sensors

Determination of Atropine in Injection with β-Cyclodextrin Modified Ion Sensitive Field Effect Transistor Sensor

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Abstract: A new atropine drug-FET sensor, which was made of an ISFET (ion sensitive field effect transistor) and a drug sensitive membrane prepared by adding electric active matter to a β-cyclodextrin solution, was developed. The pH influence, selectivity, response curve, reproducibility, stability, response time and life of this sensor were investigated. A preliminary application of the sensor was discussed as well. The experimental results indicated that the sensor showed a Nernst response to atropine with a slope of 58.0 mV/decade over the concentration range of $5.0 \times 10^{-3} - 1.0 \times 10^{-6}$ mol/L and the pH range of $5.0 - 8.5$. The sensor detection limit was 8.0×10^{-7} mol/L. The performance of this sensor was very stable when it was used to determine atropine concentration in a medicinal injection. The obtained results agreed well with the pharmacopoeia method.

Keywords: atropine, β-cyclodextrin, ion sensitive field effect transistor, drug analysis.

1. Introduction

Since the ion sensitive field effect transistor (ISFET) response to H^+ was firstly reported by P. Bergved [1], it has drawn the attention of many scientists because of the excellent characteristics of its solid state, miniature size and highly potential value. Furthermore, the ISFET can be microminiaturized, integrated and multi-functional. Owing to the peculiarities of the ISFET, such as self-impedance alteration, lower output impedance and compatibility with MOS processing technology, scientists can utilize it to prepare new kinds of intelligent analysis instruments by assembling the sensor and the determining circuit. In recent years, there have been great advances in the ISFET researches. By substituting different sensitive membranes for the MOSFET metal grid, many kinds of

ISFET sensors have been developed. These sensors of new types can be used in many fields such as chemistry, biology, medicine, environment protection, and industrial control [2-5].

As a drug resistant to choline, atropine has been widely used to cure some diseases. On one hand, it can treat muscle spasm, inhibit gland secretion, dilate eye pupils, and excite the respiratory central nerve. On the other hand, it can assist serious patients in shock caused by infection or poisoning by organic phosphorus farm chemicals. It can also relieve the pains caused by gastroenteric spasm, kidney angina and gallbladder angina. Since atropine is a poisonous drug in itself, its dosage must be strictly controlled, and its clinical determination is significant. The research of an ISFET chemical sensor, therefore, has fine prospects.

The measurements of atropine, including UV spectroscopy and HPLC methods, however, have some disadvantages [6], such as their complex equipment, troublesome operations, etc. Therefore, two methods for measuring atropine with ISFET have been reported. One was based on tri-o-e-β-CD, which is complicated in structure and difficult to be synthesized [7], another was based on sodium tetraphenylboron, as the electric active material. Both of them allowed only narrow ranges of pH, high lower-limit of the measurements [8].

In this research, a new detection method for atropine based on ISFET was studied and a new-type atropine ISFET sensor was developed. Comparing with what has been done during the past, β-CD was used as its electric active material herein, which is much easier to be obtained. It allowed wider pH ranges and possessed higher sensitivity.

To confirm the validity of this method, several operating factors such as the pH, selectivity, response curve, reproducibility, stability, response time and the life of this sensor was investigated. The preliminary application of the sensor is reported in this paper.

2. Experiments

2.1. Instruments and reagents

The instruments used in the experiments include ISFET determination meter (XG-ISFET-02, made in China), pHS-3C precision acidometer (produced by Shanghai Analytical Instrument Factory, China), and Type-217 mercurous chloride electrode (produced by Shanghai Light and Electrical Instrument Factory, China).

The reagents used in the experiments include β-CD (A.R), poly vinyl chloride (PVC) powder (purchased from Shanghai Chemical Factory), dibuty (o-) phthalate (A.R), and Atropine (A.R). Other reagents are of analytical grade and double distilled water used in all the experiments.

