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# Optical fiber sensor for tetracycline antibiotics based on fluorescence quenching of covalently immobilized anthracene

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**A new optical fiber sensor for tetracycline is fabricated with an anthracene-containing copolymer prepared from 9-anthrylmethyl methacrylate, methyl methacrylate and *n*-butyl acrylate (PAMB). The sensing relies on the fact that the fluorescence of the sensor membrane fabricated with PAMB can be strongly quenched by the tetracycline antibiotics extracted from the sample. The proposed sensor responds linearly in the measuring ranges $2.02 \times 10^{-7}$–$2.02 \times 10^{-4}$ mol l$^{-1}$ tetracycline (TC), $2.00 \times 10^{-7}$–$2.00 \times 10^{-4}$ mol l$^{-1}$ oxytetracycline (OTC) and $4.05 \times 10^{-7}$–$2.03 \times 10^{-4}$ mol l$^{-1}$ doxycycline (DC) and has detection limits of $1.00 \times 10^{-7}$ mol l$^{-1}$ for TC and OTC, and $2.00 \times 10^{-7}$ mol l$^{-1}$ for DC. The leaching of anthracene from the sensor membrane was hindered by covalent immobilization, resulting in a drastically enhanced sensor lifetime. Moreover, the sensor can rapidly respond to the antibiotics of interest (*ca.* 30 s) and exhibits good reproducibility, reversibility, and selectivity in the presence of some common pharmaceutical species as well as alkali and alkali-earth metal salts. The sensor was used for the direct assay of tetracycline antibiotics in commercial pharmaceutical preparations and urine. The results are comparable to those obtained by conventional spectrophotometry. The recovery for tetracycline antibiotics from urine samples is also satisfactory.**

***Keywords:*** *Optical fiber sensor; tetracycline antibiotics; anthracene-containing copolymer*

Optical fiber sensors based on organic polymeric membranes have been developed considerably in the last decade.[1–17] The immobilization of sensing reagents onto optical fibers is an important factor in the development of optical fiber sensors. The reagents are normally adsorbed onto,[1] or chemically bound to,[2–8] or physically entrapped [9–17] into the supporting matrices. There is a great interest in covalent immobilization of susceptible molecules, which prevents leaching of sensitive components from the organic membrane phase into the sample solutions and results in an enhanced lifetime of the sensors.

Owing to their photochemical stability, light absorption and fluorescence properties, anthracene-containing copolymers have been used as plastic scintillators[18] (organic photosemiconductors). They are widely employed in studies of photophysics and photochemistry including intra- and interpolymer interactions of polymer bound fluorophores and polymer-sized molecular interaction.[19–22] In this paper, PAMB, an anthracene-containing copolymer prepared from 9-anthrylmethyl methacrylate (AMMA), methyl methacrylate (MMA) and *n*-butyl acrylate (BA) is reported and used for the sensor membrane construction. BA and MMA are selected in the preparation of the anthracene-containing copolymer for the following reasons. The abundance of *n*-butyl groups in the copolymer molecules are supposed to act as an 'internal' plasticizer and thus reduce the necessary amount of external plasticizer added. As a result, the adhesion ability of the membrane on quartz glass increases.[23] The viscosity of the copolymer is adjusted by the amount of MMA added. The described sensor has the advantage of covalent immobilization to provide a long working lifetime, yet retains a fast response.

Tetracycline antibiotics have been employed extensively as bacteriostatic and antibiotic drugs due to their high activity against nearly all gram-positive and gram-negative bacteria. Methods for measuring the concentrations of tetracycline antibiotics include spectrophotometry,[24] fluorimetry,[25] HPLC,[26] and electrochemical detection.[27] Recently, an optical sensor for tetracycline antibiotics based on chelate reaction has been reported.[28] The major disadvantages of the sensor are the relatively long time for one measuring cycle and the leaching of $Eu^{III}$ from the resin. This paper presents a new sensing method based on the fluorescence of the sensor membrane made of PAMB copolymer quenched by tetracycline antibiotics. The simple and sensitive sensing element of the proposed sensor requires a short measuring time. In addition, the sensor exhibits good selectivity and can be used for the direct determination of tetracycline antibiotics in urine and commercial pharmaceutical preparations.

