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Low voltage electrically stimulated lab-on-a-chip device followed by Red-Green-Blue analysis: A simple and efficient design for complicated matrices

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Abstract

In the present work, a simple and portable analysis device was designed for the first time for determination of lead ion as the model analyte. The basis of lead analysis is its extraction and preconcentration in an acceptor droplet via application of electrical field. The acceptor droplet is a KI solution and therefore, formation of yellow precipitation of PbI_2 is the sign of presence of lead ion in the solution. Following that, digital picture of the final acceptor droplet was analyzed by investigating its Red-Green-Blue (RGB) components. The results show that RGB intensities of the acceptor phase is proportionate to lead concentration in the sample solution. Also, a 9.0-V battery was used to apply the electrical field and other effective parameters such as the type of organic liquid membrane, pH of the sample solution, and the extraction time were considered to reach the optimal conditions. The model analyte was determined by extracting from 100 µL sample solution across a thin layer of 1-octanol, immobilized in the pores of a polypropylene membrane sheet, and into the acceptor droplet via applying a 9.0-V electrical potential for 20 min. The device is capable of determining the lead ion down to 20.0 ng mL^{-1} with admissible repeatability and reproducibility (intra- and inter-assay precision ranged between 3.8-7.0% and 9.8-11.9%, respectively). Also, calculated Error% for the model analyte in the range of -8.5 to +4.5 depicts that the chip offers acceptable accuracy for analysis of the lead ion. The linearity was studied in the range of 50.0-1500 ng mL^{-1} and the correlation coefficient was 0.9994. Ultimately, the device designed was employed for analysis of lead in real samples.

Keywords: Electromembrane extraction; RGB analysis; Lab-on-a-chip; Lead.

1. Introduction

The *in vitro* analysis of different compounds is an attractive topic in analytical chemistry. In recent years, new designs for miniaturized devices, named lab-on-a-chip (LOC) systems, were performed to attain the goal[1]. The main advantages of LOC systems are small sample requirements, short analysis time, portability, low cost, and low consumption of power. Thus, downsized installations or LOC systems were developed to perform laboratory analyses of chemistry, biology, and medicine over a miniaturized chip[2-6]. Also, electrophoretic techniques are perfectly suited for downscaling due to their smaller separation lengths, following by increase in electrical fields.

On the other hand, reduction of matrix effect, removal of interferences, and analyte preconcentration are important and necessary steps prior to analysis of target compound in a complicated medium. To this end, some extraction or sample preparation steps are necessary. Sample preparation is an important issue in analytical chemistry, and is often a bottleneck in chemical analysis. As a consequence, during the last decade new modern sample preparation methods were developed that can generally be classified as liquid based, solid based and membrane based techniques.

Among the present sample preparation methods, hollow fiber based liquid phase microextraction (HF-LPME) is known by its high sample cleanup ability, which makes it a suitable technique for analysis of dirty samples[7, 8]. However, relatively long process time due to its passive diffusion mechanism is the main drawback of this microextraction method. Therefore, electrical driving force was employed in HF-LPME to increase the efficiency and to reduce the extraction time for ionizable compounds[9]. This electrical stimulated technique was called electromembrane extraction (EME) by Pedersen-Bjergaard et al. and is based on the fact that

ionizable species can migrate in an electrical field. Thus, two electrodes were utilized in donor and acceptor phases, respectively to lead the target analytes from sample solution across the organic liquid membrane (immobilized in the HF wall pores) and into the final acceptor solution, which was placed in the lumen of HF[9]. EME as a new and powerful method was developed rapidly during the past years[10-23]. One of the most interesting applications of EME is designing the LOC systems[15-17]. This new concept of EME technique was first introduced in 2009[15] and improved during the past years[16, 17]. However, except for an attempt to electromembrane chip coupling to a detector (UV and mass spectrometer) for online analysis[17], there is no report for designing a chip device with both extraction and detection abilities.

In the present work, an electrical field-induced LOC design followed by RGB analysis is introduced for the first time to develop a portable device for analysis of lead ion in aqueous samples. Heavy metals, especially Pb, could cause serious threat to the environment and human health. Since the toxic metals tend to concentrate in all environmental matrices; they belong to the most deleterious pollutant group. Therefore, great attention must be focused on their concentration level. Until now, different microextraction techniques have been reported for determination of lead from various matrices[24-29].

