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Novel Metronidazole Membrane Sensor Based on a 2,6-(*p*-N,N-dimethylaminophenyl)-4-phenylthiopyrylium Perchlorate

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A novel PVC-based membrane sensor based on 2,6-(*p*-N,N-dimethylaminophenyl)-4-phenylthiopyrylium perchlorate (DAPP) is described. The electrode exhibits a sub-Nernstian response to 1-(beta-hydroxyethyl)-2-methyl-5-nitroimidazole (metronidazol) over a relatively wide concentration range (1.0×10^{-1} to 1.0×10^{-5} M) with a detection limit of 8.0×10^{-6} M. The best performance was obtained with the membrane containing 30% poly (vinyl chloride), 50% dibutyl phthalate, 7% DAPP and 13% oleic acid. It has a fast response time (< 30 s) and can be used for at least four weeks without any major deviation. The proposed sensor revealed very good selectivity for metronidazole over a wide variety of common cations, anions and amino acids and could be used in the pH range of 6.0-7.5. It was successfully used for direct determination of metronidazole in an oral synthetic antiprotozoal as an antibacterial agent, in metronidazole tablets, and metronidazole injections and metronidazole gels.

Keywords: Metronidazole sensor; PVC membrane; 1-(Beta-hydroxyethyl)-2-methyl-5-nitroimidazole; 2,6-(*p*-N,N-dimethylaminophenyl)-4-phenylthiopyrylium perchlorate.

INTRODUCTION

Metronidazole is bacterocidal, active against all gram-negative anaerobes, trichomonads, and amoebas. It also has some affect on Aa even though Aa is microaerophilic and not purely anaerobic by virtue of a hydroxymetabolite. It works by penetrating the bacterial cells and inhibits replication of DNA.[1]

Metronidazole is excreted in the urine and feces. Metabolism of metronidazole occurs in the liver and one of the metabolites (hydroxy) has significant anaerobic properties. 60-80% of the drug is eliminated in the urine as metronidazole or one of the metabolites.[2] Metronidazole has been shown to be carcinogenic in mice and rats. Unnecessary use of the drug should be avoided.[1]

Chromatographic techniques are the main method for determination of imidazole and its derivatives. They include high performance capillary electrophoresis with fluorescence detection,[2] thin-layer chromatography and high-performance liquid chromatography,[3,4] derivatization with fluorescent reagents followed by chromatographic separation,[5,6] and gas chromatography.[7] Other alternatives include enzyme isotope assay[8] and carbon fiber-base electrochemical techniques.[9]

Ion-selective electrodes assure the reliability of analytical information in drug assays due to direct determination, without any prior separation, of the activity of ions in solution. Due to the accuracy of the analytical information assured by using ion-selective membrane electrodes, they can be used successfully for both in vitro and in vivo assay of pharmaceutical products,[10] as well as in clinical analysis.[11]

To the best of our knowledge there is no report on an ionophore based sensor for determination of metronidazole. In this work we wish to introduce the first membrane sensor for selective and sensitive determination of metronidazole.

EXPERIMENTAL SECTION

Reagents and Materials

Reagent grade benzyl acetate (BA), dibutyl phthalate (DBP), dioctylphthalate (DOP), acetophenone (AP), ortonitrophenyloctyl ether (NPOE), oleic acid (OA), potassium tetrakis (4-chlorophenyl) borate (KTpClPB), tetrahydrofuran (THF) and high relative molecular weight PVC were purchased from Merck Chemical Company and used as re-

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ceived. The nitrate salts of the cations and potassium salts of the anions used (all from Merck) were of the highest purity available and used without any further purification except for vacuum drying over P_2O_5. Amino acids and metronidazole were purchased from Merck. Doubly distilled deionized water was used throughout. The 2,6-(*p*-N,N-dimethylaminophenyl)-4-phenylthiopyrylium perchlorate (Fig. 1) was synthesized and purified by the usual method as described elsewhere.[12]

Electrode Preparation

Membrane solution was prepared by thoroughly dissolving 55.0 mg DBP, 30.0 mg PVC and 10.0 mg OA in 3 mL of THF. To this solution was added 7.0 mg ionophore DAPP and then mixed well. The resulting solution was evaporated slowly until an oily mixture was obtained. A Pyrex tube (3-5 mm i.d) was dipped into the mixture for about 10 s so that a nontransparent membrane of 0.3 mm thickness was formed.[13-19] The tube was then pulled out from the mixture and kept at room temperature for 1 h. The tube was then filled with an internal solution (1.0×10^{-3} M of metronidazole + 1.0×10^{-3} M of NaCl). The electrode was finally conditioned for about 24 h by soaking in 1.0×10^{-2} M metronidazole. A silver/silver chloride electrode was used as the internal reference electrode.