## Experimental

### *Materials*

MMA and BA (Tianjin Chemical Reagents, Tianjin, China) were purified by repeated washing with 2 mol l$^{-1}$ NaOH until the initially red washings became colorless with subsequent redistillation under vacuum. A monomer of AMMA was synthesized according to Ref. 29, and recrystallized from methanol as yellow crystals with an mp of 83–84 °C. The calculated analysis for $C_{19}H_{16}O_2$ was: C, 82.59%, H, 5.83%; the found composition was: C, 82.40%, H, 6.01%. The IR spectrum shows a strong absorption at 1710 cm$^{-1}$ for the carbonyl group. 2,2′-Azobis(isobutyronitrile) (AIBN, Shanghai Chemical Reagents, Shanghai, China) was recrystallized from methanol. Dichloroethane, trichloromethane, tetrahydrofuran (THF) and bis(2-ethylhexyl) sebacate (DOS) were purchased from Changsha Chemical Reagents (Changsha, China) and used without further purification.

Tetracycline (TC), oxytetracycline (OTC) and doxycycline (DC) were obtained from the Institute of Pharmaceuticals and Biologics (Beijing, China). The stock standard solutions of TC ($1.011 \times 10^{-3}$ mol l$^{-1}$), OTC ($1.001 \times 10^{-3}$ mol l$^{-1}$) and DC ($1.013 \times 10^{-3}$ mol l$^{-1}$) were prepared by dissolving an appropriate amount of the respective crystals in water. Working solutions were obtained by serial dilution of the corresponding stock solutions. $NH_3$–$NH_4Cl$ buffer (pH 9.5) was obtained by dissolving 30 g of $NH_4Cl$ and 65 ml of 15 mol l$^{-1}$ ammonia in water and diluting to 500 ml. The solution pH was checked by a pH meter and adjusted if necessary.

Unless otherwise stated, all reagents were of analytical-reagent grade and all solutions were prepared with doubly quartz redistilled water.

### *Copolymer preparation*

PAMB was prepared as follows: methyl methacrylate (313 mg), *n*-butyl acrylate (1876 mg), and 9-anthrylmethyl methacrylate (110 mg) were copolymerized in 7.0 ml of dichloroethane with AIBN (10 mg) as an initiator. The mixture was bubbled with high-purity nitrogen for 20 min and the copolymerization was carried out for 24 h under refluxing. The cooled solution was poured into 100 ml of methanol under vigorous stirring. A sticky copolymer precipitate was obtained, which was purified three times by dissolving in trichloromethane, then reprecipitated from 100 ml of methanol. The resulting precipitate was dried under vacuum at ambient temperature. The average molecular mass of PAMB was determined to be 68 580 (weight average molecular weight, $M_w$) and 63 403 (number average molecular weight, $M_n$) by gel permeation chromatography.

### *Membrane preparation*

A membrane cocktail was prepared by dissolving 74.1 mg of PAMB and 34.1 mg of DOS in 1 ml of THF. About 0.1 ml of the solution was used for fabrication of the sensor membrane by a spinning method.[30] A membrane of approximately 2–4 μm thickness was cast onto a quartz glass plate (12 mm in diameter).

### *Measurements on tetracycline antibiotics*

Fluorescence measurements were carried out using a Hitachi (Japan) M-850 spectrofluorimeter with bandpasses set at 5.0 nm for both excitation and emission and a filter of 390 nm set in the emission path. The detection of membrane fluorescence intensities was measured on the maximum excitation wavelength of 369.5 nm and the maximum emission wavelength of 412.8 nm. Tetracycline antibiotic sensing measurements were conducted on a home-made PTFE flow-through cell[31] in which the sensor membrane was located. A randomly distributed bifurcated bundle (30 + 30 quartz fibers, length 1 m, 6 mm in diameter at the common end) carried the light to and from the cell. Sample solutions were pumped through the cell at a flow rate of 1.2 ml $min^{-1}$. The sensor membrane was allowed to equilibrate with the sample solution for obtaining a stable fluorescence signal. After each measurement, the fluorescence intensity of the sensor membrane was recovered by pumping the blank buffer solution through the cell prior to the next measurement.

### *Sample preparation*

#### *Tablets*

The powder (~35 mg) of ground TC, OTC or DC tablets was dissolved in 10 ml of 0.02 mol $l^{-1}$ HCl and diluted to 50.0 ml. With the initial portion discarded, the filtrate was collected and used for analytical determinations.