For RGB analysis, an optical image of the acceptor droplet is decomposed into three components (red, green, and blue) using image processing software and is utilized as the concentration-dependent signal[30]. To this end, target ions were first extracted from few microliters of the sample solution through the organic liquid membrane, which was sustained in the pores of a sheet membrane into a droplet of the acceptor phase containing excess amounts of KI as the indicator. As the lead ions transfer into the KI solution, a yellow precipitation forms;

indicating the presence of the target analyte. Following that, the acceptor solution was online analyzed regarding the intensities of RGB components.

Herein, a simple and fast analysis technique based on the RGB image processing was employed for quantification of lead iodide in the acceptor solution. Finally, the designed LOC system was used for determination and quantification of analyte of interest in the samples of wastewater and street plant leaves.

2. Experimental section

2.1. Apparatus

The equipment used for the extraction procedure is shown in Fig. 1. A small hole with a volume of 100 µL was formed in a Plexiglass wafer and used as the sample compartment. Afterwards, the hole was coated utilizing a 1× 5-cm piece of aluminum foil with 15 µm thickness (Merck, Darmstadt, Germany) providing the electrical field. The platinum electrode used in this work, with the diameters of 0.25 mm, was obtained from Pars Pelatine (Tehran, Iran). The electrode and the aluminum foil were coupled to a common 9.0-V battery (GIL SUN, China) as the power supply. A 40-kHz and 0.138-kW (Tecno-Gaz SpA, Italy) ultrasonic water bath with temperature control and a Sepand Teb Azma centrifuge (Tehran, Iran) were used for real sample pretreatment steps.

An atomic absorption spectrometer (GBC 932 plus, Australia) equipped with a deuterium arc background corrector was used. A Pb hollow-cathode lamp was utilized as the radiation source at 217.0 nm. The optimum operating parameters were adjusted according to the manufacturer's recommendations.

2.2. Chemicals and materials

Pb $(NO_3)_2$, KI, 1-heptanol, 1-octanol, and 1-decanol were obtained from Merck (Darmstadt, Germany). All the chemicals used were of analytical reagent grades. The Accurel 2E HF (R/P) polypropylene membrane sheet (157 µm thickness, 0.2 µm pore size) was supplied by Membrana (Wuppertal, Germany). Ultrapure water was obtained from a Young Lin 370 series aquaMAX purification instrument (Kyounggi-do, Korea).

2.3. Standard solutions and real samples

A stock solution containing 1 mg mL^{-1} of lead ion was prepared in pure water and stored at 4 ˚C protected from light. Working standard solutions were prepared by dilution of the stock solution in pure water.

Wastewater samples were filtered through 0.45-µm pore size cellulose acetate membrane (Millipore, Billerica, MA, USA) filters prior to extraction.

Also, plant leaves were analyzed as the real sample. For analysis of the target heavy metal in the plant tissue, leaves were collected from city streets. The whole plant leaves were completely blended. Then, 10 mL of a 100 mM HCl solution was added to 50 g of each blended sample and it was immersed into the ultrasonic water bath. After 2 min of sonication (at 25±3 ºC), the sample was centrifuged at 2500 rpm for 10 min to separate the phases. The final liquid phase was diluted (1:9) with pure water and after pH adjustment (the pH was adjusted at 6.5 via addition of proper amounts of sodium hydroxide solution), it was transferred into the EME sample hole for analysis.

2.4. RGB analysis

Two detection modes (offline and online) were applied for analysis of the acceptor droplet. In offline mode, the droplet acceptor was collected after completion of the extraction

process and transferred into a scanning vial. The vial was scanned by a flatbed scanner (HP Photosmart C4283, Malaysia) and the variable factor of each component (red, green, and blue) was determined using Photoshop® software (Adobe systems, USA) afterwards. The final signal was calculated by additive subtraction of RGB intensities from white.

Also, the online mode was examined to design a portable device for lead analysis utilizing a smartphone (iPhone, USA) as the detector. In this mode, a smartphone was placed in 10 cm at the top of the LOC system. When the extraction was completed, a digital photo was obtained from the phone camera and analyzed using the iDropper tool, which is available for iOS smartphones. Again, the concentration-related signal was calculated by subtraction of RGB intensities from white.

2.5. Electromembrane chip procedure

One hundred microliters of the sample solution containing the model analyte in pure water was transferred into the sample vial, which was first coated by the aluminum foil. To impregnate the organic liquid membrane in the pores of the polypropylene sheet, a 1× 1-cm piece of the sheet membrane was cut out and dipped in the 1-octanol for 5 s. Also, 1 M of the KI solution was used as the acceptor droplet located at the top of the membrane sheet. Finally, the platinum electrode (the cathode) was directly introduced into the 15 µL of acceptor droplet. The electrodes (the aluminum foil and the platinum electrode) were subsequently coupled to the 9.0-V battery and the extraction unit was fixed for beginning of the process. When the extraction was completed, the final acceptor droplet (10 ± 1 µL) was analyzed by offline and online modes.

