Talanta 202 (2019) 436–442

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Capturing hemoglobin on graphene sheet from sub-microliter whole blood for quantitative characterization by internal extractive electrospray ionization mass spectrometry

Lili Song[a], Huanwen Chen[a], Mingyuan Ma[c], Zhiwen Liu[a], Feifan Jie[a], Liqun Lan[a], Rong Hua[b,**], Shuanglong Wang[a,*]

[a] Jiangxi Key Laboratory for Mass Spectrometry and Instrumentation, East China University of Technology, Nanchang, 330013, PR China
[b] School of Nuclear Science and Engineering, East China University of Technology, Nanchang, 330013, PR China
[c] Shanghai Wufeng Scientific Instruments CO., LTD, Shanghai, 200333, PR China

ARTICLE INFO

Keywords:
Quantitative blood sampler
Graphene
Internal extractive electrospray ionization
Mass spectrometry
Hemoglobin

ABSTRACT

A disposable blood sampler, which is consisted of a sub-microliter whole blood collector and a graphene filter, loading graphene sheet to selectively capture hemoglobin from sub-microliter whole blood, was developed for both qualitative and quantitative characterization hemoglobin by internal extractive electrospray ionization mass spectrometry (iEESI-MS). The blood collector was elegantly fabricated in syringe-like fashion for precisely sampling tiny amounts (1.0 μL - 2%) of whole blood, which was immediately diluted by water inside the syringe and was then pressed through the graphene filter placed between the waste outlet and the syringe reservoir to capture the hemoglobin in the blood sample. Then the graphene with hemoglobin was directly eluted by a charged (+2.5 kV) solution (mathanol/water/formic acid, 48/48/4, *v/v/v*) to produce the hemoglobin ions for mass spectrometric analysis. Low detection-of-limit (19.3 mg L^{-1} (89.5 picomol)), acceptable linear response range (300–1500 mg L^{-1}, R^2 = 0.998), relative standard deviation (0.5–6.5%, n = 3), low sample consumption (≤1.0 μL) and a relatively high speed (≤4 min per sample, including the sample loading) were achieved, demonstrating that the graphene based iEESI-MS was an alternative choice for direct detection of hemoglobin in whole blood with minimal sample consumption.

1. Introduction

Hemoglobin level in blood provides valuable information for diagnostics of abnormality of health such as anaemia, heart diseases and cancers at late stages [1]. Routine testing of hemoglobin with minimal invasion and least sample consumption appears particularly important. Traditionally, hemoglobin of whole blood is photometrically measured with the Sodium-Lauryl-Sulfate (SLS) reagent, which has been recommended by *the International Committee for Standardization* in hematology [2]. However, the SLS hemoglobin method has been suffered from the interferes such as hyperlipidemia, hyperglobulinemia, high platelets count (> 700 × 10^9 L^{-1}), high white blood cell count (> 30 × 10^9 L^{-1}) etc [3]. Immunoassay is another advanced method which was used for hemoglobin detection with high selectivity, high sensitivity (3.58 ng mL^{-1}, 2.22 × 10^{-4} picomol) [4] and low sample consumption (2 μL) [5]. However, the antibody is essential for the immunoassay test, resulting in high cost in preparation of immunoassay sensors [4–6]. Noninvasive hemoglobin monitoring techniques based on spectrophotometric methods are developed for real-time measuring [7,8], although poor sensitivity and inconsistent results were reported [9]. Ambient mass spectrometry provides premium sensitivity, high specificity and high throughput due to the requirement of no/minimal sample preparation [10–13]. Numerous applications have been successfully demonstrated by ambient mass spectrometry for direct detection of trace analytes in solid, liquid, gaseous and even viscous samples with complex matrices [14–17]. By flushing the 3-dimensional bulk substrates such as biological tissues using a charged solvent, the analytes imbedded in the bulk volume were selectively dissolved into the solvent. Driven by the syringe pump and the electric field, the charged solvent carried the analytes moving toward the ion entrance of the mass spectrometer and eventually produced the ionic plume on the sample surface under the ambient conditions, allowing the analyte ions

* Corresponding author.
** Corresponding author.
E-mail addresses: huarong80@126.com (R. Hua), jiayou1010@163.com (S. Wang).

https://doi.org/10.1016/j.talanta.2019.04.069
Received 31 October 2018; Received in revised form 25 March 2019; Accepted 27 April 2019
Available online 30 April 2019
0039-9140/

to be sampled into the mass analyzer for mass analysis. Since the extraction occurred inside the bulk sample, the method was featured with selective extraction and post electrospray ionization of the analytes distributed in a bulk 3-D volume. Therefore, the method was termed as internal extractive electrospray ionization mass spectrometry (iEESI-MS), which was introduced for characterization of interior chemicals of a bulk volume rather than the surface of solid samples [18]. Naturally, the iEESI-MS technique has been applied in characterizing biological bulk samples [19,20]. Alternatively, the bulk tissue sample could be replaced by artificial materials, solid substrates and heterogeneous subjects containing analytes of interest. Theoretically, trace analytes in a biological soup with tremendously complex matrices could be intentionally embedded with selectivity on such artificial materials, conceding a rough separation of the targeted analytes from the complex matrices. Once the analytes have been captured by the material, iEESI could be easily implemented on the analyte-loaded material for further reliable characterization by mass spectrometry.

With a perfect sp^2-bonded atomic-plane-thick 2D network of carbon atoms arranged in a honeycomb structure [21,22], graphene shows π-π interaction, carbon-π bonding, large surface area (theoretically, 2630 $m^2 g^{-1}$) [23], high stability, good flexibility and biocompatibility [24], making graphene a promising candidate for the selective adsorption [25,26] of various objects including methylated arsenic [27], polyaromatic hydrocarbon (PAHs) and their derivatives [28,29], amino acids [30], antibiotics [31], anionic dyes [32], pesticides [33]. In this study, graphene was chosen to advance iEESI-MS for quantitative detection of hemoglobin in whole blood of micro-liters. A precise quantitative blood sampler equipped with graphene was designed for accurate sampling of 1.0 μL whole blood and hemoglobin capturing. Then the graphene capturing hemoglobin with the complex matrix cleaned up was directly detected by iEESI-MS. The participation of blood sampler significantly improved the sensitivity of iEESI towards trace hemoblogin in whole blood. The method established was featured by high speed, unique sensitivity, easy sample preparation and low sample consumption.