#### *Urine*

Samples containing 1 ml of urine and 5.0 ml of pH 9.5 $NH_3$–$NH_4Cl$ buffer were prepared in 25.0 ml calibrated flasks and were diluted to volume with water.

## Results and discussion

### *Effect of acidity*

The fluorescence of the sensor membrane quenched by tetracycline antibiotics was found to be pH dependent. Fig. 1 shows the effect of pH on the fluorescence quenching efficiency $F_0/F$, where $F_0$ and $F$ denote the fluorescence intensity in the absence and presence of tetracycline antibiotics, respectively. The tetracycline antibiotic solutions were buffered at pH 2.65–11.34. It is clear from Fig. 1 that the $F_0/F$ values were nearly all constant at pH values lower than 5.58 for TC, OTC or DC, while an increase in quenching was observed on going from pH 5.58 to 9.31. The $F_0/F$ values reached the maximum and maintained a constant between pH 9.31 and 11.34. In subsequent experiments, a pH 9.5 $NH_3$–$NH_4Cl$ buffer solution was used for the determination of the tetracycline antibiotics in question.

### *Optimization of membrane composition*

The optimization of the sensor membrane composition was carried out by selecting the optimum membrane solution composition. A membrane cocktail was prepared by dissolving an appropriate amount of PAMB and DOS in 1 ml of THF. A simplex method was employed in the optimization of the amount of DOS, PAMB and anthracene bound to PAMB. Table 1 gives the results of the three-variable optimization. Because the content of the bound anthracene to PAMB copolymer was governed by the AMMA monomer participating in the copoly-

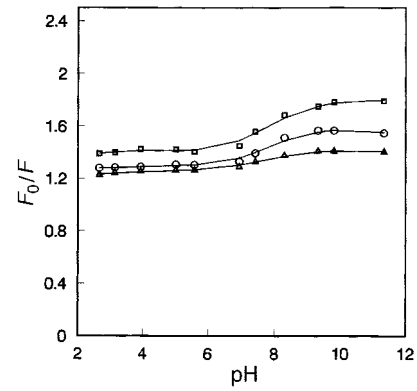


**Fig. 1** pH dependence of the response of the sensor exposed to 2.02 × $10^{-5}$ mol $l^{-1}$ TC (circles), 4.00 × $10^{-5}$ mol $l^{-1}$ OTC (squares), and 4.05 × $10^{-5}$ mol $l^{-1}$ DC (triangles).

**Table 1** Simplex optimization of the sensor membrane compositions for tetracycline antibiotics

| Experiment point | DOS/mg | AMMA/mg | PAMB/mg | $\Delta F^{*\dagger}$ TC | OTC | DC |
|---|---|---|---|---|---|---|
| 1 | 0.0 | 22 | 20.0 | 5.53 | 8.23 | 6.37 |
| 2 | 50.0 | 22 | 20.0 | 5.70 | 8.40 | 6.43 |
| 3 | 25.0 | 66 | 20.0 | 20.8 | 32.3 | 24.3 |
| 4 | 25.0 | 44 | 40.0 | 24.1 | 35.9 | 26.8 |
| 5 | 66.7 | 66 | 33.4 | 38.3 | 58.2 | 43.1 |
| 6 | 27.8 | 88(95.3) | 42.3 | 50.0 | 79.0 | 56.3 |
| 7 | 54.7 | 66 | 57.1 | 54.8 | 82.2 | 64.5 |
| 8 | 74.5 | 110 | 48.5 | 60.1 | 90.9 | 68.3 |
| 9 | 39.8 | 110 | 65.2 | 64.0 | 105.1 | 74.4 |
| 10 | 84.9 | 110 | 71.6 | 57.7 | 98.5 | 71.0 |
| 11 | 78.1 | 110(154) | 66.4 | 63.2 | 104.6 | 72.6 |
| 12 | 60.7 | 110 | 87.0 | 64.4 | 106.5 | 75.2 |
| 13 | 34.1 | 110 | 74.1 | 73.8 | 124.1 | 91.1 |
| 14 | 11.6 | 110 | 84.5 | 63.5 | 116.8 | 83.1 |
| 15 | 31.1 | 110 | 98.5 | 65.1 | 114.5 | 78.2 |

