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Interference analysis for pesticide residue photometric detection based on integrated microfluidic chip

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In order to improve the accuracy of the photometric detection of pesticide residues performed on integrated microfluidic chip, errors are analyzed and the model is built in photometric detection with different kinds of microfluidic chips. From the point of common, detection error is mainly caused by the temperature and the preparation time. The wavelength which is the causes error has became an impact factor in photometric detection with glass-based microfluidic chip, so the detective wavelength should be optimized. Because of small scale and fixed optical path, the path-length error is unique in glass-based microfluidic chip, which affect the pesticide residue sensitivity of photometric detection system.. Therefore, the relationship between error resulting from fixed optical path and optical path is established and verified by experiments. According to the particularity of photometric detection with paper-based microfluidic, the parameters of the optical wavelength and the color uniformity of different structures were evaluated and the errors analysis were carried out, then the optimal reaction conditions were determined. This research provides theoretical basis for the study of precise photometric detection of pesticide residues with glass-based and paper-based microfluidic.

Keywords: pesticide residues, photometric detection, microfluidic, error model

INTRODUCTION

The data from the state council shows that the number of cancer due to ingestion of food and vegetables with pesticide residues increases by 15% each year [1,2]. Therefore the use of portable equipment to detect residues plays an important role in guaranteeing the safety of food [3,4]. However, precision is the key factor that restricts the development of pesticide residue detection technology.

At present, the detection of pesticide residue is carried out mainly by immunoassay [5~7], sensors [8~10] and quick test card [11~13]. But the testing equipment mentioned above lacks of high sensitivity so it isn't practical for farmers to apply. Photometric detection technology provides the qualitative or quantitative analysis of pesticide residue through testing its light absorption in specific wavelength or a certain range [14]. Microfluidic technology is a new type of technology that puts the basic operating units such as reaction, separation and testing, which were involved in the detection of pesticide residues, into a chip of a few square centimeters (or less) [15,16]. The method combining microfluidic with photometric detection can resolve the problem of low sensitivity of pesticide residue detection.

Domestic and foreign scholars have studied the error in photometric detection system of pesticide residue. For example, Bera found that, in the process of detection, different ratio of the reactants in the microfluidic chip will produce volume error to different extent, while volume error will affect the accuracy of detection systems [17]. He put forward a simple and feasible method of combining micro molding and enzymatic cross-linking mechanism to make a channel of microfluidic chip, and he also pointed out that the processed error of the channel will have an influence on the test result [18]. Zou found out that the fluctuation of temperature would cause variation of spatial distribution in biochemical reaction, and eventually led to serious error in detection systems [19]. The researcher above shown that it is relatively mature to classify error and research model in photometric detection in the traditional macro scale, the theory of traditional photometry error is not directly applicable in errors caused by fixed optical path in the test condition of micro-nano liter in photometric detection of pesticide residue with integrated microfluidic and the random error existing in paper-based microfluidic chip [20]. For this reason, in this article the error caused by external factors in photometric detection of pesticide residue with microfluidic [21], such as the test temperature and preparation time is discussed. The relationship between error resulting from fixed optical path and optical path is established and verified by experiments.

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AL REACTION AND PHOTOMETRIC DETECTION MECHANISM

The principle of chemical reaction

The reaction principle of enzyme inhibition is that indophenol acetate ($C_{14}H_{11}NO_3$) can be hydrolyzed into indophenol ($C_{12}H_9NO_2$) and acetic acid (CH_3COOH). And acetyl cholinesterase (AchE) can catalyze the hydrolysis of indophenol acetate. Indophenol ($C_{12}H_9NO_2$), the product, is blue. Organophosphate and carbamate in crops will have certain inhibitory effect on acetylcholinesterase (AchE), leading to destroy of the catalysis, hydrolysis and discoloring. The concrete is shown in figure 1:

$$C_{14}H_{11}NO_3 + H_2O \xrightarrow{AchE} C_{12}H_9NO_2(\text{Blue}) + CH_3COOH$$

Fig. 1. Principle of chemical reaction

Detection mechanism of absorbance of pesticide residue in glass microfluidic chip

