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A photometric method for organophosphorus pesticide detection based on microfluidic chip

N. Yang[1,2], H. Mao[2*], J. Sun[1], C. Xiang[1], P. Xu[1]

[1] *School of Electrical and Information Engineering, Jiangsu University, Zhenjiang, PR China*
[2] *Insitute of Argricultural Engineering, Jiangsu University, Zhenjiang, PR China*

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Water pesticide pollution is one of the important environmental issues which need to be urgently solved, but organophosphorus pesticide detection in water environment often relies on traditional laboratory techniques which have poor timeliness and low degree of automation. The paper proposes a photometric detection based on microfluidic chip. We designed a special microfluidic chip according to the characteristics of water pollution pesticide detection and created an automation system platform including sample introducing, mixing, reacting and detecting on the basis of organic phosphorus pesticide hydrolysis reaction. The working parameters of the detection system were optimized under microscale conditions. Experimental results showed that the designed system parallels traditional chromatography in detecting the organic phosphorus pesticide content in seawater, with a correlation coefficient of 0.963. At the same time, reagent dosage of the designed system is one-thousandth of that in traditional chromatography, and the degree of automation is greatly improved.

Key words: Microfluidic chip; water pesticide contamination; photometric detection; detection limit.

INTRODUCTION

Pesticides are indispensable capital goods in modern agricultural production, they can greatly increase crop yield. But they also have some disadvantages such as posing a threat to the ecological environment and human lives. When we use pesticides in daily lives, only about 1% of them are applied to the target organisms. The rest remains in the soil or enters into the sea areas, eventually flowing into the ocean by means of rainfall, river and air transport [1]. These pesticides inhibit photosynthesis of algae, and moreover, cause a large-scale drop in the production of fish and shellfish. These pesticides can also do great harm to human through the food chain. Therefore, rapid detection of pesticides in seawater is necessary to prevent and control water pollution [2, 3].

Currently, the main pesticides detection technologies include chromatography [4], spectroscopy [5], chromatography-mass spectrometry and other instrumental analysis methods [6~8]. These methods provide a basis for determining pesticides pollution. However, the traditional methods of instrumental analysis have some shortcomings such as expensive instruments, long and tedious pre-processing. Moreover, each step of the routine detection process such as pesticides extraction, purification, preconcentration and detection, are affected by hardware facilities, personals, laboratory's technical proficiency, level of management and other aspects. These factors also lead to inaccurate measurement results [9]. Because enzyme immunoassay has many advantages: high detection speed, high specificity, high sensitivity, large analytical capacity, low cost analysis, safety and reliability, it has become the first choice for on-line detection based on antigen antibody specificity recognition and association reaction [10, 11]. Currently, enzyme immunoassay mainly relies on test strips or test cards. The method has three shortcomings: (1) a large amount of reagents is consumed; (2) the accurate detection readout is hard to acquire by the naked eye; (3) the automation level of this method is low.

As a new detection technology, a microfluidic chip was developed in recent years [12]. It integrates samples' reaction, separation, detection and other processes concerning chemistry, biology and other fields into a single chip. Because the technology of the microfluidic chip has features of miniaturization and automation, therefore it has been considered as one of the seven technologies that will change our world. In 2006, the Nature journal published the technology of the microfluidic chip in a special issue [13]. The scale of microfluidic chip channel is usually tens to hundreds of microns. Meanwhile, a detection process only requires nanoliters or picoliters of samples. Undoubtedly, it is particularly suitable for

* To whom all correspondence should be sent:
E-mail: yangning_ujs@163.com

meeting the needs of reducing pesticides consumption for detection. Therefore, the present paper proposes a photometric detection system to water pesticides pollution based on the microfluidic chip. As an example, microfluidic chip based organophosphorus pesticides detection to the study of water pesticides pollution is taken. The working parameters of the detection system under microscale conditions were optimized. The research provided theoretical bases for developing a rapid detection system for the assessment of water pesticides pollution.