* $\Delta F = F_1 - F_2$, where $F_1$ and $F_2$ denote the fluorescence intensity of the sensor membrane exposed to blank and tetracycline antibiotic solutions, respectively. † Mean of three measurements. [TC] = 1.62 × $10^{-5}$ mol $l^{-1}$; [OTC] = 3.00 × $10^{-5}$ mol $l^{-1}$; [DC] = 3.04 × $10^{-5}$ mol $l^{-1}$.

merization, the bound anthracene optimization was performed using AMMA and was simplified as follows. It is rather difficult to prepare a PAMB copolymer sample containing all possible anthracene levels; therefore, only five PAMB copolymers containing different levels of anthracene were prepared by fixing 313 mg of MMA and 1876 mg of BA copolymerizing with 22, 44, 66, 88 and 110 mg of AMMA monomer, respectively. If an amount of AMMA necessary for the subsequent experiment, as calculated by the simplex method (*e.g.*, 95.3 mg at point 6 in Table 1), is not consistent with any aforementioned values, the sample with the AMMA amount most close to the calculated value (*e.g.*, 88 mg for point 6) was used instead. So for point 6 the sensor membrane prepared from the PAMB copolymer corresponding to 88.0 mg of AMMA monomer was used for the sensing experiment. In the case of a calculated AMMA amount (*e.g.*, 154 mg at point 11 in Table 1) higher than 110 mg, 110 mg of AMMA monomer was employed because of a low polymerization conversion under this condition.

The value of change in fluorescence intensity ($\Delta F$) of the sensor membranes prepared from the membrane cocktails in Table 1 was used for the evaluation of the sensor membrane composition when the sensor membranes were exposed to blank and tetracycline antibiotic solutions.

Point 13 represented the largest response obtained by the sensor membrane to all TC, OTC and DC. Thus, a membrane composition was determined by the optimum THF membrane cocktail containing 34.1 mg ml$^{-1}$ of DOS and 74.1 mg ml$^{-1}$ of PAMB copolymerized from 110 mg of AMMA monomer.

### *Lifetime*

To investigate the immobilization efficiency of anthracene onto the PAMB copolymer, we fabricated a sensor membrane trapped with AMMA in a copolymer of methyl methacrylate (313 mg) with *n*-butyl acrylate (1876 mg) (PMB) polymerized similarly to PAMB. A blank solution was continuously pumped through the flow-cell to contact with the sensor membrane (flow rate: 2 ml min$^{-1}$). The membrane fluorescence intensities were recorded over a period of 10 h with an interval of 30 min. The results are shown in Fig. 2. A decrease of 12.5% in fluorescence intensity was observed, indicating that a signficant leaching of AMMA from the PMB membrane occurred during the measurement process.

Under the same experimental conditions, the decrease in fluorescence intensity was not observed (Fig. 2) from the sensor membrane fabricated with the PAMB copolymer to which anthracene was covalently bound. This experiment clearly shows that the proposed sensor has a good short-term stability when contacting with a blank solution. Moreover, after the sensor membrane was continuously exposed to $6.07 \times 10^{-5}$ mol l$^{-1}$ TC, $4.00 \times 10^{-5}$ mol l$^{-1}$ OTC or $4.05 \times 10^{-5}$ mol l$^{-1}$ DC for 24 h, the fluorescence readings of the sensor membrane also maintained their initial values. The covalent immobilization of a membrane active component can substantially improve the lifetime of an optical sensor by hindering the leaching of such a component.

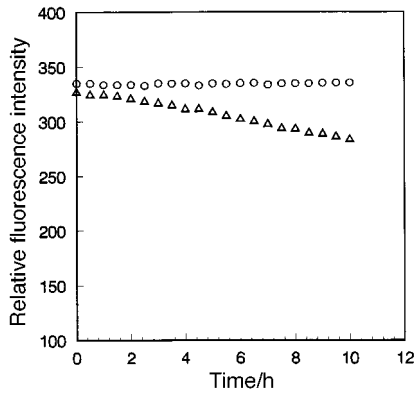


**Fig. 2** Stability of fluorescence readings of the sensors fabricated with PMB (triangles) trapped with AMMA and PAMB (circles) in continuous contact with a buffer solution.

### *Response time, reversibility and reproducibility*

The time-dependent responses of the sensor membrane on exposure to solutions of different concentrations of tetracycline antibiotics were tested. The response times to reach a stable fluorescence signal for TC, OTC, and DC were all approximately 30 s. This is a very important feature for the present sensor in comparison with previously reported sensors based on dyes covalently bound to polymeric materials, which usually show much slower response behavior.[8] The rapid response of the proposed sensor seems to be related to the vast amount of *n*-butyl group which acts as an 'internal' plasticizer and exists in the PAMB copolymer structure to which anthracene was bound. As a result, fast diffusion of tetracycline antibiotics within the membrane matrix can be guaranteed.