Lambert Beer's law is the theory basis of the detection of pesticide residue in microfluidic chip [22]. This law is also called the basic law of light absorption, which is used to show the relationship among the absorbance of pesticide residues (A), the concentration of a material that can suck light (c_i) and the thickness of liquid material that can also suck light (l). The relationship among them can be expressed following:

$$A = \alpha \cdot c \cdot l = -\lg T = -\lg (I/I_0) \quad (1)$$

Take parathion pesticide solution as an example to study the formation mechanism of error caused by fixed optical path, in the formula, A is absorbance value when parathion solution is detected in the photometric detection of pesticide residue, α is the absorption coefficient of parathion solution (the light absorption coefficient of parathion solution is 1.736•10 4L•cm-1•mol-1), c is the the concentration of pesticide residues in parathion solution, T is transmittance, I_0 is the intensity of the incident light, I is the intensity of transmitted light. The formula (1) shows that the intensity of transmitted light I also can be written in the following form:

$$I = I_0 \cdot 10^{-\alpha \cdot c \cdot l} \quad (2)$$

There are many ways to define the sensitivity of photometric detection system of pesticide residue, and the sensitivity of photometric detection system SEN was defined as the change of intensity of transmitted light caused by the change of unit concentration of components to be tested (Parathion solution), namely ($SEN = |dI/dc|$), the derived process is as follows:

$$SEN = |dI/dc| = I_0 \cdot \ln 10 \cdot (l \cdot \alpha_j \cdot 10^{-\alpha \cdot c \cdot l}) = \ln 10 \cdot I_0 \cdot l \cdot \alpha \cdot 10^{-l \cdot \alpha \cdot c} \quad (3)$$

Take derivative of the sensitivity of photometric detection system of pesticide residue SEN in formula (4) with respect to optical path l, and optical path with maximum sensitivity is:

$$l_0 = 0.434/\alpha \cdot c \quad (4)$$

The detection mechanism of reflective absorbance of pesticide residue in paper-based microfluidic chip

Since that the paper-based chip is opaque, when the monochromatic light irradiates to the surface of solid, there will be reflection and absorption, according to the theory of KUBELKA-MUNK, reflection of light follows the formula:

$$A_R = K/S \quad (5)$$

In the formula, A_R is reflective absorbance, S is reflective coefficient, K is the linear absorption coefficient. According to the definition of the linear absorption coefficient, the relationship among K, the molar absorption coefficient of light on solid phase (ε) and the concentration (C) is as follow:

$$K = \varepsilon C \quad (6)$$

So it can be deduced:

$$A_R = C\varepsilon/S \quad (7)$$

Reflection coefficient S is only related to the character of interfacial medium of incident light, this part of light intensity can be offset by a blank reference. So there is quantitative relationship existing in the detective signal and concentration of the object to be tasted. If reflection coefficient S remains the same, there is linear relationship existing in A_R and C in a certain concentration range, that is to say, reflective absorbance value is in proportion to the concentration of solution.

THE DESIGN OF TEST SYSTEM

According to the principle of enzyme inhibition reaction and effect of light absorption, a photometric detection system is established. Light is disposed differently because of the different detection mechanism of glass-based microfluidic and paper-based microfluidic.

The design of glass microfluidic detection system

Figure 2 represents schematic diagram of pesticide residue photometric detection system. The test light source 2 was placed on the test area a of microfluidic chip 1, and photosensitive diode 4 under the test area a, to receive the light emitted by the detective light. Spill-resistant cover plates 3 makes microfluidic packaged firmly. Photoelectric detection device will convert light signal into electrical signal, and achieve detecting pesticide

residues through the process of signal acquisition, data collection etc.

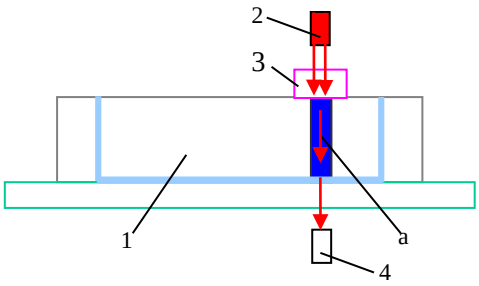

Fig. 2. Schematic diagram of pesticide residue photometric detection system: 1- microfluidic chip, 2- test light source, 3-spill-resistant cover plates, 4- photosensitive diode.