2.2. The preparation of atropine sensitive field effect transistor

Based on the plane technology, the n-channel depletion mode field effect transistor (FET) was prepared by depositing a layer of insulating material of Si_3N_4/SiO_2 on the crystal face (100) of p-type silicon with the conductivity of 6~8 S/cm [9].

The FET was isolated in a plastic or glass pipe with a sealing material (silicon rubber or epoxy resin), two metal fibres were drawn from the source (S) and drain (D), and the Si_3N_4/SiO_2 layer of the

insulated-gate was exposed. A layer of active membrane was painted on the Si_3N_4 surface. The preparation process of atropine sensitive Drug-FET was as follows: a suspension was firstly prepared by adding 500 mg of PVC as base polymer, 200 mg dibuty(o-)phthalate as plasticizer, 8 mg β-CD as receptor, and 5 ml of tetrahydrofuran as solvent respectively. The suspension was then smeared on the surface of the Si_3N_4/SiO_2 gate of the FET and dried at room temperature for 24 hours. Finally, the atropine sensitive drug-FET was activated by immerging it in the 1.0×10^{-3}mol/L atropine solution for ten hours, so that the FET could respond to Atropine. The structure of ISFET is shown in Figure 1.
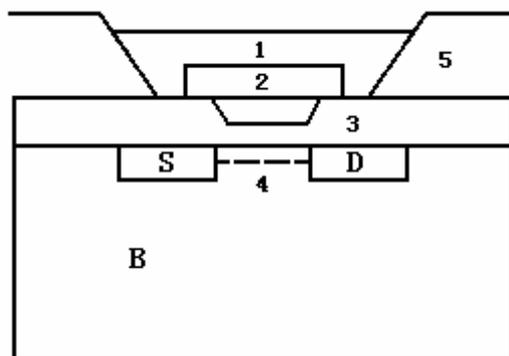

Figure 1. Structure of ISFET. S: source; D: drain; B: p-silicon; 1- drug sensitive membrane; 2 - Si_3N_4; 3 - SiO_2; 4 - n-channel; 5 - silicone rubber.

2.3. Measurement of the sensor's performances

The atropine ion sensitive FET chemical sensor was prepared by connecting the atropine sensitive drug-FET and a mercurous chloride electrode with a double salt-bridge (an outer salt bridge in a solution of 1.0mol/LKNO_3) to become a loop. When this sensor was used, the voltage V_{DS} and the current I_{DS} between the source (S) and the drain (D) should be kept constant. The relationship between the output voltage (V_{Gout}) of the gate (G) and the concentration of ions obeyed the Nernst equation. The working curve could be obtained by drawing the V_{Gout} and pC (minus logarithm of the concentration of Atropine) [10-11].

If the current I_{DS} and voltage V_{DS} between drain (D) and source (S) are kept constant, the voltage of gate (G) V_{Gout} varies with the change of the component concentrations to be determined. The relationship between them is in accordance with the Nernst formula as shown below:

$$E_M = (RT/ZiF)ln a_i \tag{1}$$

Where, a_i is the activity of atropine.

In this case, the sensor's gate voltage V_{Gout} was the algebra sum of the reference electrode potential E_{Ref}, the membrane potential E_M and the applied voltage V_G. The current between drain (D) and source (S) is shown as follows:

$$I_{DS}=K[(V_G+E_M-V_T)-V_{DS}/2] \tag{2}$$

Where, K is a parameter related to the sensor, and V_T is the grid voltage related to the performance of sensor and the reference electrode potential E_{Ref}. In this case:

$$I_{DS}=0, \quad V_{DS}=0, \tag{3}$$

Introducing Eq. (3) into Eq. (2) and arranging, we obtain

$$\triangle V_{Gout}=E_M=(RT/Z_iF)ln a_i \qquad (4)$$

During the determination, the ISFET and reference electrode (Type-217 mercurous chloride electrode) formed a measuring current loop. An Operational amplifier was used to bring a stable criterion voltage. The operating state (V_{DS}, I_{DS}) of ISFET was calibrated by adjusting W_1 and W_2. When the concentration of solution varied, the V_{Gout} also altered. The relative curve of V_{Gout} versus–log C (C was the concentration of solution to be determined) could be obtained according to the experimental results. The determination principle of atropine ISFET sensor is shown in Figure 2.
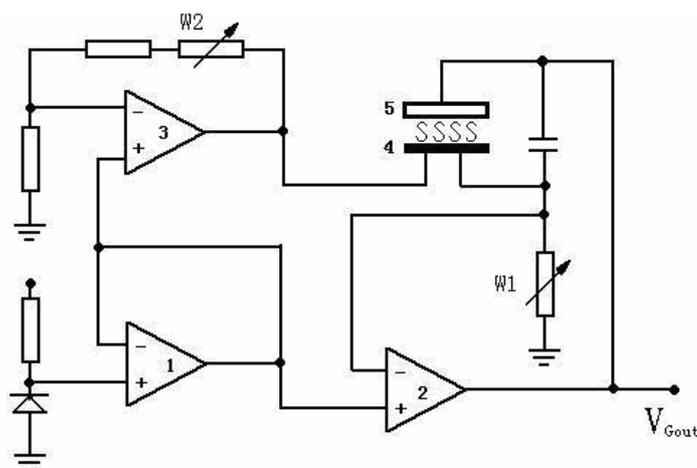

Figure 2. Determination principle of ISFET.

3. Results and Discussion

3.1 The influence of the β-CD content

The sensitivity of measurement is greatly influenced by the content of β-CD, which can be used as the electro-active substance responding to atropine, the response between the V_{Gout} with content of β-CD thus was investigated and the results are shown in Table 1. It can be known from the results that the maximum response appears when the β-CD content was 8mg in the membrane.

Table 1. The influence of the β-CD content.

Membrane constituents	PVC（mg）	β-CD（mg）	V_{Gout}（mV）
1	500	4	40.5
2	500	6	51.7
3	500	8	58.1
4	500	10	55.2

3.2. The effects of pH

Sets of solutions were prepared by mixing 1.0×10^{-3} mol/L atropine solutions, 1.0 mol/L HCl and 1.0 mol/L NaOH. The pHs of the mixture solutions were in a range from 3.0 to 9.0. The effect of pH on V_{Gout} was investigated and the results are shown in Figure 3. The figure showed that the V_{Gout} did not change significantly in the pH range from 5.0 to 8.5, however, as a weak base at the low side of pH, atropine could be combined with acid into salt when the acidity was increased, the effective concentration of atropine was decreased, the response V_{Gout} thus was decreased. When the basicity in the solution became stronger, however, β-CD could change in structure as electric active matter, thus leading to augmentation of the response V_{Gout}.
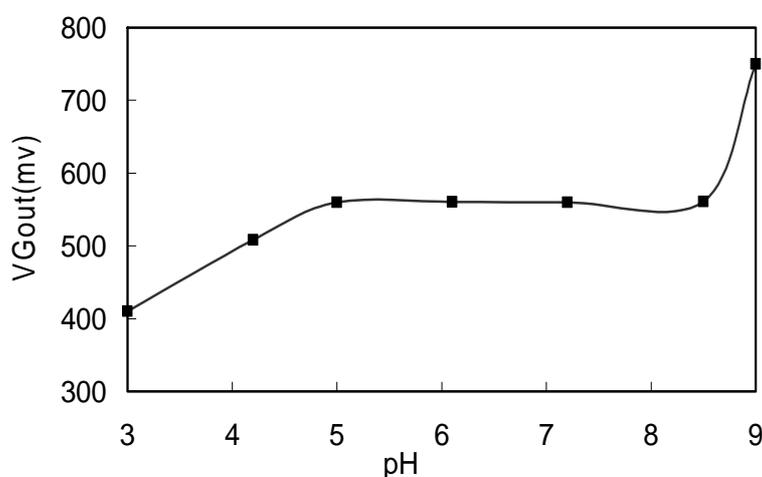
Figure 3. The effects of pH.