2. Experimental section

All the experiments were carried out employing a linear trap quadruple (LTQ) mass spectrometer (Thermo Scientific, San Jose, CA, USA) coupled with a homemade material based-iEESI source. All the chemical reagents and mass spectrometer parameters were detailed in the supporting information (SI).

2.1. Design and characterization of the blood sampler

A precise quantitative blood sampler was designed based on the classical piston principle for whole blood sampling. The accurate sampling was guaranteed by the fine processing of the size and piston stroke of the piston rod and the innovative sample collecting method via replacing water instead of air. After sampling, the graphene acted as the adsorbent, which selectively adsorbed hemoglobin from the whole blood for quantitative analysis. In more details, the whole blood sampler was composed of 3 key components as shown in Fig. 1a: 1) a disposable syringe (volume of 2.5 mL), 2) the graphene which is in the syringe, 3) the blood volume control (BVC) part for precise sampling. This syringe was cheap, commercially available. For easy sampling with tiny amount of whole blood, the pointed pin-needle was replaced by a flat head needle (inside diameter of 0.4 mm, length of 20 mm). The BVC part was self-developed for accurate blood collection (1.0 μL). It connects the cavity of the syringe and the flat head needle via compression joint. As shown in the cutaway picture (Fig. 1a), BVC consisted 3 sections: a T-junction, a piston rod I and a locknut. For matching the head of piston rod I, a side hole on the T-junction was precisely machined with the manufacturing error around 0.01 mm. Once the piston rod I reached the right position, it was fixed in the T-junction by the locknut. To guarantee the well-tight sealed condition, the piston rod I was made of stainless steel, while the T-junction and the locknut were made of polyvinylidene fluoride and poly(ether-ether-ketone), respectively. Besides, the interference fit between the internal wall of the side hole and piston rod I was enabled, so that the side hole wall have a good tightness with piston rod I. 5.00 mm away (L) from the top of piston rod I, a shaft shoulder was set as the positioning element to define the piston stroke (h) of piston rod I with 3.00 mm (Fig. 1a). Before working, push piston rod I to the end, the head goes into the cavity of T-junction vertically like we showed in Fig. 1b–i. The diameter (d) of piston rod I head was 0.65 mm with the manufacturing error under 0.02 mm (resulted in the max volume error about 0.06 μL), which could be carefully adjusted with a spiral micrometer. According to formula ($V = \pi \times (d/2)^2 \times h$, where d was the diameter of the head of piston rod, h was the piston stroke of piston rod I), by pulling piston rod I to the bottom 1.0 μL in Fig. 1b–ii whole blood was intaking from the needle head. Then puling the piston rod I water will be uptaken in order to carry blood to the syringe. now blood mixed with graphene and water as Fig. 1b–iii shows.

A standard addictive technique was employed to characterize the accuracy of the whole blood sampler. Rhenium standard solution (1000 mg L^{-1}) was added into blood samples meanwhile the sampler was used to pretreat this well prepared sample. Then the concentration of rhenium was detected by inductively coupled plasma-mass spectrometry (ICP-MS). So the volume of the blood can be calculated by the concentration of the value. For quantification of rhenium, nitric acid (4%, v/v) as solvent, a series of rhenium standards at different levels such as 0, 0.0004, 0.001, 0.004, 0.01 and 0.02 mg L^{-1} were prepared for creating calibration curves. Then, whole blood sample contained known contents of rhenium was prepared by adding specific amount rhenium. First, rhenium standard solution (1000 mg L^{-1}) was neutralized by ammonia solution (5%, v/v) with the same volume. Second, the treated rhenium standard solution (500 mg L^{-1}, 8.0 μL) was added into whole blood (92.0 μL). Third, the whole blood (1.0 μL) contained rhenium was gathered by the sampler with no graphene involved. The operating steps were introduced for details in the next section. Fourth, the sample solution was transferred into a volumetric flask and diluted to 10 mL with nitric acid (4%, v/v). Finally, content of rhenium in the solution was determined by ICP-MS. Then the volume of the sampled blood can be calculated according to the formula: $V_{sampled} = \frac{V_{detected} \times C_{detected}}{C_{standard} / 2 \times 0.08}$, where $V_{sampled}$ was the volume of the blood sampled by the sampler, $V_{detected}$ was the volume of blood solution (10 mL), $C_{detected}$ was the value obtained by ICP-MS, $C_{standard}$ was the concentration of rhenium standard (1000 mg L^{-1}).

2.2. Graphene-iEESI-MS analysis

The experimental protocol of Graphene-iEESI-MS was schematically shown in Fig. 1c. 1) At the beginning piston rod I must be pushed to the end, the head goes into the cavity of T-junction vertically at this moment. Then Graphene (0.4 mg) was added into the cavity of the syringe. And the syringe was connected with BVC and flat head needle in turn. 2) Deionized water (around 100 μL) was uptaken into the sampler *via* pulling piston rod II of syringe like we showed in Fig. 1b–i. Bubbles were removed by pushing piston rod II slightly. 3) To avoid the graphene getting into the needle, the sampler was turned upside down while whole blood was collecting. When piston rod I was pulled to the bottom, blood (1 μL, about 1.2 μL of blood in total squeezed from the fingertip was needed) was sampled. The blood which was clinging on the needle was wiped off. At this time blood fraction stays in the head of the needle hollow as we see in Fig. 1b–ii. 4) The needle tip was inserted in deionized water again and the rest of the water from step 2 was uptaken into the sampler *via* pulling piston rod II. The water would carry the blood into the syringe. Now we can see blood in the syringe as showing in Fig. 1b–iii. Lightly red colored solution gathered in syringe.