The design of detection system with paper-based microfluidic

Since that paper is not pervious to light, the detection mechanism of reflective absorbance is adopted to detect the pesticide residues. The equipment is same as the one mentioned above and shown in figure 3, in the equipment, paper-based chip 1 was placed on the chip placement agencies 4, and test light source 2 irradiate vertically to the color area b of the chip to make it be irradiated well, and the photosensitive diode 3 also placed vertically in the color area b to receive the reflective light signal. Similarly, the detection of pesticide residues is achieved through the process of signal acquisition, data collection etc.

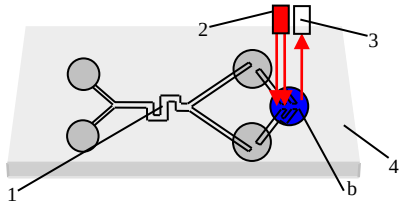

Fig. 3. Schematic diagram of the paper based microfluidic system: 1-paper-based microfluidic chip, 2-test light source,3- photosensitive diode, 4- chip placement agencies

ANALYSIS OF SYSTEM ERRORS IN MICROFLUDIC

While the system errors are analyzed, both the glass-based microfluidic and the paper-based microfluidic are influenced by the external interference, but there is also interference caused by the chip structure. So, the analysis of common error and the error caused by structure should be done separately in two types of microfluidics.

Analysis of general error

In the process of photometric detection of pesticide residue, both absorbance detection and reflective absorbance detection would be influenced by external interference such as temperature, preparation time etc. So, the interference of error caused by these factors should be analyzed.

Temperature error

Temperature is one of the factors that affects the microfluidic chip based on the chemical reaction, which would lead to differences in color, while color uniformity degree of detection precision is of great significance. In order to achieve the appropriate reaction temperature to obtain chip color uniformity, experiments were carried out. at different reaction temperatures (15˚C, 20˚C, 25˚C, 30˚C, 35˚C, 40˚C, 45˚C) with 0 mg/L pesticide reagents in microfluidic chip for enzyme inhibition. The absorbance value was detected at different temperature, and the optimum temperature was determined.

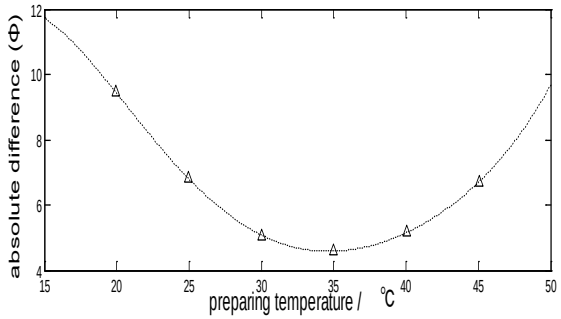

Fig. 4. Absorbance value at different temperature

Preparation time error

Multiple sets of pesticide reagent in multiple concentration are tested at 16˚C, with different preparation time (1 min, 6 min, 11 min, 16 min, 21 min, 26 min and 30 min). The average of the results of different concentration of reagent is calculated, and the error between test absorbance and standard absorbance (16˚C, 1 min) under different temperature conditions is analyzed.

Figure 5 shows the relationship between preparation time and the error of reflected intensity. In order to reduce the error arising from different preparation time, the compensation formula is

$$M_1 = 0.0001244\,T^2 - 0.0062T - 0.0009832$$

where T is prepared time (for preparation?VB)

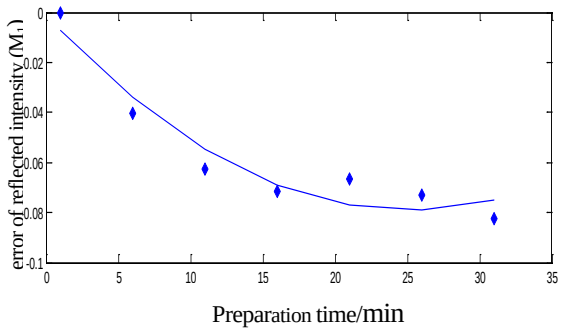

Fig. 5. Relationship between preparation time and absorbance error.

Analysis of errors in glass-based microfluidic system

Error of light wavelength

Light wavelength reflects the relationship between solution concentration and light absorption most veritably. The error of light wavelength is analyzed by color reaction of enzyme inhibition in the detection area in microfluidic chip with five different concentration of parathion solution which are10^{-4} mol/L、2×10^{-4} mol/L、3×10^{-4} mol/L、4×10^{-4} mol/L and $5\times^{-4}$ mol/L in experiments.

