

Design of an automatic pipettor for ISE based on soil nutrient detection

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Abstract: An automatic parallel pipettor was developed to achieve the programmable solution transportation for ion-selective electrode based on soil nutrient detection. A self-designed iterative model, involving variables of sample volume and residual volume, was designed to replace the cleaning operation. 140 soil samples were used for the system evaluation. Five soil nutrients contents, including nitrate-nitrogen, available potassium, calcium, chloride and pH, were simultaneously measured with the automatic pipettor in parallel. The iterative compensation effectively improved the detection accuracy. The average liquid transfer error was less than 0.1 mL. The total testing time was reduced 70% than the manual operation. The research could provide a balanced approach for the enhancement of automation, accuracy and efficiency for soil nutrient detection conducted by ISE.

Keywords: automatic pipettor, soil nutrient detection, ion-selective electrode, cross-contamination

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1 Introduction

Soil nutrient detection is the basis of site-specific fertilization. Traditional soil test was consisted of 3 steps, including field sampling, soil pretreatment and soil detection (Bai et al., 2006).

Ion-selective electrode (ISE) possessed advantages on cost, detection range and robustness (Heinen et al., 1991). Plenty studies were conducted by ISE for nutrient detection of soil and hydroponic solutions (Wang and Scott, 2010; Tully and Weil, 2014; Jung et al., 2015; Vardar et al., 2015). Kim et al. (2007) developed an ISE nutrient detection platform. The correlation coefficient of nitrate-nitrogen was found to be 0.98 between ISE and

FIA automated analyzer in 37 sets of soil detection. Kim et al. (2013) further modified the previous platform to fit the requirement of hydroponic nutrient detection of nitrate-nitrogen (NO_3^- -N) and available (K^+). The ISE measurements were strongly related to those determined using standard laboratory instruments with the correlation coefficient of no less than 0.85. But, on-line soil nutrient detection was still a challenge issue, which expected breakthroughs on sensor design, system automation and integration (Yildirim et al., 2006; Ji et al., 2015).

Manual pipettor was commonly used in soil extractant injection and extracts transfer before the detection operation. Commercial high throughput pipettor was recently manufactured to achieve parallel solution delivery in biological and life science studies (American RAININ Liquidator 96). Considering about improvements on detection efficiency, programmable liquid transportation could be employed for soil sample preparation. However, most of the automatic fluidic

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control faced the problem of cross-contamination, which was usually caused by sample residue. Additional rinsing procedure should be considered. Detection time was unavoidable extended (Eacute et al., 2006; Petrek et al., 2008; Su et al., 2014).

In order to verify the feasibility of free-cleaning operation for soil test, a cross-contamination compensation model and a multi-channel automatic pipettor were designed. The testing accuracy and efficiency were analyzed with laboratory experiments.

2 Material and method

2.1 Theoretical basis

The linear relationship between the potential produced by ISE and the logarithm molar content of target ion could be described by the Nernst equation. The concentration of the target ion was calculated by Equations (1) and (2).

$$c_i = 10^{\frac{E_i - E_0}{S}} \quad (1)$$

$$c_{iw} = 1000NA_r \times c_i \quad (2)$$

where, E_i was the potential of ISE, mV; E_0 was related to the ion-selective membrane and detection environment, mV; c_i was molar concentration of the target, mol L⁻¹; S was response slope, mV decade⁻¹. The ideal S for monovalent/bivalent ions were 59.16/28.58 mV decade⁻¹ at 25°C. Decade was defined as 10 times variation of molar concentration; c_{iw} was the mass concentration of the component, mg kg⁻¹; N is the ratio of soil and extractant solution. The ratio was selected according the national recommendation with 10 g soil: 25 mL extractant (Zhang et al., 2013); A_r was relative atomic mass. The values were 14.0 and 39.0 for NO₃⁻ and K⁺, respectively; i was the sample number. S and E_0 were usually derived from standard calibration experiments prior the sample detection with artificial solution.