PHOTOMETRIC DETECTION SYSTEM OF MICROFLUIDIC CHIP

The basic principle

Methyl parathion is a common organophosphorus pesticide. Therefore, the paper takes methyl parathion as the research subject. From methyl parathion, p-nitrophenol can be obtained under the action of hydrolytic enzyme, the solution of p-nitrophenol being of yellowish color. The reaction process is shown in **Fig. 1.**

When exposed to the sun, the molecule of p-nitrophenol can undergo polarization, a forced oscillation appears with the frequency of incident light. On the basis of the Helmholtz equation, we obtain the following expression:

$$2n^2\kappa = \frac{Nze^2}{\varepsilon_0 m}\frac{\gamma\omega}{\left(\omega_0^2-\omega^2\right)^2+\gamma^2\omega^2} \tag{1}$$

In the equation, n is the planar optical refractive index，κ is attenuation coefficient, m is electronic mass, γ is damping constant, e is electron charge, E is electric field intensity of incident light, ω is the frequency of the incident light, N is the molecule number of p-nitrophenol per unit volume, z is the number of electrons in an atom，ω_0 is the natural electron frequency.

At the same time, p-nitrophenol has the ability to absorb light, the absorption index is:

$$\alpha = \frac{4\pi}{\lambda}n\kappa = \frac{4\pi\omega}{V_0}n\kappa \tag{2}$$

In this equation, λ is wavelength of light in vacuum. V_0 represents the speed of light. By substituting equation (2) in equation (1), the following equation is obtained:

$$\alpha = \frac{2\pi Nze^2}{nV_0\varepsilon_0 m}\frac{\gamma\omega^2}{\left(\omega_0^2-\omega^2\right)^2+\gamma^2\omega^2} \tag{3}$$

It may be concluded that when the absorption coefficient α reaches a maximum, then resonance and absorption occur as ω goes to ω_0. In other words, the substance will absorb light when the frequency of light approaches the electron natural frequency. Furthermore, N is the number of molecules of p-nitrophenol per unit volume and reflects the concentration of methyl parathion, the concentration value is C, while the other parameters are inherent parameters. Therefore, absorption index can be conveyed as:

$$\alpha = kC \tag{4}$$

In this equation, k is proportionality coefficient of methyl parathion, which integrates several parameters of methyl parathion. The value of k is associated with the molecular character of the absorbing material and the frequency of detection light; k is irrelevant to the concentration of the solution. According to Lambert's law, we know that:

$$I = I_0 \cdot 10^{-\alpha gl} \tag{5}$$

In this equation, I is the light output intensity, I_0 is the incident light intensity. Substituting equation (4) in equation (5)，we obtain:

$$I = I_0 \cdot 10^{-kCl} \tag{6}$$

To describe sample‘s absorbance by the intensity of light this paper introduces the concept of absorbance with the following equation:

$$A = -\log_{10}\frac{I}{I_0} \tag{7}$$

Substituting equation (6) in equation (7), we obtain the mathematical expression of the absorbance A:

$$A = kCl \tag{8}$$

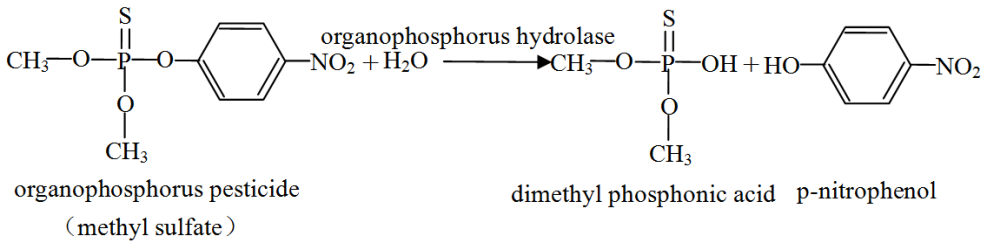

Fig. 1. Free sketch of the hydrolysis of organophosphorus pesticide.

In this equation, l is the optical length. Thus, absorbance is closely associated with the coeffcient of proportionality k, the concentration of methyl parathion C and the optical length l. If we can ascertain the numerical values of k and l, so absorbance A is proportional to the concentration of the material C and we can determine the concentration by measuring the absorbance.

Design of the microfluidic chip

The designed microfluidic chip consists of four parts, which are shown in figure 2. A is the area of sample introduction, B is the area of mixing, C is the area of reaction and D is the area of detection. The chip is made of polydimethylsiloxane (PDMS).

