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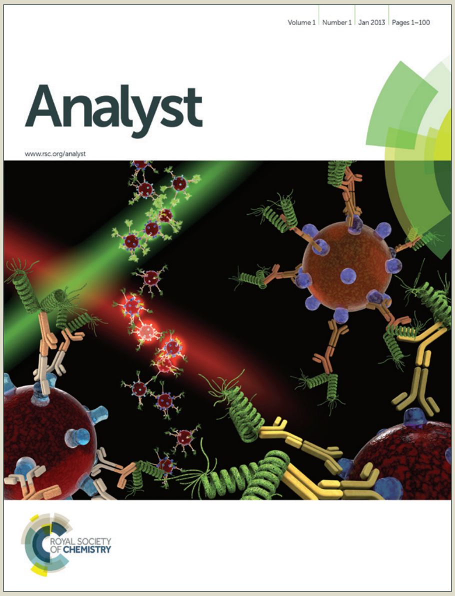

ROYAL SOCIETY OF CHEMISTRY

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Communications

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2015,
Accepted 00th January 2015

DOI: 10.1039/x0xx00000x

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"One-Drop-of-Blood" Electroanalysis of Lead Level in Blood Using Foam-Like Mesoporous Polymer of Melamine-Formaldehyde and Disposable Screen-Printed Electrodes

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Foam-like mesoporous polymer of melamine-formaldehyde (mPMF) was synthesized and further modified on the disposable screen-printed electrodes (SPEs) for the electroanalysis of Pb^{2+} ions in blood. Investigation results indicate that the so prepared mPMF was ultrastable in water showing mesoporous structure and amine-rich composition, as characterized by electronic microscopy images and IR spectra. Importantly, it could provide highly-selective chelating ability and powerful absorbent capacity of Pb^{2+} ions. By way of the solid-state $PbCl_2$ voltammetry, the mPMF-modified sensor could allow for the detection of Pb^{2+} ions in one drop of blood with high detection selectivity, sensitivity (down to about 0.10 μg L^{-1} Pb^{2+} ions), and reproducibility. Such a simple "one-drop-of-blood" electroanalysis method equipped with disposable SPEs and portable electrochemical transducer can be tailored for the field-deployable or on-site monitoring of blood Pb^{2+} levels in the clinical laboratory.

Lead (Pb) as a toxic heavy metal is commonly found in the natural environment and some consumer products.[1] The exposure of human body to lead usually occurs through ingestion, inhalation, and dermal contact; so that almost everyone has some measurable lead levels in blood.[2] When lead enters the human's bloodstream may elevate their blood lead level towards the lead poisoning and some serious diseases. For example, children are prone to the health effects of lead, causing potentially permanent learning and behavior disorders.[3] As of 2012, the Centers for Disease Control and Prevention (US) has set the standard levels of poisoning or elevated blood lead for adults and children to be about 100.0 μg L^{-1} and 50.0 μg L^{-1}, respectively.[4] How to evaluate the blood lead levels for the on-time treatment so as to avoid the lead poisoning is of great importance. Up to date, many detection methods have been applied for testing the blood lead ions, most known as atomic absorption spectroscopy (AAS)[5] and inductively coupled plasma mass spectroscopy.[6-8] However, these sensitive methods generally require expensive instruments, professional skill, and complicated sample pretreatment, which may be limited for the in-site evaluation or monitoring of lead poisoning. Therefore, developing a simple, fast, sensitive, selective, and field-deployable detection method for probing the blood lead levels has become an interesting and attractive target to pursue.