2.2 Cross-contamination compensation

The cross-contamination was defined as the prediction deviation caused by the solution residue. It could be inferred that the current tested sample was formed with 2 parts. One was a unit volume of the real soil sample extract. The other was an uncertain amount of the previous sample residue. Based on the performance

test (Kong et al., 2015; Ren et al., 2016), a simplified compensation model was assumed as iterative Equation (3). Equation (4) was its equational transformation.

$$C_{i,T} \times (V + \Delta V) = C_{i,C} \times V + C_{i-1,T} \times \Delta V \quad (3)$$

$$C_{i,C} = C_{i,T} \times \frac{(V + \Delta V)}{V} - C_{i-1,T} \times \frac{\Delta V}{V} \quad (4)$$

where, $C_{i,T}$ was the content calculated according to the ISE tested potential by Equation (1); $C_{i,C}$ was the content derived after compensation; V was the sample volume; ΔV was the volume of the sample residue; the lower case mark of i was the sample number; ($i-1$) represented for the number of the previous sample.

In this study, the compensation concentration of $C_{i,C}$ was regarded as the expected result produced by ISE. V was chosen of 12.5 mL, which was almost the maximum volume of soil extracts after filtration process; ΔV was determined as 0.8 mL by experiments introduced in 3.1.

2.3 Apparatus and reagent

The NO₃⁻ ISE (No. 9707BNWP, Thermo, USA), K⁺ ISE (No. 9719BNWP, Thermo, USA), Ca²⁺ ISE (No. 9720BNWP, Thermo, USA), Cl⁻ ISE (No. 9617BNWP, Thermo, USA), and pH ISE (SC200, pHoenix, USA) were employed to predict the soil nutrient status, respectively. The K⁺ and Ca²⁺ were extracted by Kelowna (0.25M CH₃COOH + 0.015M NH₄F) (Kim et al., 2007). Other elements were extracted by DDW (distilled de-ionized water), respectively (Adamchuk et al., 2003 and 2005; Sethuramasamyraja et al., 2008). All chemicals purchased from Beijing Lanyi Chemical Products Company were of analytical grade.

The ultraviolet spectrophotometer (UV-2450, Shimadzu, Japan) and Flame photometer (M410, Sherwood, US) were employed as standard analytical methods to measure soil NO₃⁻-N, K⁺ and Ca²⁺ concentrations, respectively.

2.4 Automatic pipettor

The automatic pipettor system was mainly composed of the horizontal/vertical manipulator, 4 peristaltic pumps, 2 stepper motors and 6 proximity sensors, as is shown in Figure 1. The mechanical structure received the programmable commands from a pipettor controller. The horizontal displacement range was 0-50 cm. The vertical displacement range was 0-12 cm. The displacement speed

was 2 cm/s. The flow rate of the peristaltic pump was 50 mL/min. The proximity sensors, located on the horizontal/vertical manipulator, acted as the limit switch of maximum displacement. The prototype system was demonstrated in Figure 2.