2.6. Data analysis

RGB analysis were performed using Adobe Photoshop® CS6 software and iDropper tool version 1.1, which is available for iOS smartphones.

3. Results and discussion

Considering previous experiences[9-23], parameters effective on efficiency of electromembrane systems are composition of organic liquid membrane, donor and acceptor phases, applied voltage, and extraction time. In this work, a common battery was used for electric field formation. Hence, applied voltage was excluded from effective variables and 9.0 V was utilized for all the experiments. Also, different signals related to red, green, and blue components were used to measure the final concentration of PbI_2 in the acceptor droplet. Fig. 2A confirms that maximum sensitivity was gained employing the blue color and therefore this RGB component was used in all the experiments.

3.1. Selection of organic liquid membrane

An adequate liquid membrane for extraction of a target analyte should have some properties. The supported liquid membrane (SLM) should have a suitable viscosity and polarity and the analyte should have an acceptable solubility in the SLM. Also, the main characteristic of the SLM is its immiscibility in water. Therefore, long-chain alcohols were studied as the organic solvent. To this end, 1-heptanol, 1-octanol, and 1-decanol were tested for extraction of lead ion from the aqueous medium. The results obtained showed that 1-octanol is the best choice for this purpose. Better behavior of 1-octanol in comparison with 1-heptanol may be justified by its higher electrical resistance that decreases the electrical current and increases the stability of the chip system. Generally, the current level through SLM in EME is a key parameter which should

be kept at relatively low levels during extraction. This issue is provided by organic solvents with proper electrical resistance. For linear alcohols, the polarity is increased and the viscosity is decreased via decreasing the number of carbon atoms. On the other hand, the electrical resistance is decreased and the electrical current is increased by increasing the polarity of alcohols as SLM. Therefore, more repeatable results were obtained by 1-octanol. Moreover, high viscosity of 1-decanol as well as its tendency for solidification due to its low boiling temperature makes transportation of the analyte into the organic phase difficult and decreases the extraction efficiency. Thus, 1-octanol was selected as the SLM for rest of the experiments.

3.2. Investigation of extraction time

EME is a non-exhaustive extraction method. Therefore, extraction time is one of the most important influential parameters, which should be investigated. The RGB analysis was carried out after extraction of the model analyte for various durations in the range of 120 min. Results in Fig. 3A illustrate that the best extraction efficiency was obtained by performing the EME process for 20 min. Since stagnant conditions were applied for lead analysis (neither donor phase nor acceptor phase were agitated), relatively long extraction time is required for best extractability achievement and RGB signals were improved by increasing the extraction time. More increase in the extraction time decreases the repeatability due to evaporation of the acceptor droplet and change of its volume. It can be seen from Fig. 1 that the setup used has no shielding for the acceptor phase and it could gradually evaporate. This is while SLM evaporation may occur by increasing of the extraction time, which disturbed the EME process. Since SLM is the main electrical resistance of the system, local loosing of the liquid membrane as a result of its evaporation leads to rising of electrical current level, increase in the electrolysis reactions, and bubble formation. Therefore, 20 min would be the optimal extraction time.

3.3. Investigation of composition of donor and acceptor phases

Since formation of PbI_2 precipitate is the indicator for lead existence, 1 M of the KI solution was used as the acceptor droplet. Considering the donor phase, it should have a suitable pH; such that the analyte of interest carries a net positive charge and migrate toward the cathode in the electrical field. So, the effect of HCl concentration was investigated in the range of 0-100 mmol L^{-1}. Previous studies proved that the flux of analyte in EME system has a relationship with the ion balance, which is defined as ratio of the total ionic concentration in the sample solution to that in the acceptor phase[31]. Also, it was shown that the extractability would reduce by increasing the ion balance. Therefore, it was anticipated that the final signal of lead analysis will decrease by increasing the concentration of H^+ or decreasing the pH value in the sample solution. Results in Fig. 3B confirm this and as can be seen, the best recoveries were obtained by extracting the lead ion from pure water with pH = 6.5 and into the KI solution.