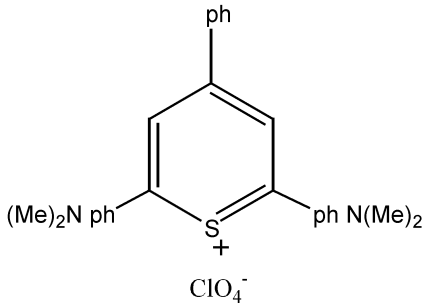

Fig. 1. Structure of DAPP.

Emf Measurements

All emf measurements were carried out with the following assembly:

Hg_2Cl_2, KCl (satd.)//sample solution/membrane/internal solution 1.0×10^{-3} M metronidazole/Ag-AgCl

A Corning ion analyzer 250-pH/mV meters was used for potential measurements at 25.0 ± 0.1 C. The emf observations were made relative to a double-junction saturated calomel electrode (SCE, Philips) with the outer chamber filled with an ammonium nitrate solution.

RESULTS AND DISCUSSION

The ionophore DAPP and the other thiopyrylium can react with various amines[20] as Scheme I.

Metronidazole as an amino compound exists predominantly as neutral molecules at about neutral and higher pH. On the other hand, the protonated metronidazole ring cannot react with the DAPP, because no lone pair of electrons is available for the interaction. Since the pK_a of metronidazole is very close to the pk_a of imidazole (6.95) and more than 50% of its molecules are unprotonated at neutral pH, it can therefore react with the DAPP. Thus, we decided to check the suitability of the DAPP as an ionophore in the preparation of a membrane sensor for the monitoring of metronidazole.

In primary experiments the ionophore DAPP was applied in construction of a number of membrane sensors for a wide variety of cations, anions, amino acids and metronidazole at about neutral pH. The results showed that except

Scheme I

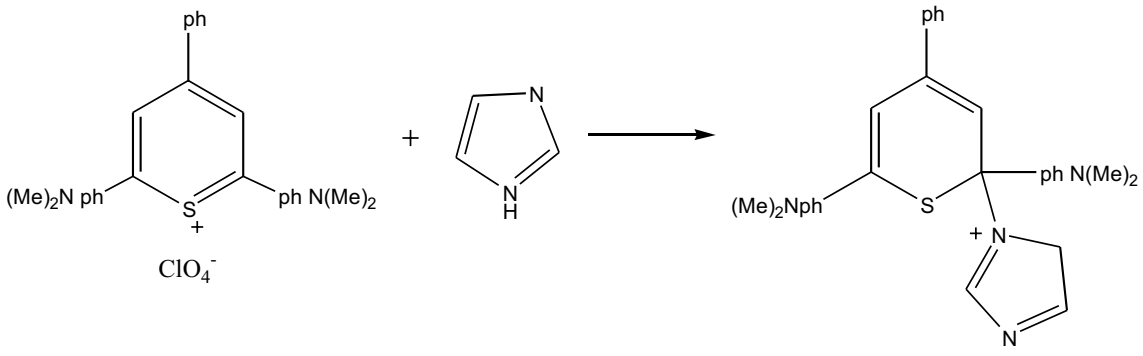

Table 1. Optimization of membrane ingredients

No.	Composition %				Slope (mV/decade)	Detection limit (M)
	PVC%	Plasticizer%	DAPP%	Additive%		
1	30	67, DBP	3	-	10.2 ± 0.3	4.0×10^{-5}
2	30	65, DBP	5	-	12.3 ± 0.2	4.0×10^{-5}
3	30	63, DBP	7	-	15.4 ± 0.4	3.5×10^{-5}
4	30	61, DBP	9	-	14.3 ± 0.2	3.0×10^{-5}
5	30	53, DBP	7	10, OA	33.1 ± 0.3	9.0×10^{-6}
6	30	50, DBP	7	13, OA	33.3 ± 0.1	8.0×10^{-6}
7	30	60, DBP	7	3, KTpClPB	15.2 ± 0.2	2.0×10^{-5}
8	30	59, DBP	7	4, KTpClPB	15.3 ± 0.1	2.5×10^{-5}
9	30	53, BA	7	10, OA	3.5 ± 0.1	4.0×10^{-5}
10	30	53, AP	7	10, OA	4.3 ± 0.1	3.5×10^{-5}
11	30	53, DOP	7	10, OA	9.6 ± 0.2	3.0×10^{-5}

for the metronidazole membrane sensor, for all other common cations, anions and amino acids used, the corresponding potential responses are very weak, and much lower than the expected Nernstian slopes of 59, 29.5 and 20 mV per decade for the univalent, bivalent and trivalent species, respectively. This is due to the specific interaction of metronidazole and DAPP over other species as well as the rapid exchange kinetics of the resulting metronidazole-DAPP complex.