Electrochemical detection technologies, which possess low cost, high sensitivity, easy operation, and portable device for the on-site applications, have been widely applied for the determination of Pb^{2+} ions.[9,10] For example, the anodic stripping voltammetry in combination with mercury drop electrodes or mercury film electrodes were established for the sensitive Pb^{2+} detection.[11-14] However, the toxicity of mercury might make it undesirable for wide sensing applications. Additionally, most of the solid electrodes might encounter with some drawbacks so far, such as the troublesome modification procedure, cross-contamination risk, electrode poisoning, and cost ineffectiveness. Alternatively, screen-printed electrodes (SPEs) have been increasingly employed in the electrochemical analysis strategies, due to their outstanding advantages over these conventional solid-state electrodes of being cost effective, disposable, ease to carry, simple to modify, and potential for mass production.[15-18] Particularly, the miniaturized size of SPEs can allow for the on-site tests by using volume-saving samples like one drop of blood. Moreover, a variety of functional materials, such as bismuth,[19,20] gold,[21,22] antimony,[23] diazonium salts,[24,25] graphene[26] and multiwalled carbon nanotubes,[27] etc., have been employed to modify the SPEs aiming to facilitate the sensitive detection of Pb^{2+} ions in different matrices. However, these surface modification methods might suffer from the limited stability and detection performances in addition to the complicated preparation route and / or expensive reagents.

In recent decade, porous organic polymers have rapidly emerged for the diverse applications, such as catalysis, adsorption, separation, sensors, and biotechnologies.[28] As an important example, mesoporous polymer of melamine-formaldehyde (mPMF), which was generally prepared using melamine and formaldehyde via the polycondensation reaction, can possess many interesting physicochemical properties, such as high aqueous stability, large surface area, great catalysis, and fast absorption kinetics.[29-32] Specially, mPMF has been recognized to present considerably high absorption capacity for Pb^{2+} ions.[32] To our best knowledge, however, such an outstanding absorption ability of mPMF has not yet been applied for the determination of the meaningful Pb^{2+} ions. In the present work, mPMF was successfully synthesized and further modified onto the disposable SPEs for directly probing lead levels in one drop of blood with high sensitivity and selectivity by the electrochemical square-wave voltammetry (SWV) of solid-state $PbCl_2$.

The morphological structure of mPMF was first examined by transmission electron microscope (TEM) imaging, showing the foam-like mesoporous characteristics of mPMF scaffolds (**Fig. 1A**). Meantime, the scanning electron microscope (SEM) image discloses that mPMF modified on the SPE could construct the mesoporous matrix and rough surface (**Fig. 1B**), so that a large surface area could be expected for the vast absorption of Pb^{2+} ions to be targeted. Furthermore, the chemical composition of mPMF was examined by FT-IR spectra (**Fig. S1**). One can find that the IR bands at 1570 cm^{-1} and 800 cm^{-1} can belong to the C=N stretching vibration and triazine ring, respectively, and the 3467 cm^{-1} and 3419 cm^{-1} belong to the –NH antisymmetric stretching vibration. Accordingly, triazine rings and aminal (–NH–CH_2–NH–) functional groups could exist throughout the mPMF matrice, thus serving as the strong chelating ligands for capturing the meaningful Pb^{2+} ions.[32] Therefore, mPMF, with the mesoporous structure, large surface area, and high amine-chelating capacity, could be applied as the ideal absorbent to be modified on the electrodes for sensing Pb^{2+} ions.

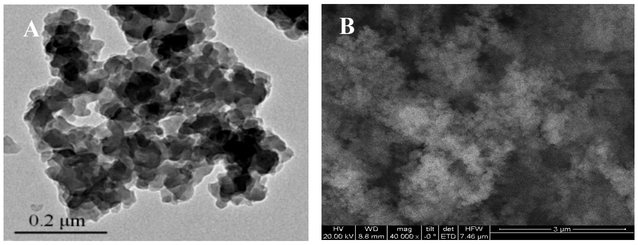

Fig. 1 (**A**) TEM image and (**B**) SEM image of mPMF prepared in suspension and modified on the SPE, respectively.