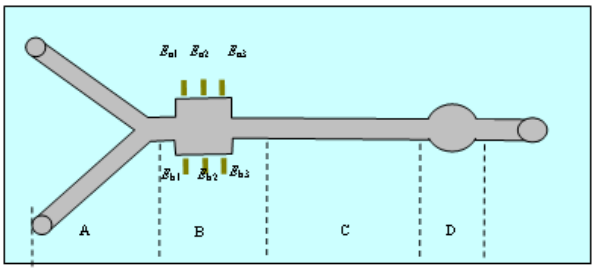

Fig. 2. Structure model graph of the design of microfluidic chip.

The area of sample introduction A is used to introduce the sample of organophosphorus pesticide (methyl sulfate) and the solution of hydrolytic enzyme. The area of mixing B is designed into chaotic mixing structure driven by the electrical field. In the picture, E_{a1}, E_{a2}, E_{a3} and E_{b1}, E_{b2}, E_{b3} are microelectrodes, which are used to produce chaotic reaction. The microelectrode material is ITO conducting glass. The area of mixing B is mainly used for mixing the organophosphorus pesticide and the solution of hydrolytic enzyme in order to trigger a complete organophosphate hydrolysis reaction. Because the progress of organophosphate hydrolysis reaction needs some time, the reaction area C can provide the space for mixing organophosphorus pesticide and organophosphorus hydrolase. There will be photometric detection after hydrolysis reaction has happened between the two fluids. The structure of the photometric detection zone D is shown in figure 3 which is the front view of the microfluidic chip model structure. From the picture we can conclude that the photometric detection zone penetrates the whole microfluidic chip, thus it can bring 5 mm detection optical path for absorbent detection. The cover is used to prevent overflowing of liquid from the detection zone. The cover is also made of PDMS. Figure 4 is a photograph of the microfluidic chip. The mixed zone extracts microelectrode. The material of the microelectrode is ITO conducting glass. The latter is welded with dupont line by silver paste so as to connect to the outside.

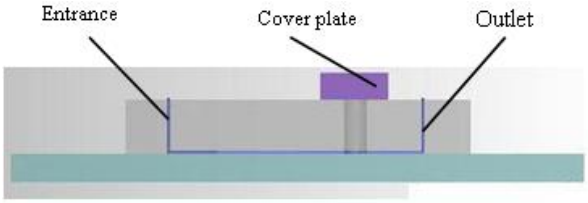

Fig. 3. Model structure front view of microfluidic chip.

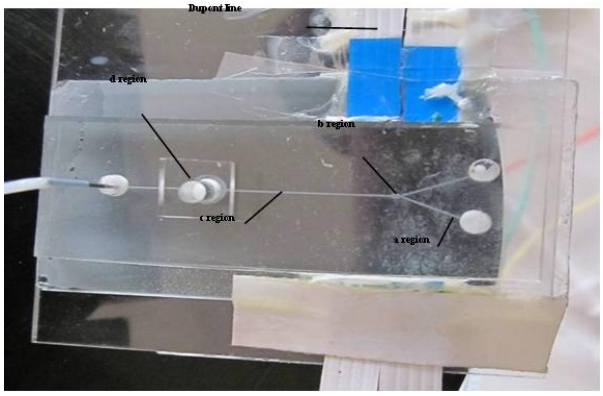

Fig. 4. Photograph of the microfluidic chip.

Experimental platform of the detecting system

Figure 5 is a photograph of the photometric detection system based on a microfluidic chip. The photometric check system is equipped with two channel squirt sampling pumps (0.098 μm/step, flow inaccuracy of CVs less than 1%) and two trace microinjectors (Terumo®, Terumo Corporation, Tokyo, Japan) (capacity of 1 mL), used to introduce seawater containing organophosphorus pesticide and organophosphorus hydrolase. The microfluidic chip is placed in the bracket of transmittance measurement, there is an optical fiber probe on the bracket and the optical fiber probe clings to the detection port of the microfluidic chip. There is detection fiber under the bracket. The detection fiber clings to the lower surface of the chip. The launching fiber is also connected with the optic precision light source (HL-2000-HP-FHSA). The optical fiber probe is connected with the USB2000 fiber optic probe spectrometer, and the light-emitting optical fiber probe is connected with a precise light source. The host computer, DSP system board and power drive plate form chaotic electric field controller. The chaotic electric field controller connects with the microfluidic chip electrode which can exert chaotic electric field to enable intensive mixing between organophosphorus pesticide and organophosphorus hydrolase.