The pK_a values for the amino, carboxyl, and imidazole groups are 9.2, 1.8 and 6.0, respectively. Therefore, metronidazole, existing mainly (about 90%) as a zwitterion at neutral pH, does not contribute to the potential response of the electrode system. Most other amino acids at pH 7.0 or lower, exist as zwitterion forms, and cannot operate as amines and therefore do not respond to the membrane containing thiopyrilium compounds.

As with many carrier-modified membrane electrodes, the total potentiometric response of the electrode towards metronidazole is dependent on the concentration of the DAPP, nature of solvent mediator and additional compounds incorporated within the membrane.[21-27]

As can be seen from Table 1 and Fig. 2, the increasing level of DAPP results in membranes that display larger slopes and lower detection limits. Using 7% of DAPP in the membrane resulted in an electrode with a sub-Nernstian slope towards metronidazole. As seen from Table 1 and Fig. 2, among three plasticizers used, DBP shows the best sensitivity (no. 6). It should be noted that the nature of the plasticizer influences both the dielectric constant of the membrane and the mobility of the ionophore and its complex.

The optimization of perm-selectivity of any membrane sensor is known to be dependent on the incorporation of additional membrane components.[27] In this work, the influence of OA and KTpClPB as suitable anionic additives[13-19,25,26] on the potential response of the metronidazole sensor was tested, and the results are given in Table 1 and Fig. 2. As is obvious, addition of 10% or 13% OA increases the sensitivity of the electrode response considerably (from a slope of 15.4 to 33.3 mV per decade). It is well understood that the presence of lipophilic anions in cation-selective membrane electrodes not only diminish the ohmic resistance and enhance the response behavior and selectivity but also, in cases where the extraction capability is poor, increase the sensitivity of the membrane electrodes.[28] It should be noted that we have recently reported the first use of a fatty acid such as oleic acid as a very suit-

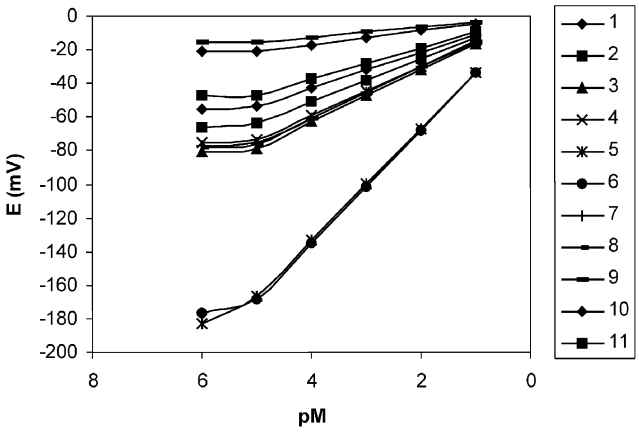

Fig. 2. The potential responses of the membrane electrodes based on DAPP with different compositions.

able lipophilic additive in inducing permselectivity to some PVC membrane selective electrodes.[29-31] It is interesting to note that, in recent years, some chemically modified forms of PVC containing carboxylic group (i.e., PVC-COOH) were used as more effective polymer matrices in construction of ion-selective electrodes.[33] It has been shown that the dissociation process of PVC-COOH depends on the polarity of the plasticizer as well as on the pH of the test solution.[34] However, the best response characteristics were obtained with a membrane composition of 30% PVC, 50% DBP, 7% DAPP, and 13% oleic acid (No. 5).

The optimum response of the electrode was tested after conditioning for different periods of time in 0.01 M metronidazole. The slope obtained using 24 h of conditioning was 33.3 mV per decade. Longer conditioning times produced no further improvements in response. The optimum conditioning solution was determined to have a concentration of 0.01 M.

The potential response of the membrane no. 6 at varying concentrations of metronidazole was investigated. Fig. 3 indicates a rectilinear range from 1.0×10^{-5} to 1.0×10^{-1} M. The slope of the calibration curve was 33.3 ± 0.1 mV/decade of metronidazole concentration. The detection limit, as determined from the intersection of the two extrapolated segments of the calibration graph, was 8.0×10^{-6} M.