The electroanalysis performances of the mPMF-modified SPEs in sensing Pb^{2+} ions were investigated comparing to the one coated with chitosan (**Fig. 2**), a famous absorbent with amine groups used for adsorbing some metal ions including Pb^{2+} ions.[33,34] As shown in **Fig. 2A**, the mPMF-modified SPE could exhibit much larger SWV response to Pb^{2+} ions (curve **d**) than that of the chitosan-modified one (curve **c**) or the bare one (curve **b**). Also, the control test indicates that the mPMF-modified SPE showed no response in the absence of Pb^{2+} ions (curve **a**). These data confirm that mesoporous mPMF with Pb^{2+}-chelating amine groups could present the highly selective and large Pb^{2+}-absorption capacity so as to endow the mPMF-modified SPEs the greatly enhanced electrochemical responses to Pb^{2+} ions. Moreover, it was experimentally found that the supporting electrolytes could also play a vital role in the electrochemical analysis of Pb^{2+} ions. **Fig. 2B** manifests the SWV comparison of the mPMF-modified SPEs in several supporting electrolytes, including phosphate buffer saline (PBS), KCl, NaCl, NaAc, and KNO_3. Obviously, larger stripping currents and sharper peaking definitions were obtained for the Pb^{2+} responses by using the chloride-containing electrolytes, i.e., sodium chloride and potassium chloride. The bettered electrochemical signal output might presumably result from the formation of slightly-soluble $PbCl_2$ that might conduct more sensitive solid-state stripping voltammetry, as reported previously for AgCl.[35-37] Also, the peak currents could depend on the NaCl concentrations (data not shown), showing the highest peak current at 0.10 M NaCl. Of note, this testing electrolyte media is in well consistence to the physiological saline (0.9 % or about 0.10 M NaCl). Therefore, the mPMF-modified SPEs can be tailored for the direct determination of Pb^{2+} ions in blood samples.

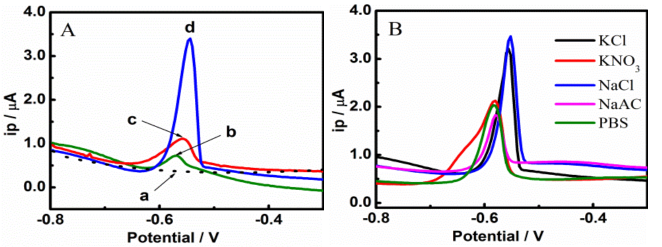

Fig. 2 (**A**) SWV responses to Pb^{2+} ions (20 μg L^{-1}) in 0.10 M NaCl (pH 6.0) at the SPEs modified separately with (b) bare, (c) chitosan, and (d) mPMF, with (a) the control (no Pb^{2+} ions); (**B**) SWV responses of the mPMF-modified SPEs to Pb^{2+} ions (20 μg L^{-1}) separately in different supporting electrolytes (0.10 M).

Moreover, the voltammetric conditions of the mPMF-modified SPEs were studied for probing Pb^{2+} ions (**Fig. 3**). As can be found from **Fig. 3A**, the Pb^{2+} stripping signals could increase with the increasing mPMF concentrations up to 2.0 mg mL^{-1}, over which they could decrease slightly, presumably due to that an increase in electron-transport resistance might undergo. Further, the pH effects on the SWV responses were explored, with the biggest response obtained at pH 6.0 (**Fig. 3B**). This could be explained by the fact that amine groups of mPMF might be protonated at the lower pH values, thereby increasing the electrostatic repulsion of Pb^{2+} ions approaching to the mPMF-modified SPEs. A decrease in the stability of the mPMF absorbent could also occur. On the contrary, too high pH values might cause Pb^{2+} ions to hydrolyze and precipitate in the end. Additionally, the SWV responses to Pb^{2+} ions could depend on the electrochemical deposition potentials (**Fig. 3C**) and deposition time (**Fig. 3D**). By compromising, the optimal deposition potential of - 1.2 V and time of 120 s were thus selected for the electrochemical Pb^{2+} sensing.