3.4. Evaluation of method performance

Figures of merit of the designed system were considered in pure water to evaluate the practical applicability of electromembrane chip for quantification of lead ion. Thus, optimal conditions were applied and calibration curves were plotted. The results summarized in Table 1 show that the proposed device is capable of determining the lead ion down to 20 ng mL^{-1} and the limit of quantification (LOQ) was 50 ng mL^{-1}. Linear range between 50-1500 ng mL^{-1} and acceptable correlation coefficient (R^2 = 0.9994) were obtained. Precision, defined as the relative standard deviation (RSD%), was determined by intra- and inter-assays by five replicate measurements at three concentrations (70, 500, and 1000 ng mL^{-1}). Regarding the RSD% in low, middle, and high concentrations, intra- and inter-assay precision in the range of 3.8-7.0% and 9.8-11.9%, respectively, were achieved (Table 2).

To validate the obtained results by the proposed methods, the acceptor phase was analyzed by both RGB and electrothermal atomic absorption spectrometry (ETAAS) after EME. For this purpose, a same sample solution spiked at the concentration of 100 ng mL^{-1} was extracted using EME-based LOC system. In the case of ETAAS, the acceptor droplet was diluted 50-fold with ultrapure water since the dynamic linear range of ETAAS system was in the range of 0-40 ng L^{-1},. Determination of Pb by each detection technique was replicated three times and the calculated averages were compared by means of t-test at 95% confidence limit. The results in Table 3 indicated no significant differences between calculated concentrations using both detection techniques ($t_{(4, 0.05)} = 1.626$, $t_{Table} = 2.776$).

Comparison of the designed electromembrane chip with other existing methods for determining of the lead in different plant tissues is provided in Table 1. The aim of this work is to introduce a simple, inexpensive and portable system to analyze Pb in complicated matrices due to its ability to providing sample preconcentration and cleanup using a membrane sheet. The proposed LOC system demonstrated wide linearity range, high sensitivity, and acceptable repeatability and reproducibility in a relatively short time.

The limit of Pb in drinking water is 50 µg L^{-1} that is higher than the obtained LOD by the proposed method[43]. On the other hand, the provided LOD is comparable with some traditional methods such as conventional EME followed by CE-UV[39].

Lower detection limits can be obtained by more sensitive detection techniques such as ETAAS, inductively coupled plasma optical emission spectrometry (ICP-OES) or mass spectrometry (ICP-MS) and x-ray fluorescence spectrometry (XRF), that are very expensive, not applicable in field analysis and also some of them are not available in common laboratories.

Application of a smartphone as the detector not only eliminates the need for expensive and unavailable analytical instruments, but also provides a simple, inexpensive, fast, and portable analysis device. Furthermore, the LOC system offers a ligandless analysis method required only 100 µL of sample to provide the sensitivity for Pb analysis down to 20 ng mL^{-1}.

It should be noticed that other metal ions can be extracted from yellow precipitates or color solution. However, the present work is the first attempt to design a portable analysis device via coupling an electromembrane chip to a smartphone and Pb was selected as the model ion to introduce the set-up. Moreover, leaves of street plants were selected as real samples due to the highest probability of lead existence in this matrix in comparison with other metal ions reacting with I^-, which is attributed to fuel consumption by vehicles. A lot of reports can be found in the literature related to lead accumulation in street plants due to petrol-fueled vehicles[44, 45].

Moreover, one of the interesting points that can be considered for the introduced method is simultaneous extraction of different metal ions and analysis by multivariate curve resolution techniques. Additional experiments are undergoing and the results will be published in the future.

3.5. Analysis of real samples

The designed electromembrane chip was employed for analysis of a model heavy metal in different real samples including wastewater samples and some plant tissues to consider its practical applicability. Wastewater samples were filtered via a cellulose acetate membrane filter and their pHs were adjusted to 6.5 prior to analysis. The whole plant leaves collected around the city were blended, sonicated, and centrifuged as it was described in section 2.3. The pH of the final 10-fold diluted liquid extract was adjusted to 6.5 via addition of proper amounts of sodium hydroxide solution and the optimal conditions of EME process were applied for quantitative

analysis. Finally, the iDropper tool of an iOS smartphone was utilized for analysis of the final droplet. The RGB analyses of the acceptor solution, which were obtained from the wastewater sample is shown in Fig. 4. Since some real samples (plant tissues) were transformed into miry complicated solutions, some experiments were necessary to determine whether the calibration curves could be directly used for analysis of real samples or not. A matrix effect is the direct or indirect alteration or interference in response due to the presence of unintended analytes or other interfering substances in the sample. Thus, accuracy (Error%) was determined by addition of 50 ng mL^{-1} of each analyte into real samples and applying EME afterwards. The relative recovery (*RR%*) and accuracy (Error%) were calculated by the following equations:

$$RR\% = \frac{C_{found} - C_{real}}{C_{added}} \times 100 \quad (1)$$

$$Error\% = RR\% - 100 \quad (2)$$

where C_{found}, C_{real}, and C_{added} are the concentrations (ng mL^{-1}) of analyte after addition of known amount of standard into the real sample, the concentration of analyte in real sample, and the concentration of known amount of standard spiked into the real sample, respectively.