The pH dependence of the membrane electrode was tested over the pH range 1.5-12 at a 5.0×10^{-3} M metronidazole concentration, and the resulting curve is shown in Fig. 4. As can be seen, the potential is fairly constant in the pH range 6.0-7.5; beyond this range, a drift was observed. The observed drift at lower pH values is due to protonation of the metronidazole. The behavior of the sensor at higher pH can be explained in terms of the increased interference from hydroxide ion by the decomposition of ionophore DAPP in the membrane. On the other hand, at pH values higher than 7.5 (with a $10^{-6.5}$ M OH), decomposition of ionophore in the membrane will start and therefore a drastic drift in the potential will appear.

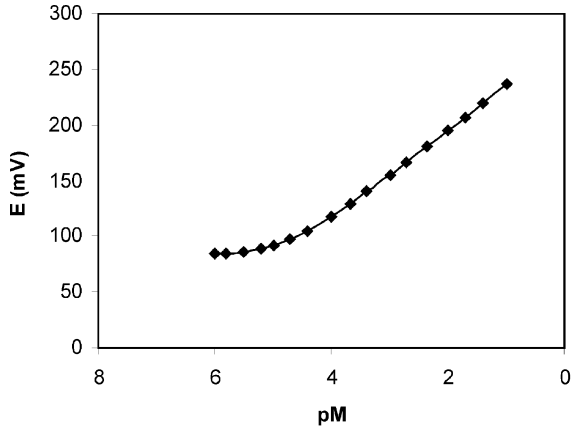

Fig. 3. Calibration curve of metronidazole ion-selective electrode based on DAPP.

For analytical applications, the response time of a sensor is an important factor. The response time of the electrode, tested by measuring the time required to achieve a steady potential (within ± 1 mV), was < 30 s and was sustained for at least 5 min over the entire concentration range (Fig. 5.). The detection system is very stable and could be used over a period of four weeks without any significant change in response characteristics being observed. The potential readings were highly reproducible within ± 0.5 mV at several metronidazole concentrations.

The influence of interfering ions on the response be-

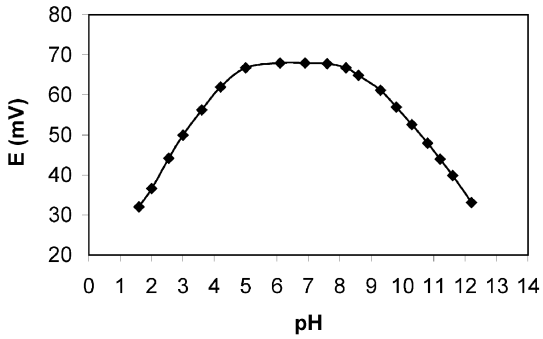

Fig. 4. Effect of pH of the test solution on the potential response of the metronidazole sensor.

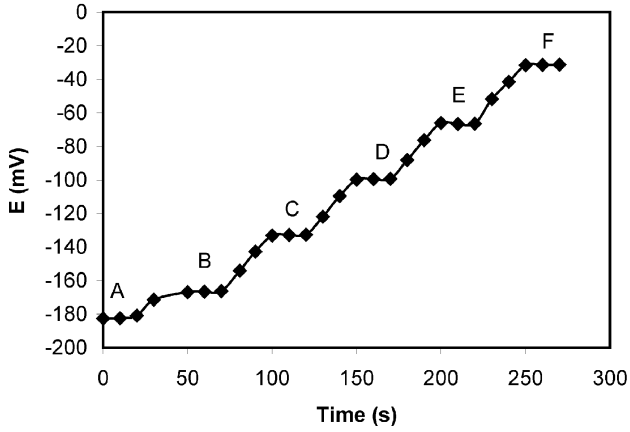

Fig. 5. Dynamic response time of metronidazole membrane electrode for step changes in concentrations of metronidazole; A) 1.0×10^{-6} M, B) 1.0×10^{-5} M, C) 1.0×10^{-4} M, D) 1.0×10^{-3} M, E) 1.0×10^{-2} M, F) 1.0×10^{-1} M.