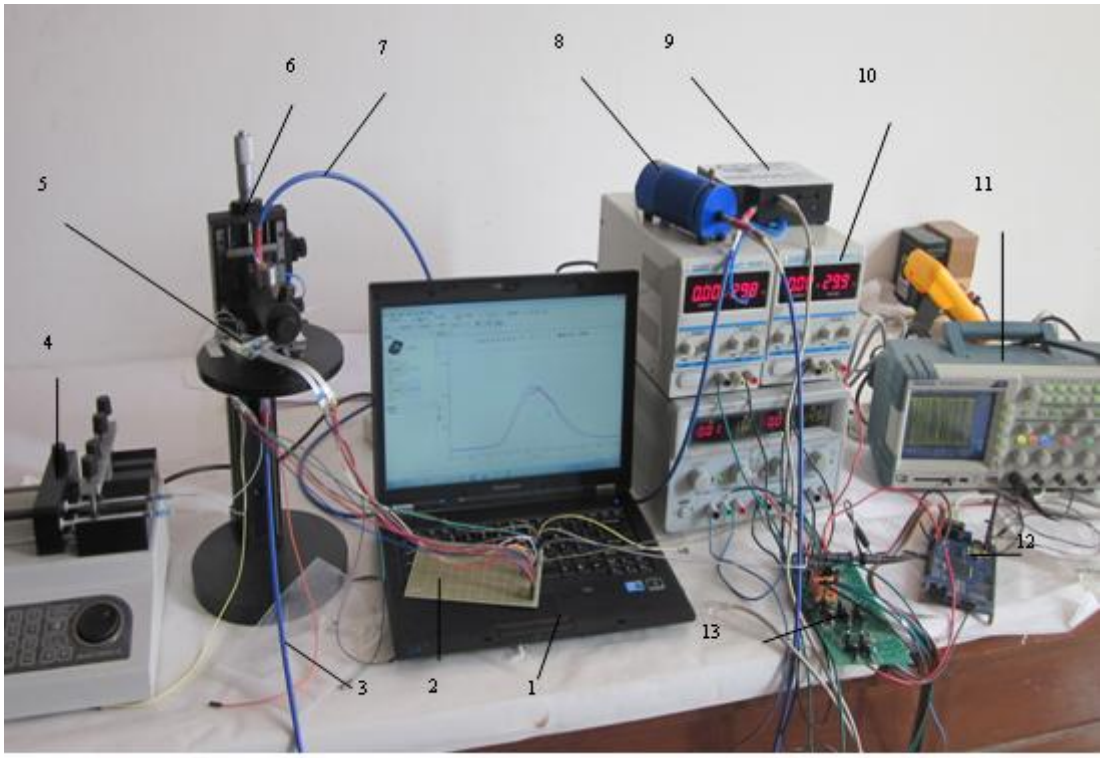

Fig. 5. Photograph of the detection system. 1-Host computer, 2-Expansion board, 3-Launching fiber, 4-Injection pump, 5-Microfluidic chip, 6-Transmission detector bracket; 7-Absorbance detection fiber, 8-UV-visible light, 9-Spectrometer, 10-Adjustable power supply, 11-Oscilloscope, 12-DSP system board, 13-Power driver board

Systematic approach to determine the optimal parameters

There are two main working parameters for rapid organophosphorus pesticide detection in microfluidic systems including optimum chaotic electric field parameter and optimal detection wavelength. The working parameters of the chaotic electric field are determined by simulating the mixing process between organophosphorus pesticide and organophosphorus hydrolase. As we know, different wavelengths have different sensitivity to the absorption of the hydrolyzate, so it is necessary to detect the absorbance of seawater samples on the basis of different concentrations of the pesticides. We can choose the most sensitive absorption wavelength as detection wavelength.