The results in Table 4 show that no significant matrix effect was observed for the real samples studied and admissible precision values were obtained. Therefore, in analysis of real samples, calibration curves could be used directly.

4. Conclusions

An EME-based LOC system followed by RGB analysis was introduced for the first time for analysis of lead as the model analyte in different matrices. It was shown that the intensity of red, green, and blue components of the final acceptor droplet is proportionate to the lead concentration. Therefore, RGB analysis of the digital picture taken from the acceptor solution

could be used for quantification of the target analyte. Combination of EME-based LOC system and a smartphone with the ability of RGB decomposition constructed a portable device for lead analysis in different matrices. However, although there are some reports for designing electromembrane chips, further studies are required to gain a fully portable and practical device.

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Figure captions

Fig. 1. The equipment used for the electrically induced LOC system (A) and detection of yellow participate with RGB analysis (B).

Fig. 2. (A) Comparison of sensitivities of different red, green, and blue components for lead quantification in the acceptor phase. RGB analysis of (B) acceptor droplet for extraction from sample contains 500 ng mL^{-1} lead ion and (C) white obtained by Photoshop® software.

Fig. 3. Optimizations of (A) extraction time and (B) composition of donor phase. Lead ions were extracted from sample solution across 1-octanol as the SLM and into 1M KI solution by application of 9.0 V electrical field.

Fig. 4. EME of wastewater sample contains 87.5 µg L^{-1} of lead ion followed by RGB analysis using iDropper tool of an iOS smartphone.

Table 1
Comparison of the proposed method with other analytical techniques for determination of Pb^{2+} in different samples.

Analytical technique [a]	Sample	Extraction time	Linear range	R^2	LOD	RSD%	Ref.
MW-ICP-OES	Dried fruit	> 31 min	10-320 µg L^{-1}	0.99994	-	< 11.4	32
USS-ETV-ICP-MS	Herbs	> 10 min	-	> 0.9992	0.2 ng g^{-1}	<4.0	33
Dried digestion-CdTe QDs	Spinach and citrus leaves	> 7-8 h	1.96-25.9 nmol L^{-1}	0.9960	4.7 nmol L^{-1}	<4.8	34
AD-VM	Vegetables	> 2 h	-	> 0.9989	43-51 ng g^{-1}	< 5.0	35
USAD-XRF	Anatomical part of plants	> 20 min	-	-	2.9 µg g^{-1}	< 9.0	36
VLLME-FAAS	Hair and urine	> 10 min	5–200 µg L^{-1}	0.992	0.307 µg L^{-1}	4.09	37
SM-DLLME-SFOD-ETAAS	Food and water	> 5 min	0.1–30 µg L^{-1}	0.9995	27 ng L^{-1}	> 2.5	38
EME-CE-UV	Biological fluids and lipstick	> 15 min	0.1–10 mg L^{-1}	0.9935	19 µg L^{-1}	4.9-15.6	39
DLLME-D-µ-SPE-FAAS	Water, plant and hair	> 2.5 min	1.9-? µg L^{-1}	0.991	0.57 µg L^{-1}	< 7.5	40
TC-IL-ME-FAAS	Hair	> 14 min	19.3-? µg L^{-1}	-	5.8 µg L^{-1}	-	41
HF-SPME-ICP-MS	Gasoline and diesel samples	> 40 min	0.8–100 µg L^{-1}	0.9967	0.3 µg L^{-1}	2.5	42
LOC-RGB	Plant leaf	> 22 min	50-1500 ng mL^{-1}	> 0.9994	20 µg L^{-1}	< 7.0	This work

[a] Microwave (MW), Ultrasonic slurry sampling (USS), Electrothermal vaporization (ETV), Quantum dots (QDs), Acidic digestion-Voltammetric method (AD-VM), Ultrasound assisted digestion (USAD), Vortex-assisted liquid–liquid microextraction (VLLME), Flame atomic absorption spectrometry (FAAS), Supramolecular solvent (SM), Dispersive liquid–liquid microextraction (DLLME), Solidification of floating drop (SFOD), Electromembrane extraction (EME), Capillary electrophoresis (CE), Dispersive micro solid-phase extraction (D-µ-SPE), Temperature controlled ionic liquid based microextraction (TC-IL-ME), Hollow fiber-solid phase microextraction (HF–SPME).