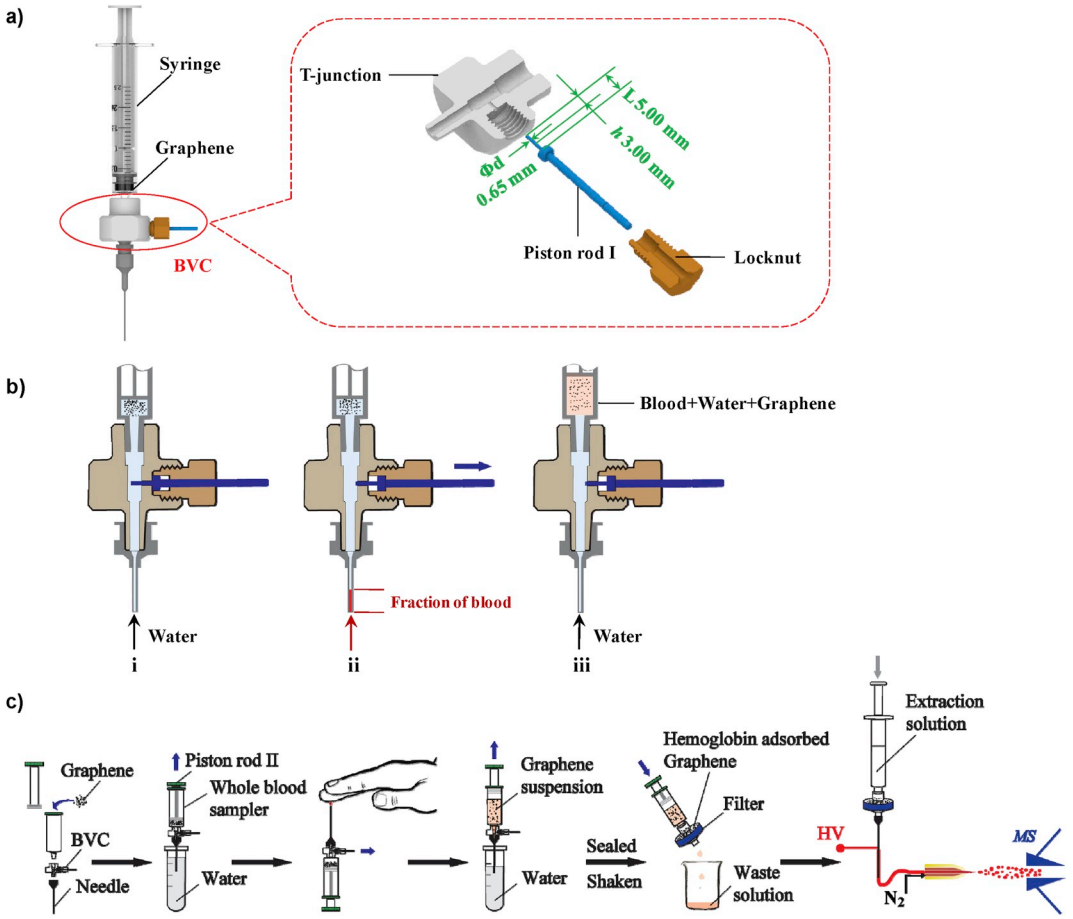

Fig. 1. Design and applying of the whole blood sampler. a) Composition of the sampler, b) Working schematic of the sampler, c) The sampling process of the sampler for Graphene-iEESI-MS analysis.

5) Remove BVC and the needle, then the syringe was equipped with a filter (with 0.45 μm filtration). Mixture of the diluted blood sample and graphene was heavily vortexed for 60 s. During vortexing, graphene could capture hemoglobin from the blood. After that *via* pushing piston rod II, the graphene loaded with hemoglobin would be gathered in the filter. Sample attached on the filter were washed three times by deionized water to largely remove the matrixes of the blood. 6) This well prepared filter above was assembled to the iEESI-MS source [34], hemoglobin adsorbed on material was extractively ionized online using extraction solution for MS analysis. The whole process was accomplished within 4 min (including the analyte capture and sample collection).

3. Result and discussion

3.1. Characterization of the whole blood sampler

The concentrations of rhenium were detected and the equation of calibration curves performs $y = 21.49x + 0.00$ ($R^2 = 0.9999$, Fig. S-1). All the standard samples were detected in triplicates. After that, samples were tested in five times and shown in Table S-1. The volume of the whole blood which was collected by the sampler could be calculated through the formula mentioned in section "Design and characterization of the blood sampler" and also shown in Table S-1, respectively. The experimental result indicated that the average of the sampling volume with five replicates was 0.98 μL, therefore relative error was −2%. And relative standard deviation between the five replicates was 13% (Table S-1). It showed high sampling accuracy of the whole blood sampler.

3.2. Optimization of the experimental conditions

The hemoglobin is a physiologically important globular protein, consisted by heme groups and subunit chains, of which two are *α*-chains and two are *β*-chians [35]. It has been well documented that the hydrophobic residues of protein, that is, the tryptophan/tyrosine residues in the hydrophobic interior of protein and the porphyrin rings of the heme group could connect with the conjugate π-electron moieties of graphene material *via* π-π stacking, leading to favorable adsorption [36,37]. There are four heme groups in the hemoglobin structure and no heme group is involved in other proteins and other compounds contained in whole blood. The hyrophobic interaction between Hb and graphene material is much stronger than that between the complex matrix and graphene, resulting in favorable adsorption efficiency for hemoglobin. This investigation provides a potential application of graphene for the selective isolation of hemoglobin from complex matrices. In this respect, graphene was employed as an attractive medium for the direct analysis of hemoglobin in whole blood by mass spectrometry. And for better performance, several experiments were designed to optimize the related parameters on the adsorption, desorption and ionization of hemoglobin in mass spectrometric analysis.

