Microfluidic chip with interdigitated ultra-microelectrode array for total phosphorus detection

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The fabrication and characterisation of an integrated microfluidic chip for phosphate detection are described. A micro three-electrode-system embedded in a microfluidic channel was used for the analysis of samples. Working electrodes and counter electrodes were arranged as an interdigitated array to increase the current response. Owing to the nonlinear diffusion effect of ultra-microelectrodes and the high generation-collection mode of interdigitated electrode arrays, the current density and sensitivity of phosphate detection were improved effectively compared with normal disk microelectrodes. The current density sensitivity for phosphate detection is $-0.00537\,\mu A/mm^2\cdot(\mu mol/l)^{-1}$, which is three times that of the disk microelectrode. The current density was averagely 6.5 times that of the disk microelectrode. The microfluidic chip was then used for total phosphorus (TP) detection. The TP detection results showed good consistency with nominal values of standard solutions.

1. Introduction: Phosphorus is one of the main sources of eutrophication of surface waters. Total phosphorus (TP) is defined as the measure of both inorganic and organic forms of phosphorus. It is set as one of the main indicators of eutrophication. The detection of TP is usually realised by detecting the concentration of phosphate in digested water samples. Phosphate detection is also an important research topic in the fields of biogeochemistry and biology. Thus, the measurement of phosphate concentration is very important.

Many phosphate detection methods have been developed, including the chromatographic method [1, 2], electrochemical methods [3] and the spectrophotometric method [4, 5] and so on. The electrochemical method uses electrodes, thus it is suitable for the requirement of miniaturisation and for the distributed sensor system in the development of *in-situ* monitoring of TP in surface water. Since Fogg and Bsebsu developed the flow injection method for phosphate detection in 1981 [6], several phosphate detection electrodes have been developed based on the reduction current of molybdophosphate on inert electrodes, such as modified glassy carbon electrodes [7], carbon paste electrodes [8] and gold microelectrodes [9, 10]. This method has good selectivity, and the electrodes need no modification, which makes the fabrication and preservation of electrodes quite easy. However, the low current response of this method limits its application. Previous work to improve the current response and sensitivity of microdisk electrodes by modifying three-dimensional (3D) gold nanoparticles on the gold electrode surface has been reported [11]. The performance was improved, but the current was not proportional to the surface area of the modified electrode, which agrees with the results reported [12]. Despite the improvements in performance, the uniformity of gold nanoparticle modifications on different electrodes cannot be ensured. To develop a TP detecting chip that can both improve current density and ensure uniformity in batch fabrication, a microchip with ultra-microelectrode interdigitated array (IDA) and microfluidic channel is developed. The main goals of the work reported in this Letter is to improve the performance by changing the structure of the electrode, and to develop a device that can be easily integrated.

In this Letter, we present the fabrication and characterisation of a microfluidic chip with an interdigitated ultra-microelectrode array for TP detection. The performance of this chip is compared with microdisk gold electrodes. This chip was utilised in the detection of TP in standard water samples to validate its use for *in-situ* monitoring of an automated, distributed sensor system combined with microdigestion chips [13].

2. Response mechanism

2.1. Molybdophosphate complex formation: Phosphate is a non-electroactive species, thus the electrochemical determination of this ion involves the treatment of the sample with an acidic molybdate solution to form a Keggin anion, $P(Mo_{12}O_{40})^{3-}$:

$$PO_4^{3-} + 12MoO_4^{2-} + 27H^+ \rightarrow H_3PMoO_40 + 12H_2O \quad (1)$$

2.2. Electrochemical response mechanism: The voltametric response of the molybdophosphate complex shows two reduction waves and two oxidation waves on the gold electrode, as shown in Fig. 1. The peak value of the reduction wave showed good correlation with concentrations of phosphate, but the linearity and limit of detection (LOD) could not meet the need of TP detection. To enhance sensitivity, amperometry was selected for the detection of phosphate. To avoid the influence of background current, the voltage at the valley of the reduction wave was chosen for the work potential.

As reported by Carpenter *et al.* [12], the amperometric response was not proportional to the electrode radius, which confirms that the rate of molybdophosphate reduction is mainly controlled by the preceding chemical reaction. However, the steady-state currents are always proportional to the concentration of phosphate in the acidic molybdate sodium solution in an appropriate range. So the steady-state currents at the gold microelectrode can be the basis of an analytical method of phosphate in water.