Table 1 was built based on different absorbance value in the same wavelength, according to the parathion solution with different concentration, and displays the change of the linearity value along with the wavelength in the range of 300-550nm. The better the system linearity is, the higher precision is and the interference of error is less.

Table 1 The value of linearity with wavelength in the range of wavelength 300-550nm.

wavelength	300	350	400	450	500	550
linearity	0.741	0.783	0.861	0.841	0.749	0.698

In the table, when the wavelength is about 300-400nm, the value of linearity in photometric detection system of pesticide residue increases along with the increase of the detective wavelength. But it is not optimized. However, when the wavelength is about 450-550nm, the value of linearity in photometric detection system of decreases along with the increase of the detective wavelength. Eventually, when the wavelength is about 400-450nm, the value of linearity in photometric detection system is obviously higher than that in other scope, and the accuracy is better, with smallest error interference.

Above all, the wavelength of 412nm is chosen as the detective wavelength by experiments, so that the interference of error in photometric detection system of pesticide residues is reduced.

Validation of the error caused by fixed optical path in glass-based microfluidic

After the experiment of light source wavelength, the light-emitting diodes with the wavelength of 412nm are chosen as the light source of photometric detection system for pesticide residues. The model is verified with the example of 10^{-4} mol/L parathion solution, which is the relationship between error caused by fixed optical path and optical path which has been deduced.

In the photometric detection system for pesticide residue, the main sources of error are those caused by transmitted light noise and reference light, while the measuring error caused by the reference light can be neglected. The error caused by transmitted light noise σ_I in photometric detection for pesticide residue can be indicated by the following expressions:

$$\sigma_c=\sigma_I/|SEN| \qquad (8)$$

If replace the expressions of the sensitivity of photometric detection system *SEN* from formula (3) into formula (8), the following expressions of the relationship between error caused by fixed optical path σ_c and optical path l is received.

$$\sigma_c=\sigma_I/|SEN| =\sigma_I/(\ln10\cdot I_0\cdot l\cdot\alpha\cdot10^{-l\cdot\alpha\cdot c})$$
$$=[\sigma_I/(\ln10\cdot I_0\cdot\alpha)]\cdot10^{l\cdot\alpha\cdot c}/l \qquad (9)$$

$\sigma_I/(\ln10\cdot I_0\cdot\alpha)$ in formula (9) is believed to be equal to m for convenience due to the fact that σ_I, I_0, α are all fixed figure. As a result, formula (9) can be simplified as :

$$\sigma_c=\sigma_I/|SEN| =m\cdot10^{l\cdot\alpha\cdot c}/l \qquad (10)$$

It can be seen that $\alpha\cdot c$ is equal to $0.434/l_0$ from formula (4) (l_0 is the optical path length with maximum sensitivity). So formula (10) can also be expressed as formula (11). The relationship between error caused by fixed optical path in photometric detection σ_c and optical path length l is shown in figure 7.

$$\sigma_c=\sigma_I/|SEN| = m\cdot10^{(0.434/l_0)\cdot l}/l \qquad (11)$$

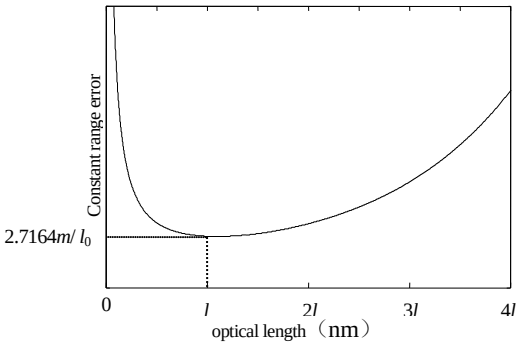

Fig. 7. The relationship between photometric detection error and constant path length in pesticide residues.

Different degree of errors caused by fixed optical path emerges during the detection. The microfluidic chips with the thickness of 0.8 mm, 1.0 mm, 2.2 mm, 2.4 mm, 2.5 mm, 3 mm, 8.5 mm, 9 mm are selected, and photometric detection system of pesticide residues, taking advantages of the chromogenic reaction principle of enzyme inhibition, is used to test the absorbance of pesticide residue in the detection area of the microfluidic chip, measuring absorbance value of parathion solution, the concentration of which is known, and get the measured value of parathion solution indirectly by combining the absorbance value that has been tested with Lambert Beer's law. The error in this optical path is the absolute value of difference between the measured value and the true value of pesticide residues in parathion solution. Figure 8 shows a curve comparison between measured value and the predictive value in formula (11) of error in optical path of any length.