WORKING PARAMETERS OPTIMIZATION FOR THE DESIGNED SYSTEM

Optimizing the parameters of the chaotic electric field

Lorenz model is a typical kinetic model to study chaos in fluid turbulence. The Reynolds number of the fluid in microfluidic systems is usually very small, therefore we use the chaotic electric field to enable the intensive mixing of the laminar flow. This paper chooses the chaotic Lorenz model as a driving source in the micro-mixing chamber. We selected Lorenz chaotic driving electric field which is coupled by three different variables x, y, z. Formulas 9, 10 and 11 are the initial electric field expressions:

$$\frac{dx}{dt} = -c(x - y) \tag{9}$$

$$\frac{dy}{dt} = ax - y - xz \tag{10}$$

$$\frac{dz}{dt} = xy - bz \tag{11}$$

x, y, z are the three control signals which are used to drive the electrode of the micro-mixing chamber. b and c are dimensionless parameters, a is the working parameter of chaotic electrical field. The paper sets an initial value as: $x(0) = y(0) = z(0) = 1$. x is the electrode control signal of E_{a1} and E_{b2}, φE is equal to βx. y is the drive signal, y is negated for $-y$ which is referred as a signal to control the electrode. The electrode includes E_{b1} and E_{a2} in figure 2, while φE is equal to $-\beta y$. The driving signal z develops into z-a as the driving signal to the electrode includes E_{a3} and E_{b3}. In figure 2, φE is equal to $\beta(z\text{-}a)$, β is the amplification factor. The dimensionless parameter b is equal to 8/3, c is equal to 10 and a is equal to 28. Figure 6 is Lorenz chaotic orbit diagram under the control of

the placed signals.

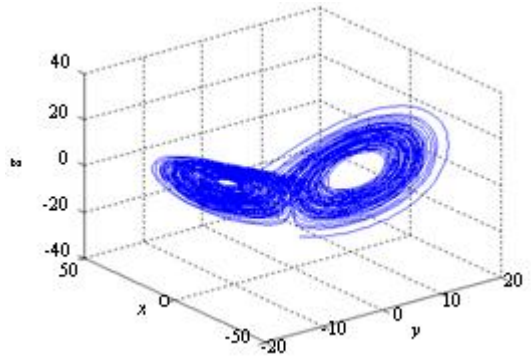

Fig. 6. Track diagram of chaotic state of the control electrodes.

Figure 7 is the microfluidic flow chart under the conditions of electric field controlled by Lorenz chaotic signal at 400 ms. According to section 3.2.1, the Lorenz chaotic signal x connects with E_{a1} and E_{b2}, while the Lorenz chaotic signal y connects with E_{a2} and E_{b1}. At the same time, E_{a3} and E_{b3} are connected with the signal of *z*. The connection type and the electric chaotic anti-control algorithm induce dynamic electroosmotic 7 vortices, wherein, the first two are large vortices and the others are small vortices. The stirring vortices promote the mixing process. Figure 8 is the scatter diagram of microfluidic concentration field under the conditions of electric field which is controlled by Lorenz chaotic signal control at 400 ms. From the picture, under the combined action of the double electrical layer electrodes on the wall surface of the microfluidic chip, an electric driving force which compels the fluid to move in, is produced. Under the configuration of electric field, the liquid near the wall of the mixing chamber moves from the high potential to low potential. Since the phase relationship between E_a and E_b is orthogonal, the wall driving force emerges under the action of the mixing chamber and forms synergy driven relationship. The relationship induces the formation of a significant convergence between the vortex circulation flow and swirl flow divergence. The marked circulation in the middle part of the micro-mixing chamber stretches and disperses the fluid. Then, a small vortex behind the micro-mixing chamber will have a secondary further dispersion after the fluid is dispersed, so as to promote the process of mixing.