2.3. Electrochemical performance of ultra-microelectrodes: The performance of ultra-microelectrodes has been studied since the 1960s. Usually, electrodes with a critical dimension smaller than 25 μm are called ultra-microelectrodes. The reactions and diffusion mode of microelectrodes and ultra-microelectrodes are basically the same. The diffusion process on the normal electrode is nearly a half unlimited planar diffusion, while the diffusion

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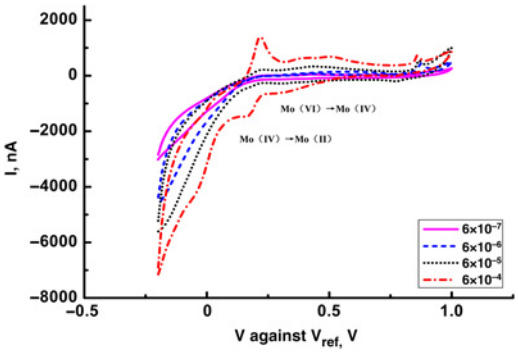

Figure 1 *Voltammetric response of molybdophosphate complex*

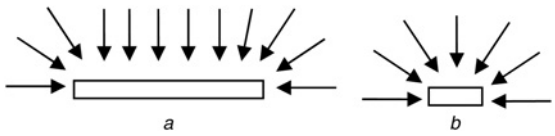

Figure 2 *Diffusion process of normal microelectrodes and of ultra-microelectrodes*
a Normal microelectrodes
b Ultra-microelectrodes

process on the ultra-microelectrode is a multi-dimensional diffusion, as shown in Fig. 2 [14].

The diffusion equation of ultra-microband electrodes used in this work is shown in the following equation

$$\frac{\partial C}{\partial t} = D\left[\frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial z^2}\right] \tag{2}$$

where c is the concentration of the species of interest, t is the time, D is the diffusion coefficient of species c and x and z are the Cartesian co-ordinates of interest [15].

The current density of the ultra-microelectrode is much higher than that of the normal electrode. However, the current response is still limited since the electrode area is very small. So ultra-microelectrode arrays are used to obtain a higher current response, and maintain the performance of the single ultra-microelectrodes.

2.4. Electrochemical performance of interdigitated ultra-microelectrode arrays: Aoki *et al.* reported that the diffusion-controlled limiting current at the IDA can be expressed as the following equation

$$|i_{L,S}|/nmbDFC^* = 0.637 \ln\left[2.55\left(1 + \frac{W_e}{W_g}\right)\right] - 0.19\left(1 + \frac{W_e}{W_g}\right)^{-2} \tag{3}$$

where W_e is the bandwidth, W_g is the gap, m is the number of band electrode pairs, n is the number of transferred electrons, b is the length of microbands, D is the diffusion coefficient, F is the Faraday constant and C^* is the concentration of analyte. The experimental values obtained for several IDA microelectrodes with different geometries fitted very well with the above equation [16].

3. Experimental

3.1. Electrode fabrication: The microdisk electrode was prepared using the standard microfabrication process, as shown in Fig. 3*a*. A 30 nm/200 nm Ta/Au composite layer was deposited and patterned on a glass substrate by lift-off process. The Ta layer is used as a seed layer and the Au layer is used as the working electrode as well as for electrical connection. After being covered

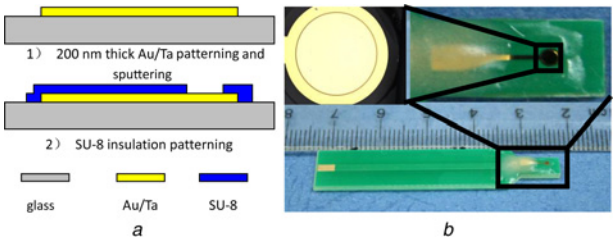

Figure 3 *Fabrication process and photographs of gold microelectrodes*
a Fabrication process
b Photographs

by SU-8 negative photoresist as the insulating layer, an electrode area of 0.45 mm^2 was discovered for detection. The microelectrode chip was attached on a PCB board for electrical connection the with probes of the electrochemical workstation. Photographs of the fabricated microelectrode are shown in Fig. 3*b*.

The microchip was fabricated with a standard microfabrication process, as shown in Fig. 4*a*. Au/Ta film was sputtered and patterned on the glass substrate by conventional lift-off process to define the ultra-microelectrodes area. The width of the working electrodes (WEs) and counter electrodes (CEs) fingers are 10 and 40 µm, respectively. The gap between the fingers is 50 µm. The reference electrode (RE) was made by Ag/AgCl paste covered on a gold microelectrode. A patterned polydimethylsiloxane membrane was then bonded to the glass substrate to form the microfluidic chip, the length of the microfluidic channel is 1 cm, and the width of the channel is 1 mm. Pictures of the chip are shown in Fig. 4*b*.