It can be concluded from figure 8 that the curve fitting which involves the error tested by experiments in different optical path length and the predictive error in formula (11) is much better. So this constant error and path length relationship model is basically correct.

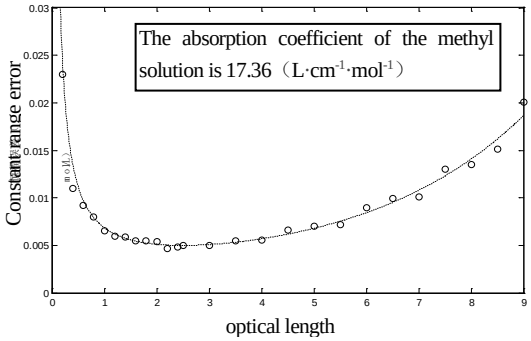

Fig. 8. The path length measurement error and forecast error curve (11)

Analysis of errors in paper-based microfluidic system

Errors of light wavelength

The system uses different wavelengths when testing pesticide residue, because that the mechanism of reflective absorbance detection is adopted. The result of the detection is different along with the different wavelength, so errors in the measurement can be produced.

According to the statement that coloring area in paper-based biosensor (blue) variously absorbs light of different wavelengths, the most sensitive wavelength to the change of pesticide residue concentration is found to be detective wavelength, which is helpful to reduce experimental error. Therefore, the pesticide reagent of different concentration (0 mg/L, 0.1 mg/L, 0.15 mg/L, 0.2 mg/L, 0.25 mg/L, 0.3 mg/L) is detected in full spectrum in experiments (wavelength: 200nm-1112.428nm). The figure 9 shows the diagram of the relationship between wavelength and illumination intensity.

In the diagram, when the wavelength is 599.753nm, the change rate of the light intensity of different concentrations of pesticides is the largest. It has the better testing linearity, with high accuracy and low error interference, so an appropriate wavelength in this range is chosen as the detective wavelength.

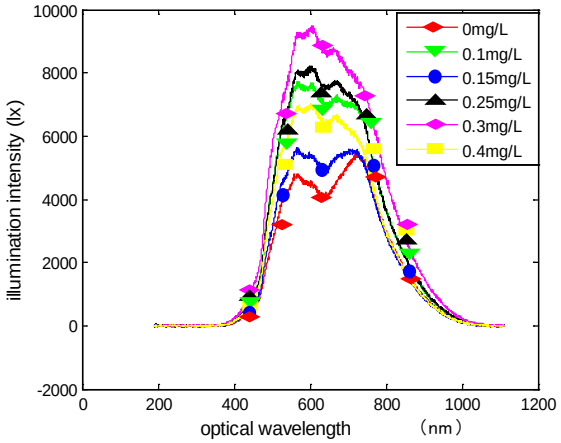

Fig. 9. Illumination intensity of different concentration.

Errors in color uniformity of different structure

Blue material can be produced after chemical reaction, the color uniformity of coloring area is the crucial testing link in paper-based microfluidic system. Uneven distribution of color will result in serious error when photometric detection has been done. So paper-based microfluidic chips of different structure are tested and the color after the reaction is processed in the form of image to determine the error. As shown in figure 10, 11 and 12, in order to compare the color uniformity, the whole color area and local color area enlarged are collected in the form of image with electron microscope and the gray-level histogram is obtained.

For the paper-based microfluidic chips of different structure, it can be concluded that the grayscale average of overall image and focused image, according to the comparison of data, the contrastive formula of color uniformity can be set as follow:

$$\Phi = |A_1 - A_0|$$

Φ is the absolute value of difference after comparing, A_1 is the grayscale average of overall image, A_0 is grayscale average of focus image. The

smaller Φ is, the higher uniformity will be and the errors in detection are smaller.