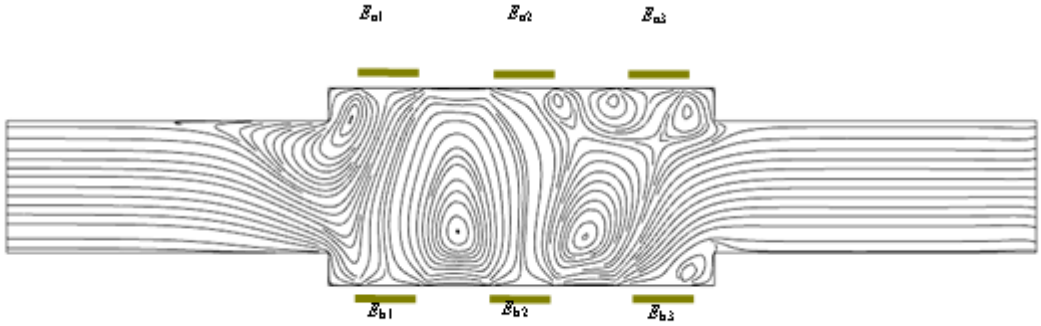

Fig. 7. Flow picture of microfluid under the conditions of electric field controlled by Lorenz chaotic signal.

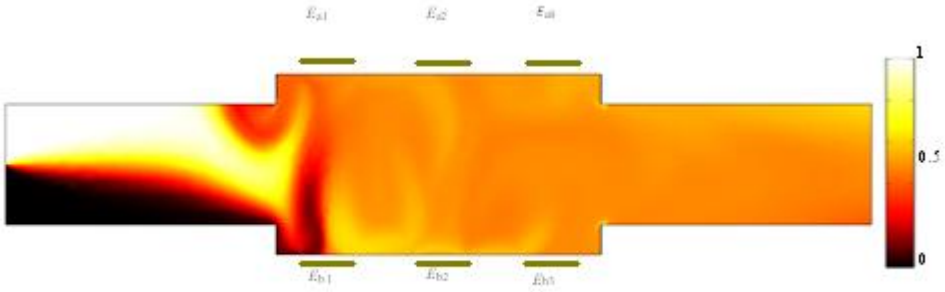

Fig. 8. Microfluidic concentration field distribution picture under the Lorenz chaotic signal control electric field conditions.

As can be seen from the figure, when the liquid flow is on the outlet of the mixing chamber, the concentration of fluid has become uniform. It is easy to conclude from the figure that the condition of laminar flow is broken and irregular movement appears when liquid enters the micro-mixing chamber.

The evaluation criteria of the mixing effect should include the uniformity coefficient of the sample solution, the concentration of tracer liquid, and the mass fraction of the tracer liquid. Therefore, we adopt the state of the concentration field to represent the variance in the gray image. The concentration distribution picture which is shown in figure 8 is converted to a gray-scale picture. The two gray-scale entrance boundaries are defined as 0 and 1, 0 representing the lowest concentration, and 1 representing the highest concentration. Other chromas are normalized as:

$$C_i = \frac{x_i - y}{z - y} \tag{12}$$

In the equation, C_i is the value of chromaticity after normalization. x_i is the value of chromaticity that will be calculated. y is the entrance of low concentration, while the low concentration can be expressed to 0 "gray-scale". z is a high concentration entrance, while the high concentration can be expressed to 1 "gray-scale". The target of mixing effect can be expressed as:

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(C_i - \sigma_0\right)^2} \tag{13}$$

In the equation, N is sample size, σ_0 is the average chromatic value after the liquid is completely mixed, while the liquid is the mixture of organophosphorus pesticide and the solution of hydrolytic enzyme, so σ_0=0.5. In terms of the numerical simulation object, the value of σ varies from 0 to 0.5, 0 indicates intensive mixing, 0.5 shows incomplete mixing. Figure 9 is the distribution graph of the mixing effect evaluation at the outlet of the micro-mixing chamber. The working parameter a is chosen from 2, 35, 120 and 240.

From the figure, when the working parameter a is equal to 2, the mixing evaluation index σ little changes over the time and the working parameter is in a large state (about 0.4) at the outlet. This phenomenon indicates that the electric field is not clearly an effective way to control over hybrid drive. When the working parameter a=35 or 120, in other words, fluid particles are under the conditions of chaotic motion, the mixing index σ changes largely over time and stops at the point of a small value (0.04) at the outlet, which indicates that the chaotic system has control over the mixing drive at the moment.