Hemoglobin (from swine) standard solution (750 mg L^{-1}, 300 μL) was detected using Graphene-iEESI-MS technique under the positive ion mode. According to the previous reports, hemoglobin molecule is prone to break down into their subunits during the electrospray ionization, i.e., *α*- and *β*-chians [38]. Therefore, native hemoglobins gave typical mass spectra, indicating cleavage of this molecule into its component parts [38]. As depicted in Fig. 2a, the *α*-chain was the dominant ion, while the *β*-chian had lower intensity. The *α*-chain

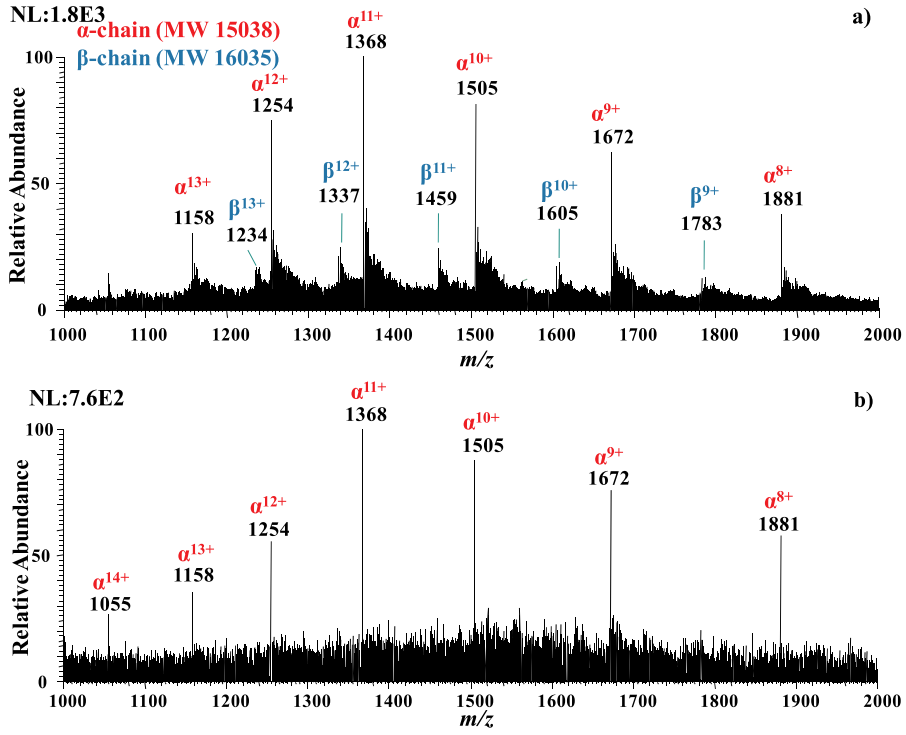

Fig. 2. Graphene-iEESI-MS spectra. a) hemoglobin (swine), b) swine whole blood.

carried between 8 and 14 charges producing ions with a mass-to-charge ratio (*m/z*) of 1000–2000. By an averaging calculation, molecular weights of 15038 Da and 16035 Da for the α-chain and β-chain of swine hemoglobin were obtained, respectively. They are in good agreement with the reported data [38]. To reduce the possibility of a false positive signal, an averaged MS/MS spectrum of ions of *m/z* 1368 detected with normalized collision energy (NCE) of 22% was shown in Fig. S-2a. The most intensity fragment ion (*m/z* 1350) was selected as quantitative ion for the quantification of hemoglobin in solution. Furthermore, the averaged MS/MS spectra of ions of *m/z* 1505 and *m/z* 1672 detected with NCE of 22% were described in Fig. S-2b and S-2c, respectively.

The parameters considered in this study are pH of sample solution, dosage of graphene and extraction solution (i.e., the solution for electrospray ionization). As illustrated in Fig. 3a, the highest signal intensity of the fragment ion (*m/z* 1350) was obtained when the graphene dosage reached 0.4 mg. And the waste solution was detected *via* ESI-MS, through which no obvious signal of hemoglobin was observed. Then, graphene powder material (0.4 mg) was used for subsequent experiments.

The pH levels of sample solution were selected around the isoelectric point of hemoglobin which was controlled using 4.0 mmol L^{-1} B-R buffer solution. Hemoglobin is a typical globular protein. It was documented that pH can lead a structural transition in hemoglobin [39]. The form of hemoglobin existing at pH < 3 was the well-known tight T form (deoxy form) which transformed into the loose R form (oxy form) above pH 3. With increasing of pH value, more hemoglobin molecules were in the R form. When the pH value was close to the isoelectric point value of hemoglobin (pH 6.8–7.0), the hydrophobic residues inside the framework of the hemoglobin molecules were prone to be exposed [40]. It facilitated the π-π stacking interactions between the oxygen-coordinated porphyrin ring and the graphene particles, which leaded to an effective adsorption of hemoglobin. In addition, when the whole blood was diluted with deionized water in the blood sampler, the solution was maintained at pH 6.8 without significant changes. For low cost and simple operation, the deionized water instead of buffer solution was employed for the sample solution in adsorption steps.

In the iEESI-MS analysis step, in order to achieve the highly efficient elution and good ionization efficiency of the hemoglobin captured on the graphene, the composite and flow rate of the extraction solution were also optimized. Similar to graphite, the isoelectric point of graphene is around pH 3.0 [41]. The surface of graphene would be positively charged during pH < 3.0. Thus, electrostatic repulsion between graphene and the positively charged hemoglobin might provide a

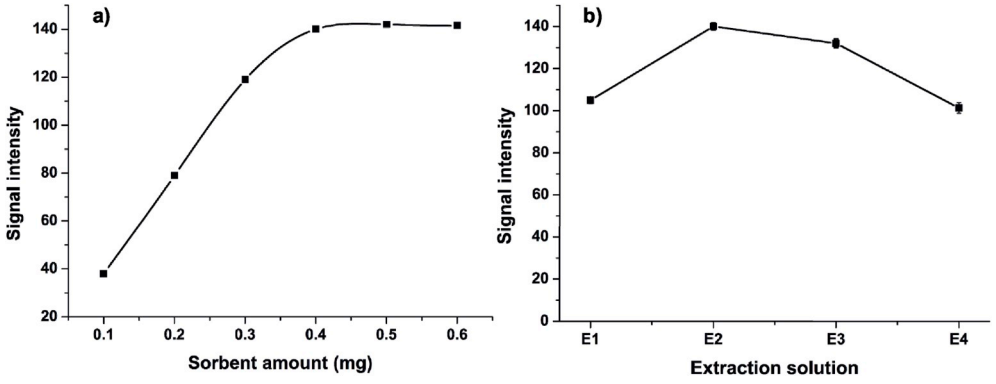

Fig. 3. Optimization of the experimental conditions. a) Signal levels with different sorbent amount, b) Signal levels obtained from hemoglobin samples against the chemical composition of the extraction solution (E1, methanol/water/formic acid (v/v/v, 49/49/2); E2, methanol/water/formic acid (v/v/v, 48/48/4); E3, methanol/water/formic acid (v/v/v, 47.5/47.5/5.0); E4, acetone/water/formic acid (v/v/v, 48/48/4).).