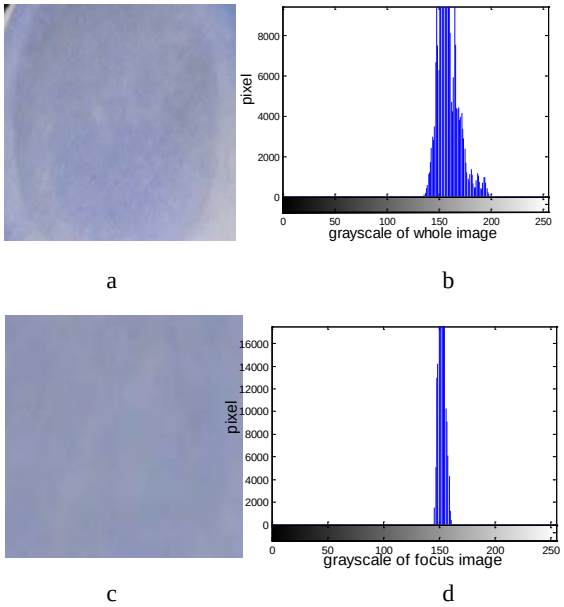

Fig. 10. Bridge structure color uniformity contrast figure: a) picture of whole image; b) gray histogram of whole image; c) picture of focus image; d) gray histogram of focus image.

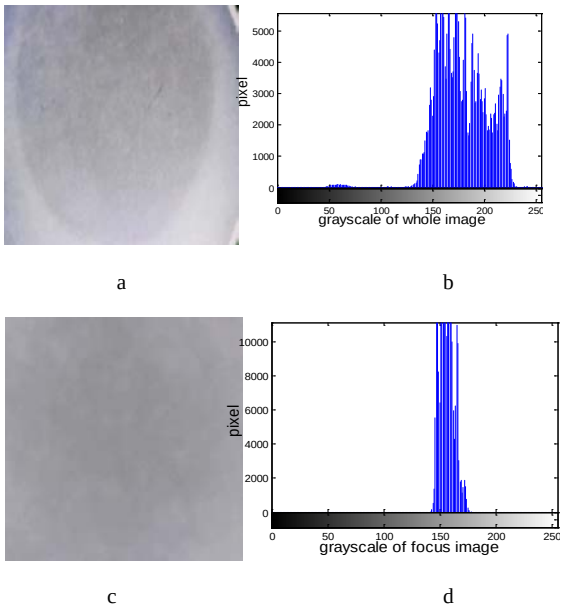

Fig. 11. Y structure color uniformity contrast figure: a) picture of whole image; b) gray histogram of whole image; c) picture of focus image; d) gray histogram of focus image.

Specific experimental data are shown in table 2. The grayscale average of overall image and focus image of bridge structure are 158.563 and 152.512 respectively, the difference is 6.501, and the data of structure Y are 178.274 and 155.686 respectively, with the difference of 22.588. According to the formula, paper-based microfluidic chip of bridge structure is chosen in photometric detection for pesticide residue, with small difference of the average of color grayscale, uniform color and smaller error.

Table 2. Different structure of the overall image and the focus of the image grayscale average difference contrast table.

structure / numerical value	Bridge structure microfluidic	Y structure microfluidic
Averge gray value of the whole	158.563	178.274
Averge gray value of the focus	152.512	155.686
D-value	6.051	22.588

SUMMARY

The error sources of photometric detection system of pesticide residues performed on microfluidic device have been analyzed. Error analysis has been done, which involves the external factors such as preparation time, temperature, and error analysis model is established. The parameters of the system can be optimized to reduce error sources. The model of the relationship between error caused by fixed optical path and the optical path was analyzed, and the model is verified by experiments from the view of Lambert Beer's law through the study of the formative mechanism of error caused by fixed optical path in glass-base microfluidic system. The results show that error caused by fixed optical path does exist in photometric detection system for pesticide residue, the model of the relationship between error caused by fixed optical path and optical path has been analyzed theoretically. At the same time a method of optimizing the testing data has been put forward for paper-based microfluidic system, reducing the errors in detection of pesticide with paper-based microfluidic chip. In this method, by comparing the testing standards in different cases, errors in light wavelength in detection is analyzed, and the model is established and the parameters of process are optimized, reducing the error in detection system of pesticide residue with paper-based microfluidic.