3.3. The selectivity of the sensor

Some chemicals were chosen as the research objects. All of them are the substances that would be met during the application of the sensor such as producing and clinic using processes of atropine, or substances that would affect the determination process of atropine.

Based on the method of fixation disturbing for mixing solution, the selectivity coefficient of the sensor was determined. The results are shown in Table 2. It showed that the sensor had high selectivity since most of the substances did not disturb the determination of atropine except for procaine.

3.4. The response curve of the sensors

Since the response of the sensor used in this work is a kind of host-guest response between the β-CD and atropine, V_{Gout} of the sensor and the concentration of the atropine showed a linear relationship as given in Figure 4.

. **Table 2.** The selective coefficients of the sensor (solution-mixing method).

Disturbing substance	K
KCl	1.2×10^{-3}
NaCl	5.0×10^{-4}
NH_4Cl	1.5×10^{-3}
$Ca(NO_3)_2$	3.0×10^{-3}
Glucose	1.5×10^{-3}
Procaine	Interfered
Caffeine	1.1×10^{-2}
VB_1	1.5×10^{-2}
Erythromycin	3.5×10^{-2}
Adrenaline	5.0×10^{-2}

In the concentration range of 5.0×10^{-3}-1.0×10^{-6} mol/L, the relationship between V_{Gout} and pC obeyed the linear correlation equation: V_{Gout} (mV) = 518.5+58.0pC (mol/L). The linear correlation coefficient of the equation $\gamma = 0.999$. The sensors detection limit was 8.0×10^{-7}mol/L, while the RSD of atropine solution determination for five times was 2.1%.
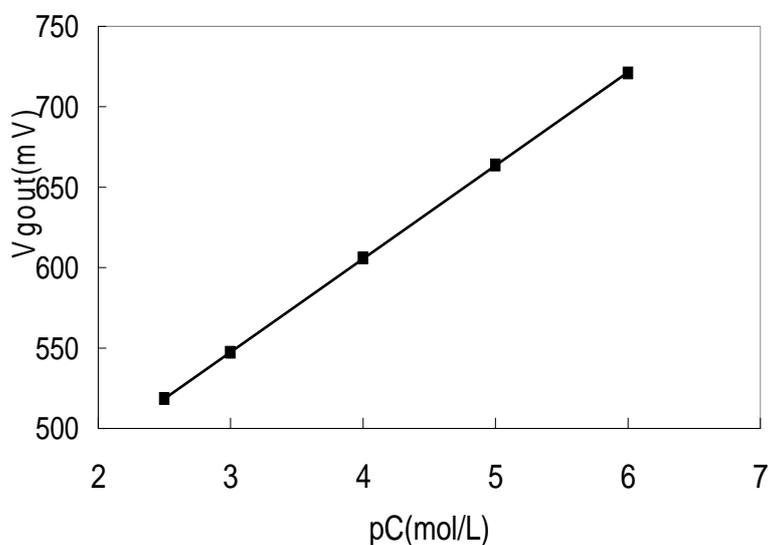
Figure 4. Calibration of curve.

3.5. The reproducibility

In the concentration range of 1.0×10^{-3}-1.0×10^{-6} mol/L atropine solutions, each sample was repeatedly determined for five times, the variation of V_{Gout} is shown in Table 3. For each sample, the change of the V_{Gout} was less than 1 mV. When the sensor was continuously used for four hours, the V_{Gout} was still less than 1 mV. Therefore, it can be considered that the reproducibility of the sensors is good and the drift is small.

Table 3. Reproducibility of the sensor.