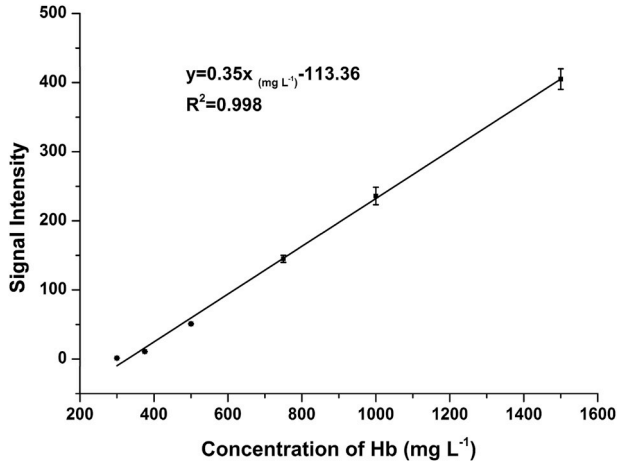

Fig. 4. Calibration curve of the hemoglobin (swine) determinated by Graphene-iEESI-MS using standard solution.

contribution to desorption. Otherwise, the graphene turned into negatively charged at pH higher than the isoelectric point, and thus electrostatic attraction leaded to a decline for protein desorption. According to the properties of hemoglobin and graphene, solvents with a relatively low pH level were preferred for the hemoglobin extraction. Acid solutions including methanol/acetone, water and formic acid were also tested (see Fig. 3b). Finally, optimized solution of mathanol/water/formic acid (v/v/v, 48/48/4, pH 2.0) at a flow rate of 5 mL min^{-1} was employed for Graphene-iEESI-MS analysis.

3.3. Quantitative analysis of hemoglobin

Series of hemoglobin standard solutions (300 μL) were detected to construct calibration curves. Under the optimum experimental condition, the signal intensity of *m/z* 1350 was linearly responded with swine hemoglobin concentrations over the range of 300–1500 mg L^{-1} (R^2 = 0.998) (Fig. 4). Each data point designated three measurements, and the relative standard deviations (RSDs) were between 0.5% and 6.5% (Table S-2). The limit of detection of hemoglobin defined by a signal-to-noise ratio (S/N) of three was 19.3 mg L^{-1} (89.5 picomol).

Furthermore, the analytical ability of the method in swine whole blood was experimentally evaluated. Apparently, the spectra showed ions corresponding to the α-globin portion of hemoglobin with matrix clean-up thoroughly. Since the concentration of the swine hemoglobin (Fig. 2b) in the prepared whole blood is lower than that in hemoglobin standard solution (Fig. 2a), which can be confirmed by comparing the intensity of α-chain of swine hemoglobin and hemoglobin standard solution. On the other hand, it seemed that the mass spectrum signal relative intensity of β-chain to α-chain from swine hemoglobin was much lower than that from human hemoglobin, both in our experiment result and reported literature [42]. Thus, β-chain of swine whole blood was hard to observe in the mass spectrum. Due to no heme groups inside the framework of the various proteins and other compounds contained in the blood, it resulted in low adsorption efficiency. Therefore, the complex matrix showed no significant interference. An averaged MS/MS spectrum of *m/z* 1368 ion was shown as Fig. S-2d. As the result, the average of hemoglobin concentration detected in the raw swine whole blood was 137.7 g L^{-1}, and the relative standard deviation (RSD) of triplicates was 6.7% (Table S-3). It was in good accordance with the result of 131.4 g L^{-1} obtained with the SLS hemoglobin method (Table S-3). Note that trace hemoglobin in whole blood could be intentionally enriched by the sampler for detection. With the sample loading, iEESI-MS can be performed to detect hemoglobin with high selectivity and excellent sensitivity.

Although, there are many methods concerning hemoglobin detection, he main purpose of methodology development for practice are targeted on time consumption and cost-saved. Compared with other reported hemoglobin detection methods (Table 1), such as lateral flow immunosensor, microarray immunoassay and aptamer-based label-free electrochemical biosensor array (in which recognition receptors obtained with comlex preparation process was required), the cost of material (graphene) is quite lower and easy to get. Moreover in this study total time consumption including sample preparation and detection are less than 4 min. Despite of the high sensitivity of the reported methods, the concentration of hemoglobin in blood are quite high. So higher sensitivity is an advantage but not very serviceable. In this case our study is believed to be more practical orientation for on-site facilitation of physical care.

3.4. SEM characterization

For comparison, SEM studies of grapene before/after adorption of hemoglobin and the one after iEESI-MS analysis were performed. All the samples were dried naturally before SEM study. Fig. 5a showed the SEM image of the pure graphene, which clearly illustrated the typically sheet-like shape with slightly scrolled edges. It was consistent with the previous report [43]. The perfect 2D graphene structures tended to alter into 3D structures for structural stability, so many wrinkles appeared on free-standing graphene [44,45]. Because of the strong Van der Waals forces between single layers, the graphene nanosheets tended to stack together [46]. Generally, the intrinsic wrinkles were essential for the structural stability of single layer graphene [44,47]. This 3D structure possessed large specific surface area and interconnected channels, and such structure features could facilitate the diffusion and adsorption of molecules in the network [48]. As anticipated, the SEM micrographs of graphene after hemoglobin adsorption exhibited drastic changes confirming the strong adsorption ability of the graphene to hemoglobin. More wrinkles were observed in the image (Fig. 5b) suggesting that adsorption with hemoglobin resulted in less flat surfaces but more grooved regions on the graphene [22]. To observe the efficient desorption of hemoglobin from this material after iEESI-MS analysis, the morphology was also interrogated. As described in Fig. 5c, the surface was much rougher than the one before iEESI-MS analysis owing to the successful desorption of hemoglobin. In addition, it was quite similar with the surface of pure graphene.