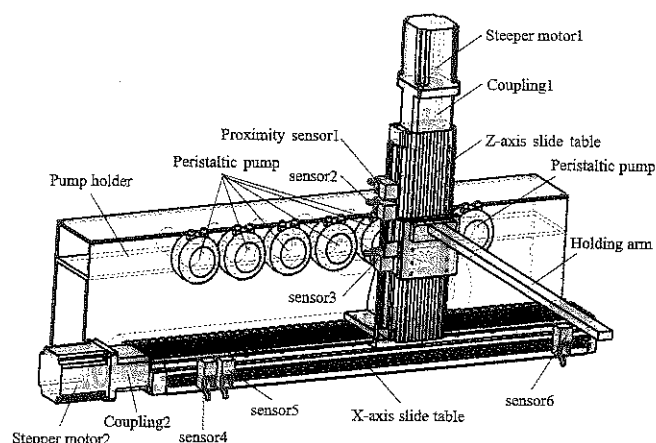


Figure 1 Mechanical structure of the multi-channel automatic pipettor

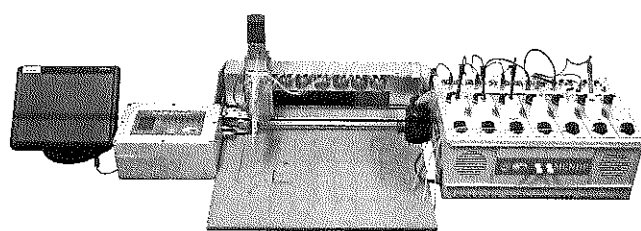


Figure 2 Prototype of the automatic pipettor

2.5 Soil information

140 soil samples were collected from 20 fields of summer maize, which were located at the northwest suburbs of Beijing. Samples covered 7 growth stages, including non-fertilization, fertilization, V3, V7, VT, maturation and harvest period. The reference information of the 140 soil samples were provided by the soil testing and formula fertilization center, as listed in Table 1.

Table 1 Basic information of 140 soil samples

Samples	Total-nitrogen, g/kg	Ammonium-nitrogen, mg/kg	Organic matter, g/kg	Moisture, %
I-140	0.59-2.33	4.33-120.57	2.41-21.20	12.75-23.46

Soil nutrients test was conducted in laboratory. Samples were processed under recommended pretreatment operation (Bai et al., 2007). After that, each sample was divided into two parts. The first part was detected by recommended spectrometric method. The test results were regarded as the standard reference value. The other one was detected by ISE with the sample pretreated with the self-designed automatic soil pipettor. The

oscillator and the centrifuge was applied for sample shaking and filtration.

3 Results and discussion

3.1 Performance of the automatic pipettor

In order to test the precision and stability of the pipettor, solution transportation experiments were carried out. Seven channels of the pipettor were tested simultaneously. In the injection test, the pipettor was controlled to transfer the pre-designated volume of DDW from the sample container to a cylinder. The injection volume could be easily recognized in the cylinder. In the second test, certain amount of DDW was filled into the testing chamber of the detection platform prior to the test. Pumps were programmed on the opposite direction. DDW was delivered from the chamber to the cylinder. The pump out volume left in the cylinder could be easily obtained. The residue was calculated by the injection and the pumped out. Pre-designated volumes of 5, 10 and 12.5 mL were evaluated. Each test was conducted 3 times. Results were summarized in Table 2.

Table 2 Performances of solution transportation by the automatic pipettor

Vol, mL	Test Operation	Test volume, mL							Mean, mL	SD, mL
		ch 1	ch 2	ch 3	ch 4	ch 5	ch 6	ch 7		
5.0	Injection 1	4.9	4.9	5	5.1	5	5	4.9	4.97	0.07
	Pump out 2	4.1	4.2	4.2	4.2	4.2	4.3	4.2	4.20	0.05
	Residue	0.8	0.7	0.8	0.9	0.8	0.7	0.7	0.77	0.07
10.0	Injection 1	10.1	10	10	9.9	10	9.9	10.1	10	0.08
	Pump out 2	9.4	9.3	9.2	9.2	9.2	9.1	9.2	9.23	0.09
	Residue	0.7	0.7	0.8	0.7	0.8	0.8	0.9	0.77	0.07
12.5	Injection 1	12.4	12.5	12.5	12.5	12.5	12.4	12.5	12.47	0.05
	Pump out 2	11.7	11.7	11.7	11.7	11.7	11.6	11.7	11.69	0.03
	Residue	0.7	0.8	0.8	0.8	0.8	0.8	0.8	0.79	0.03

As concluded from Table 2, the standard deviation of the liquid transportation was less than 0.1 mL. The accuracy was satisfactory for the extractant injection for soil pretreatment. The required ratio of soil to extractant could be accomplished by the pipettor.

Obvious errors were occurred in the pump out operation. An average amount of 0.77-0.79 mL DDW was left in the testing chamber, which would place ineligible influences on the detection precision. Therefore, the ΔV value of the iterative compensation model was determined as 0.8 mL according to the residue volume.