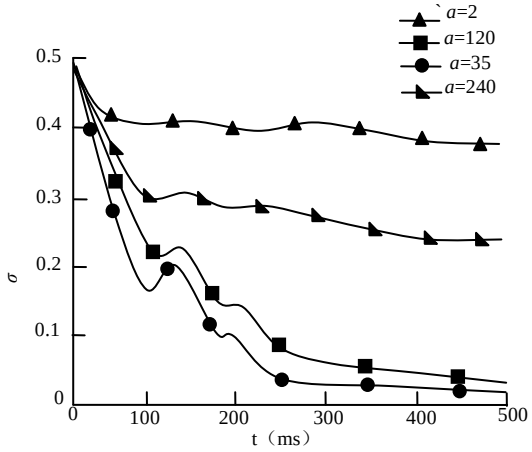

Fig. 9. Mix effect changes in different working parameters at the export.

When the working parameter a is equal to 240, in other words, fluid particles are in a non-chaotic motion, the mixing evaluation index σ has small change over time at the outlet and eventually stops at the larger state of about 0.28, which indicates that the system of Lorenz has poor control over the mixing results. Therefore, the paper selects 35 for the working parameter a of the chaotic electric field.

Determination of the optimal detection wavelength

Only when the hydrolysis product of organophosphate pesticide has resonance with the frequency of light wave, the proportional relation between the concentration and the absorptivity of light can be truly reflected. Therefore, experimental methods are needed to select the optimal detection wavelength. In the experiment, the adopted concentrations of organophosphorus pesticide are 2×10^{-5}mol/mL, 4×10^{-5}mol/mL, 6×10^{-5}mol/mL, 8×10^{-5}mol/mL, and 1×10^{-4}mol/mL, respectively. Organophosphorus pesticide will react with the hydrolytic enzyme, the absorption spectrum is shown in the figure. Different concentrations of organophosphorus solution will be obtained by diluting with sodium tetraborate. From the figure, we can also conclude that when increasing the concentration of organophosphorus pesticide, the absorption spectrum curve also increases.

The wavelength should be within the range of 200 nm~800 nm. It is necessary to screen characteristic absorption wavelength. The principle of screening is to select the wavelength which has the largest variance ratio as the characteristic wavelength. After calculating, we have found that the change rate reaches a maximum when the absorbance is at 405 nm, the maximum is at 0.08 Abs/($\times10^{-5}$ mol/mL).

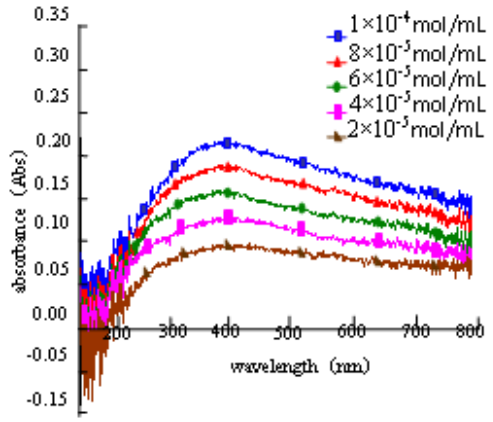

Fig. 10. Absorption spectra of different concentrations of organophosphorus pesticide hydrolyzate

ANALYSIS OF THE DETECTION SYSTEM

According to the optimal system parameters obtained in section 3, the analysis results were compared with those of the traditional chromatographic detection method.

Calibration curve

Different concentrations of organophosphorus pesticide reagent and the solution of hydrolytic enzyme were added into the syringe pump system. The measurement result is regarded as one part of the calibration curve which is shown in figure 11. A good linearity is seen between organophosphorus pesticide and absorbance, the linear equation is $y=2.995\times10^{3}x-9.74\times10^{-3}$, the correlation coefficient (R) is 0.9934.

