. Detailed detected concentration, spiked recovery, bottled water types, water sources, characteristic components and other useful information on the labels of all samples are provided in the Supplementary Materials.

Among eight brands of purified or pure drinking water, Cr(VI) was non-detectable in seven of them. This indicated that the reverse osmosis, nano-filtration and distillation techniques used in the purification processes of manufacturing bottled water effectively removed trace Cr(VI). Four brands of drinking water with artificial additives had comparable Cr(VI) levels (ND-1.5 nM) with the purified water samples. This is as expected because production of this type of water usually involves purification processes as well, as declared on the product labels (see Supplementary Material). The detected Cr(VI) concentration in 25 brands of mineral water ranged from 1.2 nM to 27.6 nM, substantially lower than the guideline value for drinking water (50 μg/L, ~0.96 μM) set by WHO and the Chinese government. However, all these samples exceeded the 0.4 nM Californian PHG, which confirmed the importance of developing high sensitivity and easy-to-use detection methods to monitor drinking water safety.

Water sources of natural mineral water are usually strictly protected. Therefore, the level of chemical components typically exists as background value. The distribution of water sources of 19 out of 25 brands of mineral water originating from China are plotted in Fig. S5. Cr(VI) as a hazardous material can certainly be an indicator for anthropogenic pollution. However, it can also be naturally occurring, especially in aquifers composed of mafic and igneous rocks [61]. Therefore, determining Cr(VI) concentration can be useful both in the field of environmental monitoring and environmental geological research or even epidemiological study. Certainly, the latter research needs far more data, and is out of the scope of this manuscript.

4. Conclusion

In this work, a rapid and reliable syringe-pump-based flow-batch analysis method has been developed for Cr(VI) detection in environmental waters and drinking water. Successful coupling of flow cell and LWCC demonstrated the ease and versatility of operation and wide linear range of this method. The accuracy of the method was validated with excellent results by measuring standard solutions, method comparison with benchtop or manual operation methods and satisfactory recovery of real samples. Compared with the standard method of ion chromatography-post column reaction, the proposed method is more suitable for routine determination and field application due to its simplicity, robustness and comparable sensitivity. If applied to a greater variety of samples with more complicated matrices, spectral and/or non-spectral interferences from natural organic matter and/or co-existing ions among others, and the elimination of them would need to be further investigated, especially for trace Cr(VI) determination. In the future, combining *i*SEA with on-line solid phase extraction or ion chromatography would provide more accurate data and serve as a more powerful tool for chromium monitoring. In addition, the proposed system can be utilized to study Cr(VI) reduction at nanomolar levels, which will lead to better understanding of the behavior of chromium in aquatic systems.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2018.12.040.

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