3.2. Reagents and equipment: Phosphate standard solutions for calibration were prepared using potassium dihydrogen phosphate (KH_2PO_4) diluted into different concentrations using deionised (DI) water, and kept in glass bottles. The molybdate sodium (Na_2MoO_4) solution was prepared with Na_2MoO_4 dissolved in DI water with a concentration of 20 mmol/l, and kept in polypropylene bottles. All the solutions were adjusted to pH = 1.0 with 98% sulphuric acid (H_2SO_4). Interfering solutions were prepared with sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$), sodium nitrate ($NaNO_3$), sodium nitrite ($NaNO_2$) and sodium sulphate (Na_2SO_4). All the above reagents were bought from Sinopharm Chemical Reagent, Beijing, China. The gold electrode clean solution was prepared with 98% H_2SO_4 diluted to 0.5 mol/l with DI water. The digesting solution was prepared with potassium persulphate ($K_2S_2O_4$, SIGMA-ALDRICH Co., USA) dissolved in DI water in 5% concentration, and kept in a brown glass bottle. Calibration and measurements with microdisk electrodes were carried out with a three-electrode-system consisting of an Ag/AgCl electrode as the RE and a platinum plate as the counter electrode. The calibration and measurements with microdisk electrodes were performed in 15 ml polypropylene bottles. The calibration and measurements with the microchip were realised by injecting phosphate standard solutions into the microfluidic channel. The measurement data were recorded when the fluid in the channel was kept static. All the electrical signal generation and detection processes were carried out using a Reference-600 electrochemical analyser (Gamry Instruments, USA).

3.3. Phosphate standard solution measurement: The electrodes and microchip were all cleaned in 0.5 mol/L sulphuric acid solution using cyclic voltammetry within −0.2–1.5 V for ten circles until the reduction peaks became uniform.

The cleaned electrode was dipped into 10 ml Na_2MoO_4 solution together with RE and CE. The detection was carried out using chronoamperometry under a voltage of 0.27 V. The solution was

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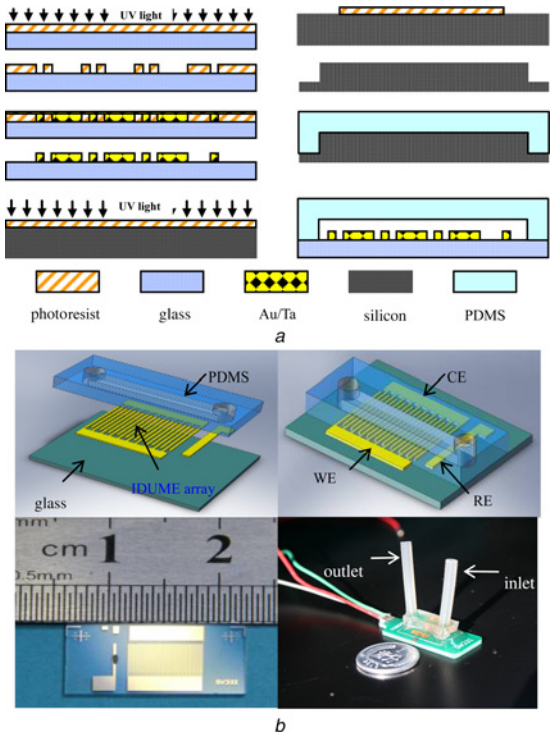

Figure 4 *Fabrication process and 3D picture and photographs of microfluidic chips*
a Fabrication process
b 3D picture and photographs

stirred during the measurement process. Phosphate solutions with different concentrations were added into the Na_2MoO_4 solution every 200 s.

Different concentrations of phosphate standard solutions were mixed with 20 mM Na_2MoO_4 solution and injected into the microfluidic channel. The solution in the microfluidic channel was kept static during the measurement process. The current response under −14 mV was recorded. DI water was injected into the channel to wash it between the measurement of different concentrations.

3.4. Selectivity determination: The selectivity of the gold microdisk electrode to the molybdophosphate complex was examined by adding disturbing ions into phosphate and molybdate mixed solutions with a concentration five times the phosphate concentration. The response current before and after adding the disturbing ions was recorded and compared.

3.5. TP determination: The diluted TP standard samples were digested using the digesting unit of the 5B-6P TP detection equipment manufactured by the Lian-hua Tech. Co. Ltd. Standard samples measuring 8 ml were first mixed with 1 ml of digesting solution in sealable reaction tubes and shaken well. The mixtures were then put into the digest equipment and heated at 120°C for 30 min. The digested samples were cooled to room temperature in cold water baths, and Na_2MoO_4 was added into the samples to obtain a concentration of 20 mmol/l. The mixed samples were then measured with a microfluidic chip.