Table 2
Accuracy, precision, and relative recovery of the designed LOC system for determination of lead ion in pure-water.

Conc. (ng mL^{-1})	Accuracy (Error %)		Precision (RSD %)		RR%
	Intra-assay (n = 3)	Inter-assay (n = 3)	Intra-assay (n = 5)	Inter-assay (n = 3)	
70	+2.6	-1.9	5.7	10.2	102.6
500	-2.7	-8.5	3.8	11.9	97.3
1000	-3.0	+4.5	7.0	9.8	97.0

Table 3
The t-test results to validate LOC-RGB method.

	Detection technique	
	RGB	ETAAS
Pb Conc. (ng mL^{-1})	95.2	92.1
	104.2	89.0
	108.5	102.7
Average	102.633	94.6
Standard deviation	6.787	7.184
S_{pooled}	6.988	
$t_{(4, 0.05)}$	1.626	$t_{(4, 0.05)} < t_{Table}$
t_{Table}	2.776	Not significant

Table 4
Lead determination in real samples.

Sample	C_{real} (µg L^{-1})	C_{added} (µg L^{-1})	C_{found} (µg L^{-1})	RSD%	Error%
Wastewater 1	<LOQ	50.0	52.1	5.9	+4.2
Wastewater 2	87.5	50.0	135.8	8.3	-3.4
Plant 1	<LOQ	50.0	47.6	6.5	-4.8
Plant 2	345.4 (0.69 µg g^{-1})	50.0	391.6	7.8	-7.6
Plant 3	148.2 (0.30 µg g^{-1})	50.0	202.9	6.4	+9.4