The reversibility of the sensor was also satisfactory. After sensing tetracycline antibiotics, the fluorescence intensity of the sensor membrane can be recovered by exposing it to a blank for about 30 s. The reproducibility of the sensor exposed to the tetracycline antibiotics of interest was evaluated by relative standard deviations of the membrane fluorescence intensities of repeat measurements. The results are listed in Table 2. It is noteworthy that the sensor has a good reproducibility for determinations of TC, OTC and DC.

### *Selectivity*

The effect of interferents on sensing tetracycline antibiotics was examined. Alkali and alkali earth metal salts and some common pharmaceutical species do not show a substantial effect on the membrane fluorescence intensities (Table 3). The sensor has a sufficient selectivity towards tetracycline antibiotics, making it feasible for practical application in pharmaceutical preparations and urine analyses.

### *Quantitative basis*

On the supposition that a 1 : 1 complex was formed between anthracene bound to the PAMB copolymer and tetracycline antibiotics, the quenching efficiency ($F_0/F$) is given by the Stern–Volmer equation

$$\frac{F_0}{F} = 1 + K[\mathrm{TCs}] \quad (1)$$

**Table 2** Reproducibility of the sensor on exposure to tetracyclic antibiotic solutions

| Tetracycline antibiotic | Concentration/ mol l$^{-1}$ | Mean fluorescence intensity* | RSD (%) |
|---|---|---|---|
| TC | $1.21 \times 10^{-5}$ | 270.9 | 0.550 |
| | $6.07 \times 10^{-5}$ | 160.8 | 0.530 |
| OTC | $1.20 \times 10^{-5}$ | 291.4 | 1.18 |
| | $6.01 \times 10^{-5}$ | 182.0 | 1.26 |
| DC | $1.62 \times 10^{-5}$ | 299.3 | 1.04 |
| | $8.10 \times 10^{-5}$ | 196.5 | 0.88 |

* Mean of ten determinations.

where $F_0$ and $F$ denote the fluorescence intensity in the absence and presence of tetracycline antibiotics, respectively, [TCs] is the respective concentrations of TC, OTC and DC, and $K$ is the corresponding quenching constant. The calibration equations are $F_0/F = 0.9544 + 2.406 \times 10^{-4}$ [TC] ($r = 0.9995$) for TC, $F_0/F = 0.9995 + 2.305 \times 10^{-4}$ [OTC] ($r = 0.9998$) for OTC, $F_0/F = 0.9544 + 1.439 \times 10^{-4}$ [DC] ($r = 0.9998$) for DC, where [TC], [OTC] and [DC] are the concentrations of TC, OTC and DC, respectively. The linear relationship between $F_0/F$ and the concentrations of TC, OTC and DC suggests that the stoichiometric ratios of the complexes between anthracene and tetracycline antibiotics are all 1 : 1. Thus eqn. (1) provides a quantitative basis for the determination of tetracycline antibiotics. Furthermore, the present sensor can respond linearly in the ranges $2.02 \times 10^{-7}$–$2.02 \times 10^{-4}$ mol l$^{-1}$ TC, $2.00 \times 10^{-7}$–$2.00 \times 10^{-4}$ mol l$^{-1}$ OTC, $4.05 \times 10^{-7}$–$2.03 \times 10^{-4}$ mol l$^{-1}$ DC with detection limits of $1.00 \times 10^{-7}$ mol l$^{-1}$ for TC and OTC, and $2.00 \times 10^{-7}$ mol l$^{-1}$ for DC.