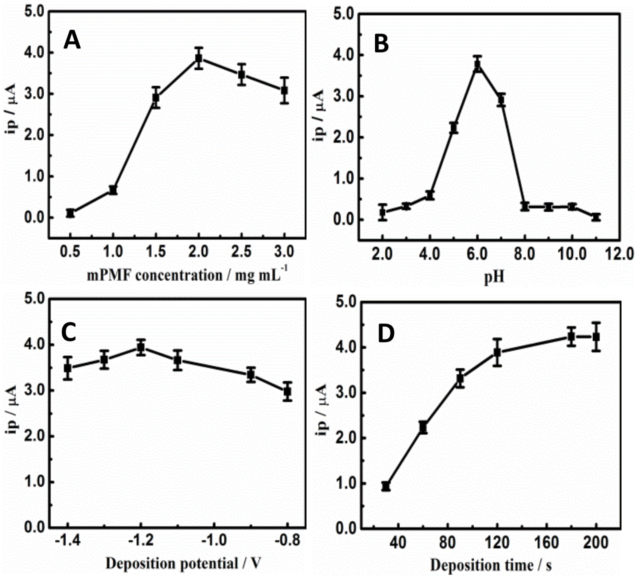

Fig. 3 The electrochemical SWV responses of the mPMF-modified SPEs to Pb^{2+} ions (30 µg L^{-1}) depending on (**A**) mPMF dosages, (**B**) pH values, (**C**) deposition potentials, and (**D**) deposition time.

Under the optimized conditions, the electroanalysis for Pb^{2+} ions was carried out, showing a linear detection range of 1.0 -100 µg L^{-1} (**Fig. 4A**). Herein, a linear equation was obtained as $I = 0.1376C+ 0.2904$ ($R = 0.9979$), and the limit of detection (LOD) was estimated to be about 0.10 µg L^{-1}. Such a LOD is lower than those of most of the SPEs modified with other sensing materials (**Table S2**). Subsequently, the mPMF-modified SPEs were applied for the detection of blood Pb^{2+} ions by using one drop of blood with different Pb^{2+} concentrations (**Fig. 4B**). Accordingly, Pb^{2+} ions in blood could be quantified in the linear range of 1.0 - 50 µg L^{-1}.

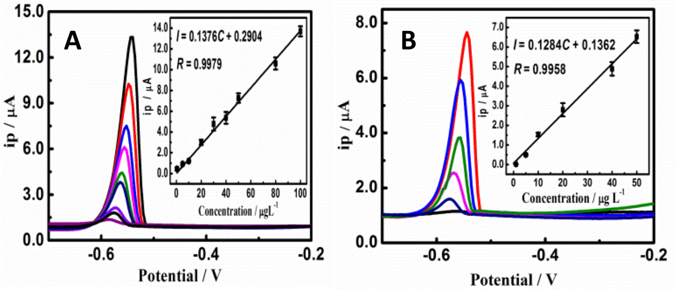

Fig. 4 SWV responses of the mPMF-modified SPEs to Pb^{2+} ions with different concentrations in (**A**) 0.10 M NaCl (pH 6.0) and (**B**) blood spiked, with the corresponding calibration plots (inset).

Furthermore, the application feasibility of the proposed mPMF sensor for the analysis of Pb^{2+} ions in the practical blood samples was assessed by the recovery tests, in comparison with the AAS technology (**Table S1**). Results indicate that the developed eletroanalysis method could be comparable to the classic method in the detection of Pb^{2+} ions in blood.

In addition, the interference of some other ions possibly co-existing in blood samples were evaluated (**Fig. 5A**). It was noted that 1000-fold excess concentrations of K^+, Mg^{2+}, Ca^{2+}, Fe^{2+}, Fe^{3+}, NO_3^-, SO_4^{2-}, and PO_4^{3-} ions caused no significant interference to the Pb^{2+} responses. Also, 100-fold excess concentrations of Cu^{2+} ions and Hg^{2+} ions could induce a deviation of electrochemical Pb^{2+} responses within ± 10 % only. In addition, the selective determination of Pb^{2+} ions could be allowed in the presence of at least 20-fold excess concentration of Cd^{2+} ions, showing a stripping peak well separated from the one of Pb^{2+} ions. Such a high Pb^{2+} detection selectivity could be attributed to the specific and powerful absorption capacity of amine-derivatized mesoporous mPMF for Pb^{2+} ions, where the nitrogens of the mPMF unit could bind with Pb^{2+} ions in a 1:4 complexation mode[32], as schematically illustrated in **Fig. 5B**.