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REFERENCES

1. Y. Fintschenko, J. Krynitsky, W. Wong. *Journal of Agricultural & Food Chemistry*, **58**, 10 (2010).
2. H. Wang, J. Yu, X. Fei, J.G. Zhang. *Guangdong Agricultural Sciences*, **40**, 8 (2013)
3. Y. Lu, S. Song, R. Wang, Z. Liu, J.Meng. *Environment International*, **55**,1 (2015).
4. G .Stefania, M. Comoglio, T. Livio. *Breast Cancer Research Bcr*, **12**, 4 (2010).
5. H. Akhavan-Tafti，D.G. Binger，J.J. Blackwood，Y Chen, RS Creager. *Journal of the American Chemical Society*, **135**, 11 (2013).
6. A. Penna, M. Magnan. *Journal of Phycology*, **36**, 6 (2008).
7. M.J. Xie, K. Fukui, M.Horie, Y. Sakihama, K Hashino. *Analytical Biochemistry*, **374**, 2 (2008).
8. V. Tran, S. Reisberg, B. Piro, *Electroanalysis*, **25**, 3 (2013).
9. D.Q. Huo，M.O. Lian，M. Xie，N.I. Bin，M. Zhang. *Chinese Journal of Analysis Laboratory*, **31,** 9 (2012).
10. S.D. Marcos, E. Callizo, E. Mateos, J. Galban. *Talanta*, **122**, 3 (2014).
11. P. Noyrod, O. Chailapakul, W. Wonsawat, S. Chuanuwatanakul, *Journal of Electroanalytical Chemistry*, **719,** 4 (2014).
12. B.M. Del, M.S. De, V. Cebolla, J .Heiland, S. Wilhelm, *Biosensors & Bioelectronics*, **59**, 13 (2014).
13. X. Dai, Q.H. Wu, P.C. Wang, J. Tian, Y. Xu. *Biosensors & Bioelectronics*, **59**, 9 (2014).
14. X. Yu, N. University, Y. Shen, *Chinese Journal of Rare Metals*, **30**, 6 (2006).
15. K. Ren, J. Zhou, H. Wu, *Accounts of Chemical Research*, **46**, 11 (2013).
16. X. Lin, X. Hu, Z. Bai, Q. He, H. Chen, *Analytica Chimica Acta*, **828** , 5800 (2014).
17. N. Bera, S. Majumder, B. Bhattacharya, *Applied Algorithms, Springer International Publishing*, **8321**, 1 (2014).
18. J. He, R. Chen, Y. Lu, L. Zhan, Y. Liu. *Materials Science and Engineering C*, **59**, 53 (2016):.
19. M. Zou, S. Cai, Z. Zhao, L. Chen,. Zhao. *Review of Scientific Instruments*, **86**, 4 (2015).
20. Y. Wang, Y.H. Lu, R. Wang, K.X. Xu. *Journal of Tianjin University*, **37**, 10 (2004).
21. N. Yang, R. Zhang, P. Xu, J. Guo, L. Cheng. *Chinese Journal of Scientific Instrument*, **34**, 6 (2013).
22. E. Ábraham, C. Hourton-Cabassa, L. Erdei, L. Szabados. *Methods in Molecular Biology*, **639**, 3 (2010).

ИНТЕРФЕРЕНЧЕН АНАЛИЗ ЗА ФОТОМЕТРИЧНОТО ОПРЕДЕЛЯНЕ НА ОСТАТЪЦИ ОТ ПЕСТИЦИДИ С ИНТЕГРАЛНИ МИКРО-ФЛУИДНИ ЧИПОВЕ

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(Резюме)

За подобряването на точността на фотометричното откриване на остатъци от пестициди се въвежда микро-флуиден чип и се съставя модел с анализ на грешките. Обикновено грешката на откриване се причинява главно от променлива температура и времето за подготовка на пробата. Дължината на вълната е фактор с основно влияние при фотометричното откриване с микро-чипове на стъклена основа, заради което дължината на вълната трябва да се оптимизира. Поради малките размери и фиксирания оптичен път, грешката от това е единствена при микро-чиповете на стъклена основа, което влияе върху чувствителността на метода. Затова тук е установена грешката при фиксирания оптичен пъти е потвърдена експериментално. Според особеностите на фотометричното определяне с микро-чипове на хартиена основа са определени дължината на вълната и еднаквостта на цвета за различни структури и са анализирани грешките за този случай. В последствие са определени оптималните условия за анализите. Настоящето изследване дава теоретична основа за прецизното фотометрично откриване на остатъци от пестициди с микро-флуидни системи на стъклена или хартиена основа.