Table 1
Comparison on the analytical performances of our method versus other technique.

	Material needed	Sample volume	Rapidity of test	Sensitivity
This study	Graphene (purchased)	1 μL	≤ 4 min	89.5 picomol
Lateral flow immunosensor	Conjugates of detecting antibody and gold nanoparticles	2 μL	Approximately 20 min	NA[a]
Microarray immunoassay	Antibody microarray on a polydimethylsiloxane (PDMS) substrate modified with fluorinated compounds	4 μL	2 h incubation prior to test	2.22×10^{-4} picomol
Aptamer-based label-free electrochemical biosensor array	DNA aptamers	1 μL	> 30 min	2.11×10^{-5} picomol

[a] NA - Not available in the study.

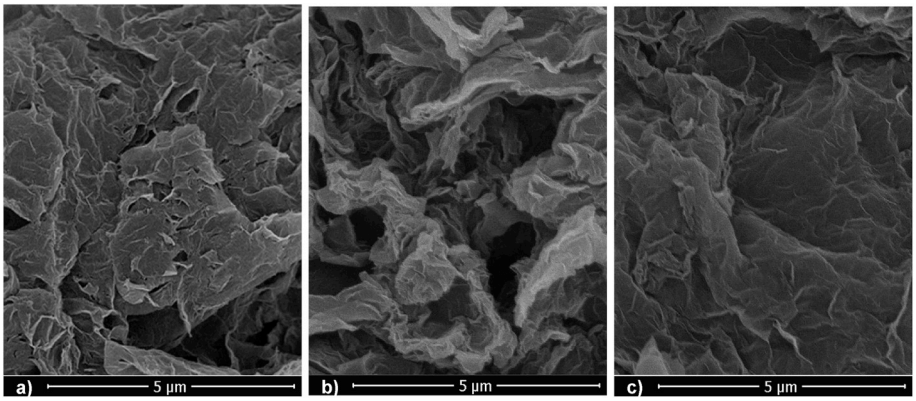

Fig. 5. SEM images of the graphene material. a) graphene, b) hemoglobin/graphene and c) hemoglobin/graphene after iEESI-MS analysis.

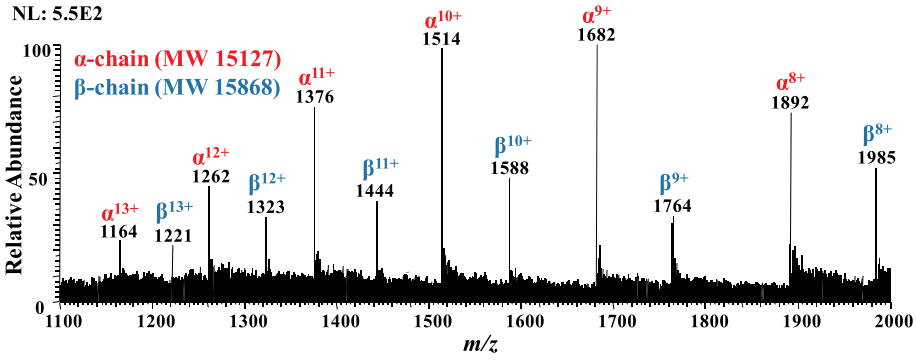

Fig. 6. Graphene-iEESI-MS spectrum of human whole blood.

3.5. Identification of hemoglobin from human whole blood

1.0 μL human whole blood was precisely sampled by the blood sampler. Then Graphene-iEESI-MS analysis technique was applied for hemoglobin analysis. As shown in Fig. 6, the spectrum showed ions corresponding to the globin portions of hemoglobin with matrix clean-up. Because the β-chain contained fewer basic amino acid residues than the *α*-chain and consequently ionizes less readily [49]. The peaks corresponding to the *β*-chain were less intense than those for *α*-chain. And the molecular weights obtained were comparable with the values in the literature, which were in excellent agreement [50,51]. Averaged MS/MS spectra of ions of *m/z* 1514 and *m/z* 1682 detected with NCE of 22% were shown in Fig. S-3a and S-3b. The most intensity fragment ions were proposed as quantitative ions for the quantification of hemoglobin in whole blood.

4. Conclusions

Conclusively, the sub-microliter sampler which was constructed performed accurate sampling volume in 1 μL with the deviation of −2%. With the help of loading graphene this equipment gives excellent performance of enriching hemoglobin and good compatibility with iEESI-MS technique. Trace amounts of hemoglobin were selectively captured on graphene from untreated whole blood sample, allowing the analytes of interest (e.g., hemoglobin) to embed onto graphene, and separating the analytes from the major part of the matrix. Once the analyte embedment was accomplished by the graphene, internal extractive electrospray ionization mass spectrometry (iEESI-MS) was easily implemented to characterize the analytes with high sensitivity and specificity. Under the optimized experimental conditions, a single sample run required less than 4 min (including the analyte capture and sample loading). The limit of detection was 89.5 picomol. This method established was featured by the high speed of analysis, unique sensitivity, easy sample preparation and low sample consumption. Furthermore, this work established a facile Graphene-iEESI-MS for advanced applications in trace analysis of other real-world samples.

Notes

The authors declare no competing financial interest and no conflicts of interest.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Nos. 21501025 and 21675021), Science and Technology Planning Project at the Ministry of Science and Technology of Jiangxi Province (No. 20171ACG70015),Jiangxi Key Laboratory for Mass Spectrometry and Instrumentation Open Foundation (Nos. JXMS201607 and JXMS201703), Research Foud of East China University of Technology (No. DHBK2017115).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2019.04.069.