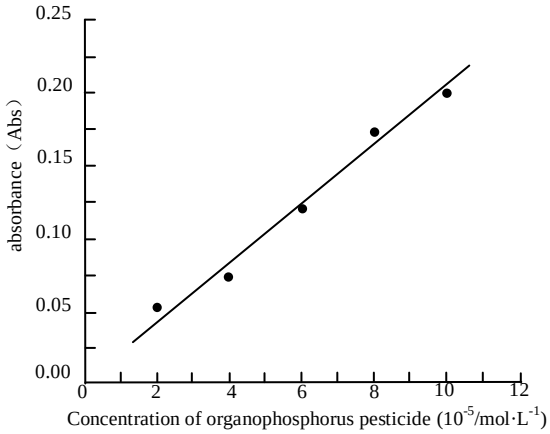

Fig. 11. Absorbance changes with the concentration of organophosphorus pesticide.

Comparative analysis of samples detection accuracy

In order to analyze the detection accuracy of the designed microfluidic chip system, nine samples were selected whose concentrations change with the step of 1×10^{-5} mol/mL in the absorbance detection experiment. The concentrations of pesticide samples in seawater were from 2×10^{-5} mol/mL to 10×10^{-5} mol/mL. Finally, a relation analysis between detection result and the result of chromatographic detection was performed. Figure 12 is the scattergram obtained by adopting two methods to detect organophosphorus pesticide. The correlation coefficient obtained by the regression analysis method was 0.963. Compared to the traditional chromatography, these data indicate that water pesticide pollution detection method based on microfluidic chip has no obvious deviations in terms of the accuracy of detection.

For the present water pesticide pollution detection method, the consumption of reagent is only 1/1000 compared to the traditional method and the level of automation of the designed system is improved.

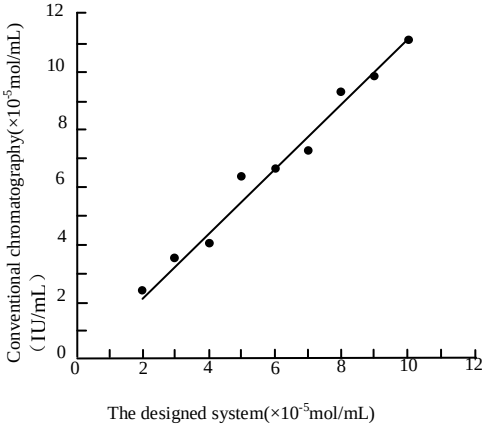

Fig. 12. Regression analysis between designed system and chromatography.

CONCLUSIONS

This paper presents a water pesticide contamination detection system based on a microfluidic chip. The organophosphorus pesticide pollution detection is taken as an example and the working parameters of the system are optimized. The experimental results show that the accuracy of the system detection is basically the same as in conventional chromatography, using only nanoliter quantities of the reagent. For the designed system, the liquid consumption is only 1/1000 of that in chromatography. Therefore, the paper can provide a theoretical basis for a portable and on-line water pesticide contamination monitoring system.

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МЕТОД ЗА ФОТОМЕТРИЧНО ОТКРИВАНЕ НА ОРГАНО-ФОСФОРНИ ЕСТИЦИДИ, ОСНОВАН НА МИКРОФЛУИДЕН ЧИП

Н. Янг[1,2], Х. Мао[2*], Дж. Сун[1], Ч. Ксианг[1], П. Ксу[1]

[1] *Училище по електро- и информационно инженерство, Университет Джиангсу, Женгджиан, Китай*

[2] *Институт по аграрно инженерство, Университет Джиангсу, Женгджиан, Китай*

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(Резюме)

Замърсяването на водите с пестициди е един от най-важните проблеми, които спешно трябва да се решат от човечеството, но откриването на органо-фосфорните пестициди във водна среда често зависи от традиционните лабораторни условия при дълго време и ниска степен на автоматизация. Настоящата работа предлага фотометрично откриване с микрофлуиден чип. За целта бяха създадени специален микрофлуиден чип и автоматизирана система, включваща въвеждане на пробата, смесване, реакция и откриване, основано на хидролиза на пестицидите. В работата също се оптимизират работните параметри на детекторната система в микромащаби. Експерименталните резултати показват, че създадената система е паралелна на традиционната хроматография за откриването на органо-фосфорни пестициди в морски води, като корелационният коефициент достига 0.963. В същото време анализираното количество е една хилядна от това в традиционната хроматография, а степента на автоматизация е много по-висока.