According to Equation (4), a larger volume of sample would reduce the cross-contamination interference. For the purpose of minimizing the residue error, the sample volume was chosen as 12.5 mL, which was also the guaranteed ultimate capacity of soil extract.

3.2 Evaluation of the compensation model

Evaluation experiment was conducted after obtaining the basic parameters of ΔV and V in Equations (3) and (4). Four sets of artificial potassium nitrate with known concentrations of 2.5, 5, 25, 100 and 250 mg/kg were employed as the testing solution. Solution of each concentration was pumped into 7 chambers through the automatic pipettor simultaneously. Four sample sets were detected continuously from the dilute to the dense in sequence. No rinse process was made during the experiment.

Results of NO_3^- ISE were recorded, as shown in Figure 3. The X-axis represented for the standard content of the solution. The Y-axis represented for the predicted concentration by ISE. The direct measurement was recorded in black. The iterative compensation model, discussed in 2.2, was applied to the average value of direct detection. The compensation result was illustrated in red.

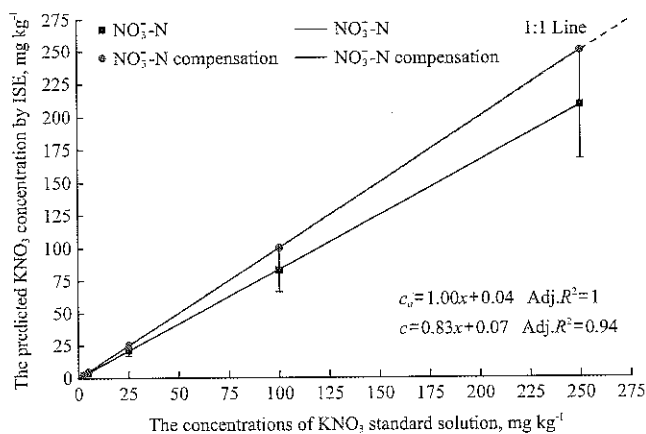


Figure 3 Comparison between the direct measurement and the compensation of standard nitrate solution

As seen from Figure 3, the direct measurement demonstrated obvious deviation with the absolute error of 0.91 - 41.04 mg/kg. The relative error was 0.97% - 18.26%. The compensation data were almost equal to the standard value. The absolute error was obtained of 0.02 - 1.33 mg/kg. The relative error was found to be 0.32% - 1.66%. In case of continuous measurement without

effective cleaning, the cross-contamination compensation model could effectively improve the prediction accuracy of nitrate detection.

3.3 Soil test

Soil samples were collected from 20 sites during the 7 growth stages of corn, including non-fertilization, fertilization, V3, V7, VT, maturation and harvest period. The nutrient contents, including nitrate nitrogen, available potassium, calcium, chloride and pH of 140 soil samples were measured. Each sample was separated into 2 parts. The first part was treated with the traditional operation of manually sample preparation and optics detection. The second part was detected with the prototype system illustrated in Figure 2. Operation of the soil solution delivery was carried out by the 7-channel automatic pipettor. The performance comparison between the manual operation and the automatic pipettor was tabulated in Table 3.

Table 3 Efficiency comparison between manual detections and the automatic pipettor

Items	Manual operation	Automatic pipettor
Sample amount	140	
Test elements	nitrate nitrogen, available potassium, calcium, chloride and pH	
Pretreatment, h	8.6	1.4
Detection, h	20	7
Detection Items	Discrete device 1 element/time	Integrated device Maximum 7 elements/time
Total time, h	30.6	8.4
Labor intensity	Intensive	Low

As illustrated in Table 3, conventional manual operation was time-consuming and labor intensive. The automatic system could achieve the programmable batch process of extractant injection, extract transfer and sample detection. It demonstrated apparent superiority on efficiency. Compared with the manual method, the pretreatment rate was reduced from 6 h/100 sample to 1 h/100 sample. The detection rate was speeded up from 8-9 min/ (sample of five elements) to 1 min/ (sample of maximum 7 elements). The total time of soil nutrient testing could reduce more than 70% under low labor intensity.