4. Results and discussion

4.1. Selectivity: The results of selectivity are listed in Table 1. Cl^-, SO_4^{2-}, HCO_3^- and NO_3^- have little disturbance to phosphate detection; only NO_2^- has a high interference ratio of up to 39.42%. Considering the digesting process prior to detection, in which oxidants are the most reductive ions in the water samples, this disturbance could be avoided. It could be concluded that the normal disturbing ions in water have little interference to phosphate detection.

Table 1 Interference test results

Ions	Cl^-	SO_4^{2-}	HCO_3^-	NO_2^-	NO_3^-
current change, %	1.71	3.42	5.59	39.42	13.39

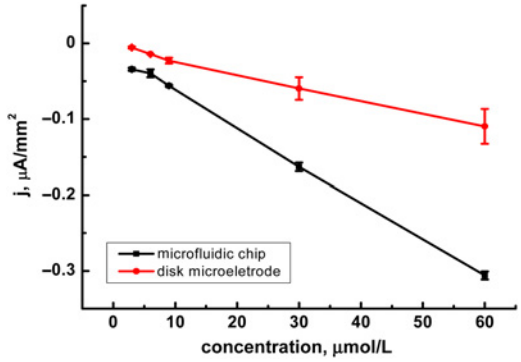

Figure 5 *Calibration curves of phosphate detection using microfluidic chip and microdisk microelectrodes*

4.2. Phosphate detection: The calibration curves shown in Fig. 5 indicate that both microchip and microdisk electrodes have good linearity in the concentration range of 3–60 μmol/l. To eliminate the influence of the electrode area, current density responses were compared. The sensitivity of the microchip was −0.00537 $\mu A/mm^2 \cdot (\mu mol/l)^{-1}$ with a LOD of 0.54 μmol/l; the sensitivity of the disk microelectrode was −0.00179 $\mu A/mm^2 \cdot (\mu mol/l)^{-1}$ with an LOD of 0.12 μmol/l. The current density response is effectively improved, with an average of 5.5 times increase.

4.3. Repeatability: To verify the reusability of the microfluidic chip, its repeatability was examined. The measurement was performed on three different phosphate concentrations, each one for seven times. The results shown in Fig. 6 indicate that the repeatability of the microfluidic chip gets better with the increase of phosphate concentration. This is because the measurement errors do not increase proportionally to the increase in detected concentration. The repeatability of the microfluidic chip can meet the requirement for non-disposable use.

4.4. TP detection: Referring to the statistical data of the environmental study [17], most water samples obtained from the land surface contained phosphorus in concentrations of less than 1 mg/l. Standard solutions for calibration have concentrations of

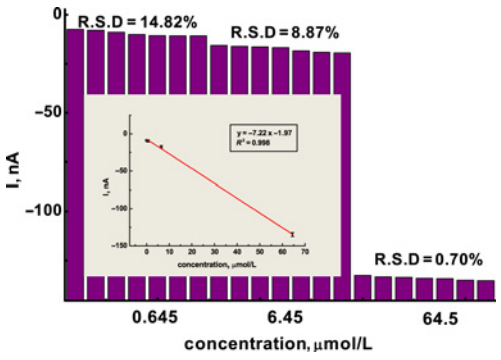

Figure 6 *Repeatability test of microfluidic chip*

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Table 2 TP detection results using microfluidic chips

Added, mg/l	Found, mg/l	Recovery, %
0.3	0.32 ± 0.015	107 ± 5.27
0.6	0.64 ± 0.003	106 ± 0.51

0.1, 0.5 and 1 mg/l. Two different concentrations of TP standard samples were tested, and the results are listed in Table 2.

The measurement results show good consistency with their nominal values. The relative error is less than 10%, and the R.S.D is less than 6%. The results indicate that the digesting process has little influence on detection. The microchip can be used for TP detection in water.

5. Conclusion: In this Letter, a microfluidic chip with an interdigitated ultra-microelectrode array has been developed to perform the detection of TP in digested water samples. A three-electrode system is contained in this chip: WE and CE are gold electrodes patterned as an interdigitated ultra-microelectrode array, and the RE is Ag/AgCl paste covered on a gold microelectrode. The performance of this chip was examined and compared with a microdisk gold electrode. Results show that the chip can improve the sensitivity effectively. Repeatability test results show that this chip can be used for the non-disposable detection of phosphate, which offers a solution for the *in-situ* detection of phosphate. The detection of digested TP solutions was also performed, and the measured result showed good consistency with nominal values. This method is more applicable than other electrochemical methods for TP detection because of its high selectivity, stability and it being easy to preserve. In future studies, we will seek to easily integrate this microfluidic chip with microdigesting devices to form an integrated autonomous microchip for TP detection.

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