References

[1] S. Naeeminejad, R.A. Darban, S. Beigoli, M.R. Saberi, J. Chamani, Studying the interaction between three synthesized heterocyclic sulfonamide compounds with hemoglobin by spectroscopy and molecular modeling techniques, J. Biomol. Struct. Dyn. 35 (2017) 3250–3267.
[2] A. Mess, J.P. Vietzke, C. Rapp, W. Francke, Qualitative analysis of tackifier resins in pressure sensitive adhesives using direct analysis in real time time-of-flight mass spectrometry, Anal. Chem. 83 (2011) 7323–7330.
[3] C.Y. Liu, C.L. Luo, Clinical Laboratory Medicine, fifth ed., People's Medical Publishing House, Beijing, 2012.

[4] H.H. Chen, C.H. Wu, M.L. Tsai, Y.J. Huang, S.H. Chen, Detection of total and A1c-Glycosylated hemoglobin in human whole blood using sandwich immunoassays on polydimethylsiloxane-based antibody microarrays, Anal. Chem. 84 (2012) 8635–8641.

[5] S.H. Ang, M. Rambeli, T.M. Thevarajah, Y.B. Alias, S.M. Khor, Quantitative, single-step dual measurement of hemoglobin A1c and total hemoglobin in human whole blood using a gold sandwich immunochromatographic assay for personalized medicine, Biosens. Bioelectron. 78 (2015) 187–193.

[6] S. Eissa, M. Zourob, Aptamer-based label-free electrochemical biosensor array for the detection of total and glycated hemoglobin in human whole blood, Sci. Rep. 7 (1016) (2017) 1–8.

[7] E. Gayat, A. Bodin, C. Sportiello, M. Boisson, J.F. Dreyfus, E. Mathieu, M. Fischler, Performance evaluation of a noninvasive hemoglobin monitoring device, Ann. Emerg. Med. 57 (2011) 330–333.

[8] J.Z. Yuan, L.U. Qi-Peng, J.L. Wang, H.Q. Ding, H.Z. Gao, W.U. Chun-Yang, L.I. Wan-Xia, Support vector regression for non-invasive detection of human hemoglobin, Chin. J. Anal. Chem. 9 (2017) 1291–1296.

[9] T. Xu, T. Yang, J.B. Kim, M.C. Romig, A. Sapirstein, B.D. Winters, Evaluation of noninvasive hemoglobin monitoring in surgical critical care patients, Crit. Care Med. 44 (2016) 344–352.

[10] M.Z. Huang, C.H. Yuan, S.C. Cheng, Y.T. Cho, J. Shiea, Evaluation of noninvasive hemoglobin monitoring in surgical critical care patients, Anal. Chim. Acta 702 (2011) 1–15.

[11] A. Venter, M. Nefliu, R.G. Cooks, Ambient desorption ionization mass spectrometry, Trac. Trends Anal. Chem. 27 (2008) 284–290.

[12] A.R. Venter, K.A. Douglass, J.T. Shelley, H.G. Jr, E. Honarvar, Mechanisms of real-time, proximal sample processing during ambient ionization mass spectrometry, Anal. Chem. 86 (2014) 233–249.

[13] G.A. Harris, A.S. Galhena, F.M. Fernández, Ambient sampling/ionization mass spectrometry: applications and current trends, Anal. Chem. 83 (2011) 4508–4538.

[14] H. Gu, X. Ning, H. Chen, Direct analysis of biological samples using extractive electrospray ionization mass spectrometry (EESI-MS), Anal. Bioanal. Chem. 403 (2012) 2145–2153.

[15] C. Guo, F. Tang, J. Chen, X. Wang, S. Zhang, X. Zhang, Development of dielectric-barrier-discharge ionization, Anal. Bioanal. Chem. 407 (2015) 2345–2364.

[16] X. Li, X. Wang, L. Li, Y. Bai, H. Liu, Direct analysis in real time mass spectrometry: a powerful tool for fast analysis, Mass Spectrometry Letters 6 (2015) 1–6.

[17] C. Wu, A.L. Dill, L.S. Eberlin, R.G. Cooks, D.R. Ifa, Mass spectrometry imaging under ambient conditions, Mass Spectrom. Rev. 32 (2013) 218–243.

[18] H. Zhang, H. Gu, F. Yan, N. Wang, Y. Wei, J. Xu, H. Chen, Direct characterization of bulk samples by internal extractive electrospray ionization mass spectrometry, Sci. Rep. 3 (2495) (2013) 1–6.

[19] H. Zhang, L. Zhu, L. Luo, N. Wang, K. Chingin, X. Guo, H. Chen, Direct assessment of phytochemicals inherent in plant tissues using extractive electrospray ionization mass spectrometry, J. Agric. Food Chem. 61 (2013) 10691–10698.

[20] H. Zhang, K. Chingin, L. Zhu, H. Chen, Molecular characterization of ongoing enzymatic reactions in raw garlic cloves using extractive electrospray ionization mass spectrometry, Anal. Chem. 87 (2015) 2878-2783.

[21] A.K. Geim, K.S. Novoselov, Graphene calling, Nat. Mater. 6 (2007) 169-169.

[22] P. Zhang, X.N. Fang, G.Q. Yan, M.X. Gao, Highly efficient enrichment of low-abundance intact proteins by core-shell structured Fe_3O_4-chitosan@graphene composites, Talanta 174 (2017) 845–852.

[23] G.C. Yang, P.L. Tang, Removal of phthalates and pharmaceuticals from municipal wastewater by graphene adsorption process, Water Sci. Technol. 73 (2016) 2268–2274.

[24] Z. Wang, F. Li, J. Xia, L. Xia, F. Zhang, S. Bi, G. Shi, Y. Xia, J. Liu, Y. Li, An ionic liquid-modified graphene based molecular imprinting electrochemical sensor for sensitive detection of bovine hemoglobin, Biosens. Bioelectron. 61 (2014) 391–396.

[25] E. Yavuz, Ş. Tokalıoğlu, Ş. Patat, Dispersive solid-phase extraction with tannic acid functionalized graphene adsorbent for the preconcentration of trace beryllium from water and street dust samples, Tanlata 190 (2018) 397–402.

[26] Y. Kanematsu, K. Gohara, H. Yamada, T. Yu, Applicability of density functional tight binding method with dispersion correction to investigate the adsorption of porphyrin/porphycene metal complexes on graphene, Chem. Lett. 46 (2017) 51–52.