Fig. 1

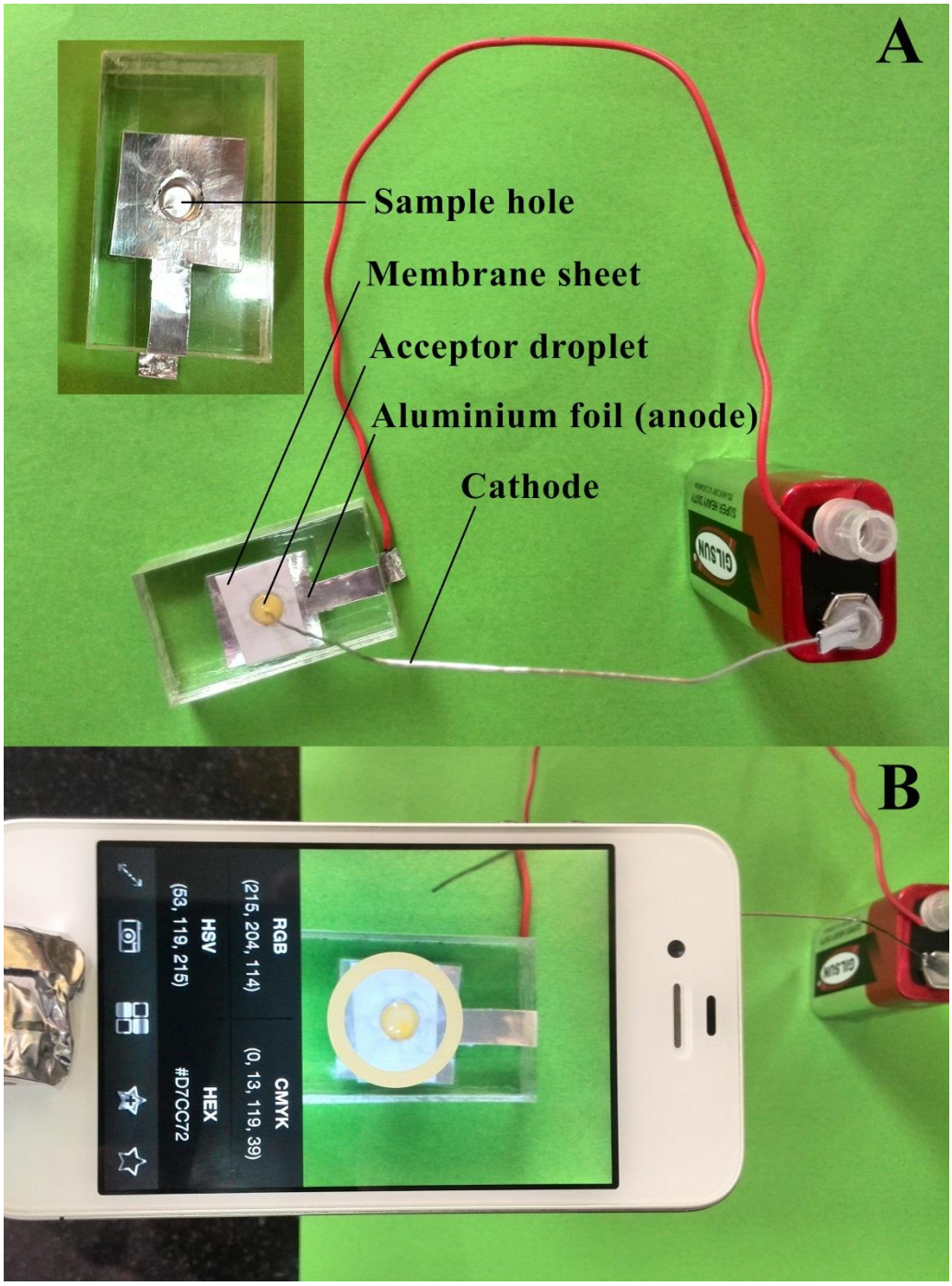

Fig. 2

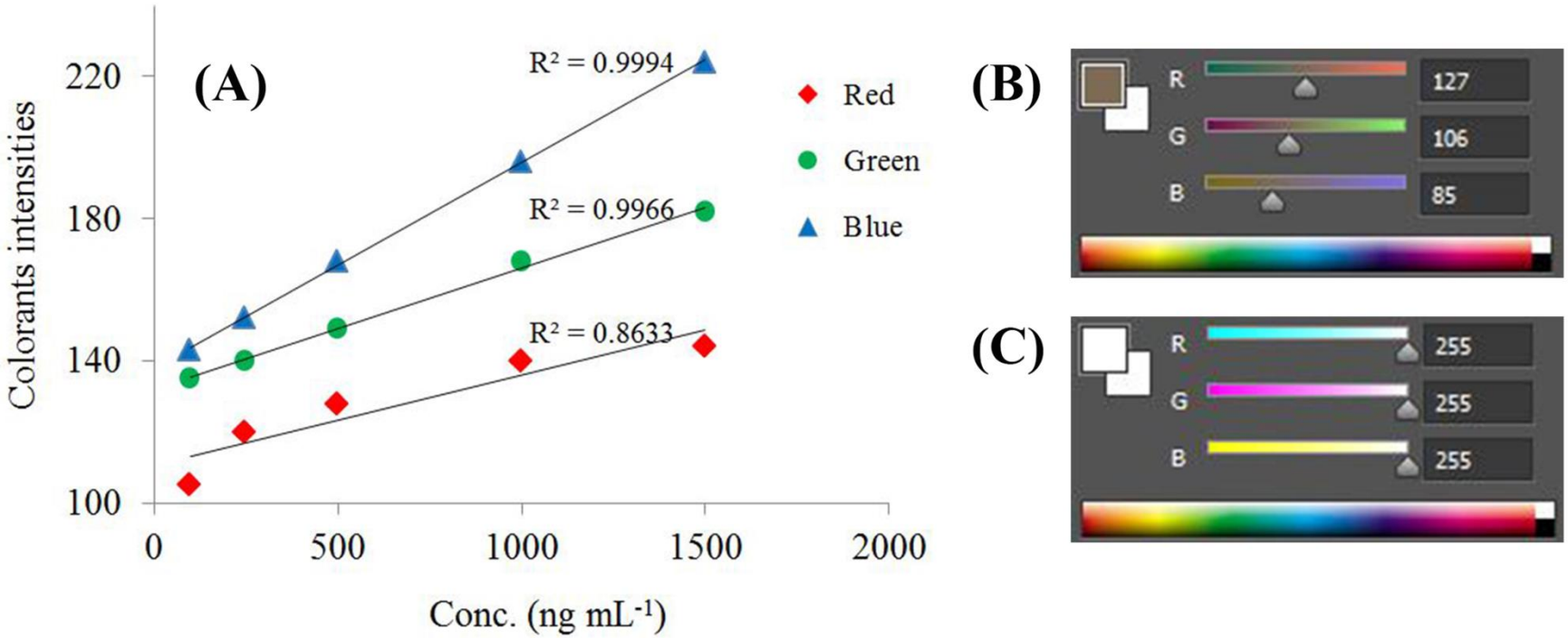

Fig. 3

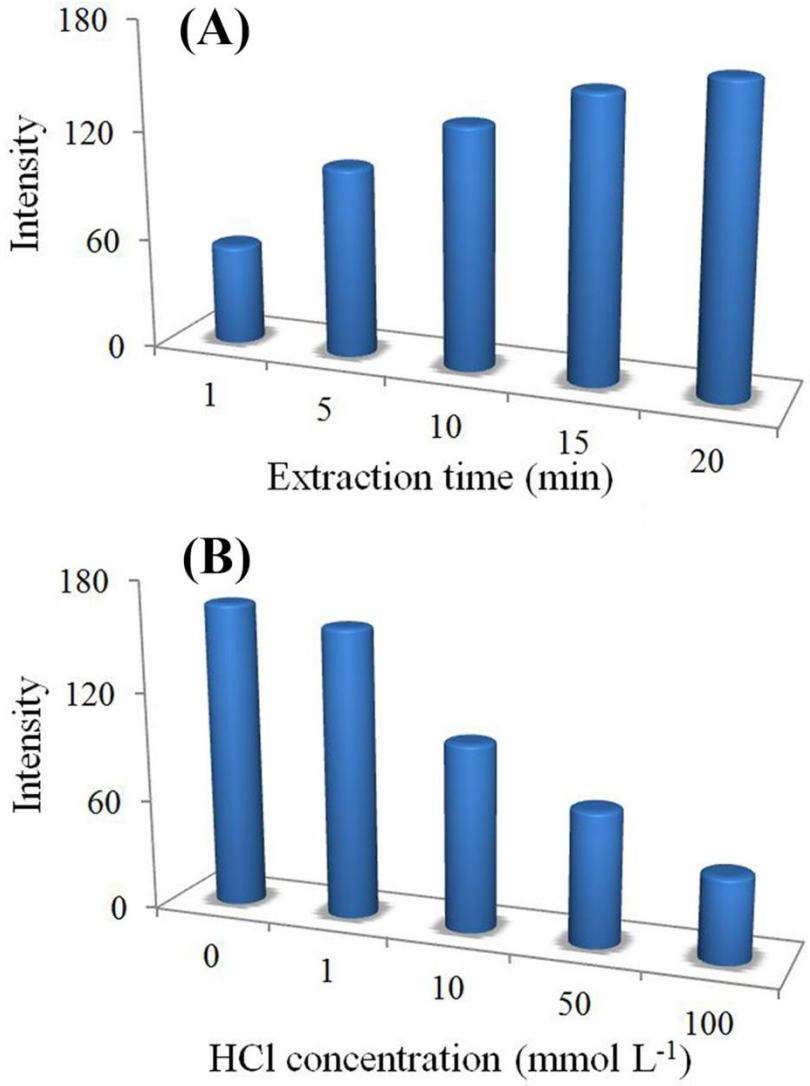

Fig. 4

Low voltage electrically stimulated lab-on-a-chip device followed by Red-Green-Blue analysis: A simple and efficient design for complicated matrices