Soil test results were shown in Figure 4. The X-axis represented the sample number. Each field site was collected seven times. For example, samples of No.1, 21,

41, 61, 81, 101 and 121 were taken from the same place. The Y-axis represented the nutrients status of 5 target ions. As seen from Figure 4, the maize field was in alkalescency condition with soil pH higher than 7.0. Soil macronutrients of nitrate nitrogen, available potassium and calcium varied from 1.24-181.15, 27.23-183.14 and 5.11-886.26 (majority below 300) mg/kg, respectively. Contents of these 3 macronutrients were at a low level in

the first stage of the non-fertilization period, as seen in sample 1-20. In periods of fertilization and V3, macronutrients started increasing, as illustrated in samples 21-40 and 41-60. The contents of macronutrients kept decreasing during the rest four growth periods, which would be caused by the nutrient absorption of the maize plant. The pH exhibited greatly fluctuation.

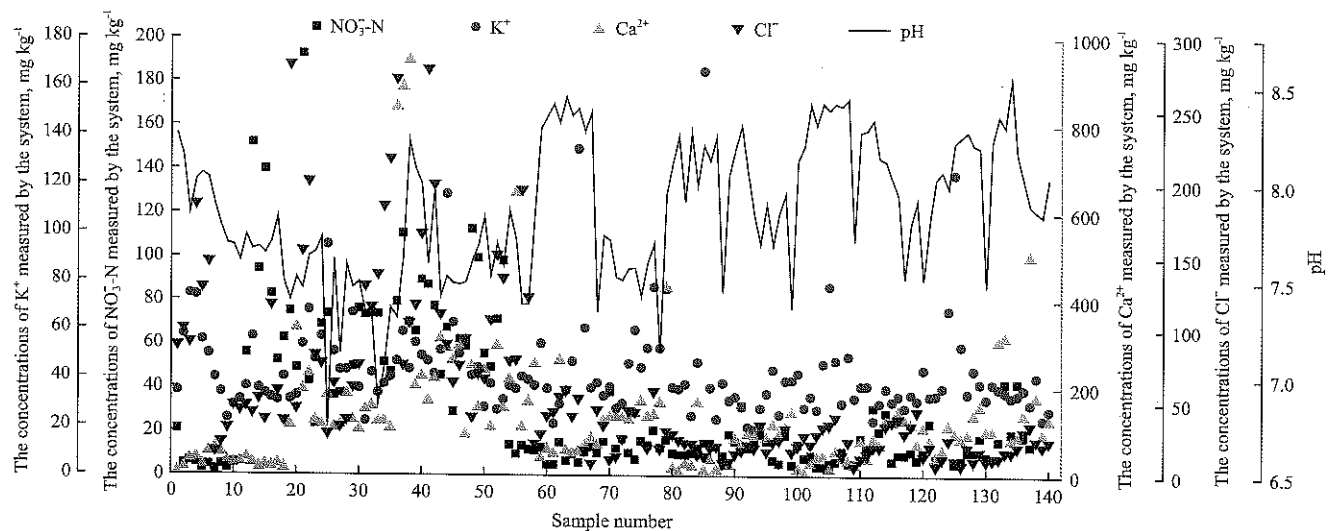


Figure 4 Nutrients variations of summer maize conducted by the automatic pipettor

4 Conclusion

In this study, a cross-contamination compensation model was designed for a self-developed automatic pipettor. Experiments were conducted to verify the feasibility of the system. Following conclusions could be made:

(1) The automatic pipettor could realize precisely liquid sample delivery with the accuracy of 0.1 mL, which could fit the operation standard of soil extractant injection.

(2) The cross-contamination compensation model could reduce the interference of samples residue. The absolute error of predicted nitrate-nitrogen was less than 1.33 mg/kg in standard solution detections carried out by ISE. The automatic pipettor would perform continuous detection without gap time of cleaning.

(3) The self-design automatic pipettor could greatly improve the detection efficiency and labor intensity. The batch measurement process reduced the test duration to less than 1/3 of the manual method.

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