**Table 3** Effect of interferents on fluorescence intensity of the sensor membrane. Each sample solution contains a fixed concentration of TC, OTC or DC, respectively

| Interferent | Concentration/ mol l$^{-1}$ | Relative error* $[(F_i - F_a)/F_a] \times 100$ TC | OTC | DC |
|---|---|---|---|---|
| KCl | 0.10 | −1.93 | −1.38 | −1.16 |
| $NaNO_3$ | 0.10 | −0.67 | −0.48 | −1.16 |
| $MgSO_4$ | 0.10 | −2.15 | −4.44 | −4.58 |
| $CaCl_2$ | $1.00 \times 10^{-2}$ | 2.93 | 3.10 | 3.57 |
| Lidocaine | $1.03 \times 10^{-3}$ | −0.67 | −1.09 | 0.00 |
| Amobarbital | $1.00 \times 10^{-3}$ | 1.22 | 1.30 | 2.32 |
| Theophylline | $1.00 \times 10^{-3}$ | 2.44 | 1.95 | 2.27 |
| Levamisole | $1.20 \times 10^{-3}$ | 1.40 | 4.72 | 1.93 |
| Vitamin $B_1$ | $9.70 \times 10^{-4}$ | 2.44 | 3.09 | 3.10 |
| Sulfaguanidine | $1.00 \times 10^{-3}$ | −4.88 | −2.52 | −0.78 |
| Sulfadiazine | $1.00 \times 10^{-3}$ | 4.23 | 3.09 | 4.12 |
| Naproxen | $1.00 \times 10^{-3}$ | −4.03 | 0.41 | 0.66 |
| Tropocanide | $1.00 \times 10^{-3}$ | −1.22 | 0.66 | −0.16 |
| KC-404 | $1.00 \times 10^{-3}$ | 4.88 | 3.09 | 3.88 |
| Clindamycin | $1.00 \times 10^{-3}$ | 0.0 | 0.26 | 0.78 |

* $F_a$ are the fluorescence intensities of the sensor membrane in contact with the analytes ($2.02 \times 10^{-5}$ mol l$^{-1}$ TC, $2.00 \times 10^{-5}$ mol l$^{-1}$ OTC or $2.03 \times 10^{-5}$ mol l$^{-1}$ DC solution), while $F_i$ are the same in the presence of interferents in the concentration level indicated.

**Table 4** Determination of tetracycline antibiotics in commercial pharmaceutical preparations using the proposed sensor and conventional spectrophotometry

| Sample | Tetracycline antibiotics content/mg (per tablet) Proposed sensor Mean* ± $s$† | Spectrophotometry Mean* ± $s$† |
|---|---|---|
| *TC*— | | |
| 1 | 70.0 ± 0.65 | 70.1 ± 0.56 |
| 2 | 160 ± 0.53 | 159 ± 0.83 |
| 3 | 211 ± 1.60 | 213 ± 1.30 |
| *OTC*— | | |
| 1 | 287 ± 1.30 | 286 ± 1.01 |
| 2 | 287 ± 1.62 | 289 ± 1.73 |
| 3 | 267 ± 1.51 | 266 ± 1.24 |
| *DC*— | | |
| 1 | 25.0 ± 0.23 | 25.2 ± 0.16 |
| 2 | 26.1 ± 0.17 | 26.3 ± 0.18 |
| 3 | 25.6 ± 0.17 | 25.6 ± 0.15 |

* Mean of six determinations. † $s$ = standard deviation.

**Table 5** Determination of tetracycline antibiotics in urine using the proposed sensor

| Sample | Added/$10^{-5}$ mol l$^{-1}$ | Found*/$10^{-5}$ mol l$^{-1}$ | Recovery (%) |
|---|---|---|---|
| *TC*— | | | |
| 1 | 2.00 | 2.10 | 105 |
| 2 | 4.00 | 3.84 | 96 |
| 3 | 8.00 | 8.00 | 100 |
| *OTC*— | | | |
| 1 | 2.00 | 2.07 | 103 |
| 2 | 4.00 | 4.24 | 106 |
| 3 | 8.00 | 8.07 | 101 |
| *DC*— | | | |
| 1 | 2.00 | 1.95 | 98 |
| 2 | 4.00 | 4.06 | 102 |
| 3 | 8.00 | 7.77 | 97 |

* Mean of three determinations.

## Preliminary application

The proposed sensor was used for the direct determination of tetracycline antibiotics in commercial pharmaceutical preparations. The sample solutions (prepared according to the Experimental) were diluted with buffer solution (pH 9.5) and analyzed using the sensor by a calibration curve method. Table 4 gives the results, which are in agreement with those obtained by conventional spectrophotometry.

The direct determination of tetracycline antibiotics in urine was also performed. A series of recovery experiments were carried out by adding standard TC, OTC and DC solutions to aliquots of a urine sample. As shown by Table 5, the recovery was also satisfactory.

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