Atropine (mol/L)	V_{Gout} (mV)					Average	Reproducibility
1.0×10^{-6}	847	848	846	847	848	847.2	$\leqslant\pm1.0$
1.0×10^{-5}	787	787	786	787	788	787.0	$\leqslant\pm1.0$
1.0×10^{-4}	729	728	727	728	728	728.0	$\leqslant\pm1.0$
1.0×10^{-3}	670	671	670	671	671	670.6	$\leqslant\pm1.0$

3.6. The response time and the life of the sensors

The response time and the life of the sensors are also important factor. Generally, the response time is defined as the time interval from the beginning of determining until the change of the V_{Gout} is less than 1 mV. The life of the sensor is the time interval from the start using until it cannot response normally.

In this work, the response time was less than 30 seconds for 5.0×10^{-3}-1.0×10^{-6} mol/L atropine solutions, and the test on the life of the sensor showed that the sensor still remained good response characteristics after three months storage.
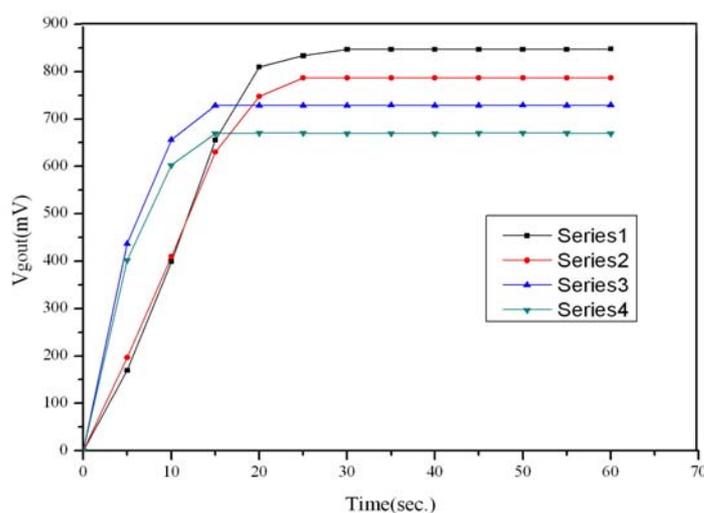

Figure 5. Response time of sensor. Series1, 1.0×10^{-6} mol/L atropine, Series2, 1.0×10^{-5} mol/L atropine, Series, 3 1.0×10^{-4} mol/L atropine, Series 4, 1.0×10^{-3} mol/L atropine.

3.7. The preliminary application of the sensors

This sensor was applied to quantitatively analyze injection atropine. The standard curve was firstly obtained: the atropine sample was weighed to prepare some reserve solution in the concentration of 1.0×10^{-3} mol/L, this solution was diluted step by step and a series of standard solutions were obtained. The V_{Gout} of these solutions were measured respectively, and a plot of V_{Gout} with minus logarithm of

the concentration for standard sample thus was obtained. When the V_{Gout} of the sensor was measured in the same condition, the amount of injection atropine thus was known from the standard curve.

This method was also compared with the spectroscopy method in Pharmacopoeia of P. R. China [12]. The results are shown in Table 4. It showed that the results obtained by this work agreed well with the data reported by the traditional method. However, since the proposed method allows wider pH ranges, possesses higher sensitivity, accuracy, rapidness, easy operation and the characteristic of the effective determination without using any expensive equipment, it is more perspective.

Table 4. The determination of atropine concentration for an injection.

Method/No	1	2	3	4	5	X ± SD
Sensor	1.02	1.00	1.02	1.06	1.08	1.04 ±0.03
Pharmacopoeia	1.05	1.03	1.06	1.04	1.06	1.05 ±0.01

4. Conclusion

A new ISFET sensor modified with β-CD for determining atropine concentrations in solutions was developed. The determination results obtained by using the new ISFET sensor agreed well with the data obtained by pharmacopoeia method.

Since the sensor is a kind of host-guest response between β-CD and atropine, it shows a high sensitivity. Meanwhile, because of the sensor's peculiarities of self-impedance alteration, lower output impedance and compatibility with MOS processing technology, it can be beneficially used to prepare a new kind of intelligent analysis instrument. The analytical method proposed in this research provides many advantages such as full-solid, miniature volume, the potential of being microminiaturized, integrated and multi-functional capability.

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