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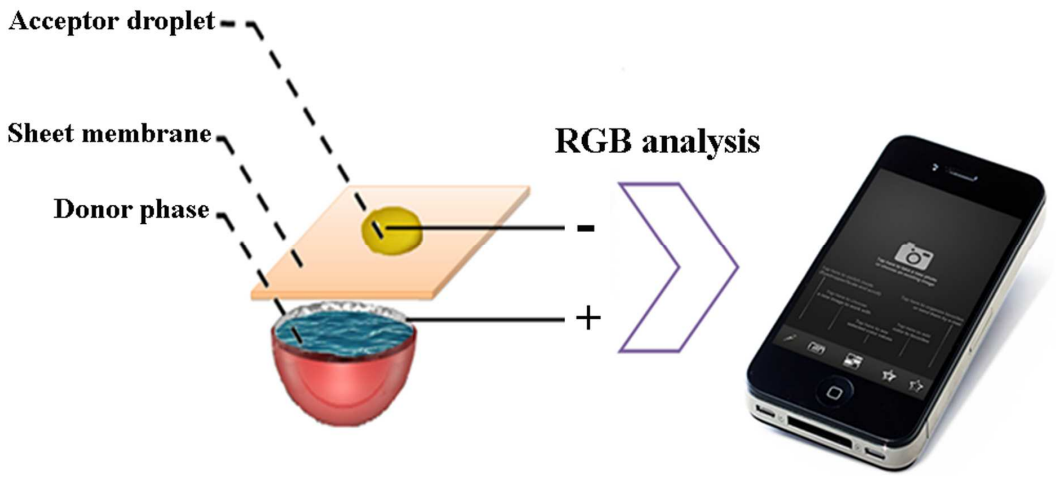

An electrical field-induced lab-on-a-chip design followed by RGB analysis is introduced to develop a portable device for analysis of Pb^{2+}.

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