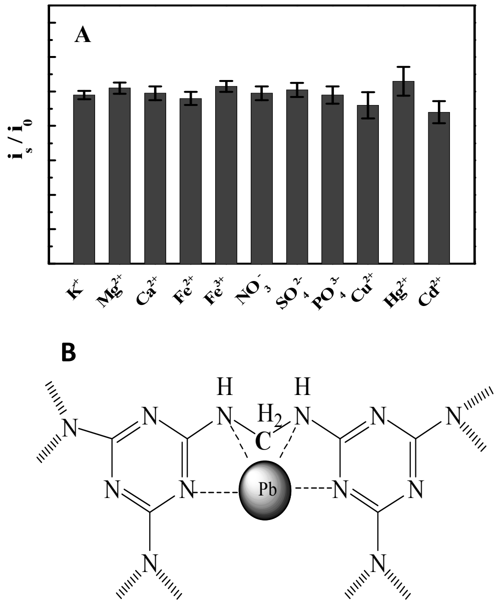

Fig. 5 (**A**) The SWV responses in current ratios (I_s/I_0) of the mPMF-modified SPEs to Pb^{2+} ions (40 µg L^{-1}) in the presence of K^+, Mg^{2+}, Ca^{2+}, Fe^{2+}, Fe^{3+}, NO_3^-, SO_4^{2-}, and PO_4^{3-} ions of 1000-fold excess concentrations, Cu^{2+} and Hg^{2+} ions of 100-fold excess concentrations, and Cd^{2+} ions of 20-fold excess concentration, where I_s and I_0 refer to the SWV current of Pb^{2+} ions in the presence and absence of interfering metal ions, respectively; (**B**) the possible interaction between Pb^{2+} ions and the mPMF modified on the SPEs.

The reproducibility of the mPMF-modified SPEs for Pb^{2+} ions was investigated by monitoring the anodic peak responses to Pb^{2+} ions using six independent SPEs which were prepared from the different batches (**Fig. S2A**). The relative standard deviation so obtained was found to be about 4.58 % for six repetitions. The storage stability of the mPMF-modified SPEs was examined over different time intervals by comparing the responses to 40 µg L^{-1} Pb^{2+} ions during 30 days (**Fig. S2B**). No observable change was witnessed over the testing period, revealing a satisfactory stability for the detection of Pb^{2+} ions due to the powerful aqueous stability of mPMF. In addition, the detection performances of the mPMF-modified SPEs were systematically compared with the SPEs modified with other sensing materials (**Table S2**). The results indicate that the developed mPMF sensor could present some advantages over most of the detection ones in sensing to Pb^{2+} ions.

In summary, mPMF was successfully synthesized with foam-like mesoporous structure, high surface area, large amine density, and aqueous ultra-stability. The mPMF-modified SPEs so developed could present some outstanding advantages over the traditional detection technologies in probing Pb^{2+} ions in blood. First, the highly selective chelating ability (1:4 complexation mode) and large absorption capacity of mesoporous mPMF to Pb^{2+} ions could endow the mPMF SPEs the practical electroanalysis for blood Pb^{2+} ions with high detection selectivity and sensitivity. Second, the aqueous ultra-stability and fast absorption kinetics of mPMF could bring the resulting sensors the rapid responses and considerably high reproducibility and stability of Pb^{2+} detection. Third, the ultra-sensitive signal output of solid-state $PbCl_2$ voltammetry could achieve greatly enhanced Pb^{2+} electroanalysis performance comparable to the classic method like the AAS technology. Forth, use of the cheap and disposable SPEs as the size-miniaturized sensing platform could allow for the Pb^{2+} analysis with really volume-saving samples like one drop of blood towards the on-site tests by combining with the portable electrochemical transducers. Therefore, the developed "one-drop-of-blood" electroanalysis candidate may promise the extensive applications for the real-time tests of blood lead levels in the clinical diagnosis.

Acknowledgements

This work is supported by the National Natural Science Foundations of China (No. 21375075, 21302109, and 21302110), the Taishan Scholar Foundation of Shandong Province, Domestic Visiting Scholar Foundation for Young Backbone Teachers of Department of Education, Shandong Province, and Qingdao Agricultural University High-Level Personnel Funds (630736), Qingdao, P. R. China.

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