[27] D. Cortés-Arriagada, A. Toro-Labbé, Aluminum and iron doped graphene for adsorption of methylated arsenic pollutants, Appl. Surf. Sci. 386 (2016) 84–95.

[28] L. Ji, W. Chen, Z. Xu, S. Zheng, D. Zhu, Graphene nanosheets and graphite oxide as promising adsorbents for removal of organic contaminants from aqueous solution, J. Environ. Qual. 42 (2013) 191–198.

[29] G. Zhao, L. Jiang, Y. He, J. Li, H. Dong, X. Wang, W. Hu, Sulfonated graphene for persistent aromatic pollutant management, Adv. Mater. 23 (2011) 3959–3963.

[30] R. Zhiani, Adsorption of various types of amino acids on the graphene and boron-nitride nano-sheet, a DFT-D3 study, Appl. Surf. Sci. 409 (2017) 35–44.

[31] Y. Gao, Y. Li, L. Zhang, H. Huang, J. Hu, S.M. Shah, X. Su, Adsorption and removal of tetracycline antibiotics from aqueous solution by graphene oxide, J. Colloid Interface Sci. 368 (2012) 540–546.

[32] W. Zhao, Y. Tang, X. Jia, J. Kong, Functionalized graphene sheets with poly (ionic liquid) s and high adsorption capacity of anionic dyes, Appl. Surf. Sci. 326 (2015) 276–284.

[33] S.M. Maliyekkal, T.S. Sreeprasad, D. Krishnan, S. Kouser, A.K. Mishra, U.V. Waghmare, T. Pradeep, Graphene: a reusable substrate for unprecedented adsorption of pesticides, Small 9 (2013) 273–283.

[34] L. Song, J. Xu, K. Chingin, T. Zhu, Y. Zhang, Y. Tian, H. Chen, X. Chen, Rapid identification of meat species by the internal extractive electrospray ionization mass spectrometry of hemoglobin selectively captured on functionalized graphene oxide, J. Agric. Food Chem. 65 (2017) 7006–7011.

[35] A.K.M. Kafi, D.Y. Lee, S.H. Park, Y.S. Kwon, Development of a peroxide biosensor made of a thiolated-viologen and hemoglobin-modified gold electrode, Microchem. J. 85 (2007) 308–313.

[36] Y. Zhang, J.W. Liu, X.W. Chen, J.H. Wang, A three-dimensional amylopectin-reduced graphene oxide framework for efficient adsorption and removal of hemoglobin, J. Mater. Chem. B 3 (2015) 983–989.

[37] J.W. Liu, Q. Zhang, X.W. Chen, J.H. Wang, Surface assembly of graphene oxide nanosheets on SiO2 particles for the selective isolation of hemoglobin, Chem. Eur J. 17 (2011) 4864–4870.

[38] A.J. Taylor, R. Linforth, O. Weir, T. Hutton, B. Green, Potential of electrospray mass spectrometry for meat pigment identification, Meat Sci. 33 (1993) 75–83.

[39] S. De, A. Girigoswami, A fluorimetric and circular dichroism study of hemoglobin—effect of pH and anionic amphiphiles, J. Colloid Interface Sci. 296 (2006) 324–331.

[40] J.W. Liu, Q. Zhang, X.W. Chen, J.H. Wang, Surface assembly of graphene oxide nanosheets on SiO2 particles for the selective isolation of hemoglobin, Chem. Eur J. 17 (2011) 4864–4870.

[41] Y.H. Yang, H.J. Sun, T.J. Peng, Synthesis and structural characterization of graphene by oxidation reduction, Chin. J. Inorg. Chem. 26 (2010) 2083–2090.

[42] J.A. Loo, R.R.O. Loo, Applying charge discrimination with electrospray ionization-mass spectrometry to protein analyses, J. Am. Soc. Mass Spectrom. 6 (1995) 1098–1104.

[43] W. Sun, Y. Guo, Y. Lu, A. Hu, F. Shi, T. Li, Z. Sun, Electrochemical biosensor based on graphene, Mg2Al layered double hydroxide and hemoglobin composite, Electrochim. Acta 91 (2013) 130–136.

[44] J.C. Meyer, A.K. Geim, M.I. Katsnelson, K.S. Novoselov, T.J. Booth, S. Roth, The structure of suspended graphene sheets, Nature 446 (2007) 60–63.

[45] J. Wang, Z. Chen, B. Chen, Adsorption of polycyclic aromatic hydrocarbons by graphene and graphene oxide nanosheets, Environ. Sci. Technol. 48 (2014) 4817–4825.

[46] S. Stankovich, D.A. Dikin, R.D. Piner, K.A. Kohlhaas, A. Kleinhammes, Y. Jia, Y. Wu, S.T. Nguyen, R.S. Ruoff, Synthesis of graphene-based nanosheets via chemical reduction of exfoliated graphite oxide, Carbon 45 (2007) 1558–1565.

[47] A. Fasolino, J. Los, M.I. Katsnelson, Intrinsic ripples in graphene, Nat. Mater. 6 (2007) 858–861.

[48] Y. Chen, L. Chen, H. Bai, L. Li, Graphene oxide–chitosan composite hydrogels as broad-spectrum adsorbents for water purification, J. Mater. Chem. 1 (2013) 1992–2001.

[49] R.L. Edwards, A.J. Creese, M. Baumert, P. Griffiths, J. Bunch, H.J. Cooper, Hemoglobin variant analysis via direct surface sampling of dried blood spots coupled with high-resolution mass spectrometry, Anal. Chem. 83 (2011) 2265–2270.

[50] T. Nakanishi, A. Miyazaki, M. Kishikawa, M. Yasuda, Y. Tokuchi, Y. Kanada, A. Shimizu, Quantification of glycated hemoglobin by electrospray ionization mass spectrometry, J. Mass Spectrom. 32 (1997) 773–778.

[51] B. Wild, B. Green, E. Cooper, M. Lalloz, S. Erten, A. Stephens, D. Layton, Rapid identification of hemoglobin variants by electrospray ionization mass spectrometry, Blood Cells Mol. Dis. 27 (2001) 691–704.