havior of ion-selective membrane electrodes is usually described in terms of selectivity coefficients. In this work, the matched potential method (MPM)[35] was used. According to the MPM, a specified activity (concentration) of primary ions (A = 1.0×10^{-4} M metronidazole) is added to a reference solution (1.0×10^{-5} M metronidazole) and the potential is measured. In a separate experiment, interfering ions (B = 1.0×10^{-1}-1.0×10^{-3} M) are successively added to an identical reference solution until the measured potential matches the one obtained before by adding primary ions. The selectivity coefficient, $K^{Pot}_{A,B}$, is determined as:

$$K^{Pot}_{A,B} = A/A_B$$

where $A = a_A - a_A$; that a_A is the initial primary ion activity and a_A is the activity of A in the presence of interfering ion, a_B. The results are given in Table 2. As is immediately obvious, for all common cations, anions and molecules used (except for I^-, SCN^-, and L^- histidine with selectivity coefficients of 2.6×10^{-2}, 3.1×10^{-3}, 4.1×10^{-3}, respectively), the selectivity coefficients are smaller than 6.5×10^{-4}, indicating they would not disturb the functioning of the metronidazole sensor. Table 2 shows, for all amino acids tested, the selectivity coefficients are in the range of 6.5×10^{-4}-1.1×10^{-5}, and they cannot have any effect on response of the sensor at various concentrations.

The selectivity coefficients for thiocyanate and iodide are larger than other species tested; this is most probably due to the relatively weak soft-soft interaction of these anions and S^+ group of ionophore DAPP.

The detection limit of the proposed metronidazole sensor was compared with the previously reported fiber optic chemical metronidazole sensor.[36] The proposed sensor is 2 times more sensitive than the fiber optic sensors with a detection limit of 1.5×10^{-5} M (2.6 μg/mL).

ANALYTICAL APPLICATION

The electrode was successfully used to determine the

Table 2. Selectivity coefficients

Interference ions, j	$K^{MPM}_{I,J}$	Interference ions, j	$K^{MPM}_{I,J}$
Na^+	1.2×10^{-5}	Acetate	1.1×10^{-5}
K^+	1.1×10^{-5}	Oxalate	1.4×10^{-5}
NH_4^+	1.1×10^{-5}	Salycilate	1.2×10^{-5}
F^-	1.0×10^{-5}	Tartarate	1.1×10^{-5}
Cl^-	1.3×10^{-5}	Citrate	1.6×10^{-5}
Br^-	1.2×10^{-5}	D,L-Alanine	6×10^{-4}
I^-	2.6×10^{-2}	L-Arginine	8.1×10^{-5}
NO_2^-	1.1×10^{-5}	L-Aspartic acid	7.3×10^{-5}
NO_3^-	1.4×10^{-5}	L-Cysteine	4.2×10^{-5}
SO_4^{2-}	1.2×10^{-5}	L-Histidine	4.1×10^{-3}
CO_3H^-	1.0×10^{-5}	Glycine	6.3×10^{-5}
ClO_4^-	1.4×10^{-5}	D,L-Methionine	7.4×10^{-5}
PO_4H^{2-}	1.2×10^{-5}	L-Phenyl Alanine	1.2×10^{-5}
SCN^-	3.1×10^{-3}	Triptophane	3.1×10^{-5}
Azide	2.2×10^{-5}	L-Tyrosine	1.1×10^{-5}

Table 3. Composition of simulated serum

Compound	Conc. (mM)	Compound	Conc. (mM)
D,L-alanine	0.41	D,L-methionine	0.034
L-arginine	0.21	L-phenylalanine	0.16
L-aspartic acid	0.88	L-serine	0.16
L-cysteine	0.071	D,L-tryptophan	0.085
Glycine	0.14	$NaHCO_3$	8.0
L-histidine	0.14	NaCl	88.8
L-lysine	0.20	Citric acid	0.17

Table 4. Recovery test of metronidazole added to the synthetic serum sample by using the metronidazole PVC-based membrane electrode

Amt. added (mmol/L)	Amt. found (mmol/L)	Recovery (%)	Amt. added (mmol/L)	Amt. found (mmol/L)	Recovery (%)
0.10	0.09	90.0	1.50	1.47	98.0
0.15	0.14	93.3	3.70	3.60	81.0
1.20	1.17	97.5	6.20	6.25	100.8
1.30	1.27	97.7	7.10	7.05	99.39
1.40	1.36	97.1	9.50	9.55	100.5

Table 5. Determination of metronidazole in pharmaceutical preparation samples

Stated content	Amt. found by ISE	Amt. found by HPLC
Tablet (500 mg per tablet)	495.5 ± 0.1	501.5 ± 0.4
Gel (1.0% w/w)	1.05 ± 0.02	1.01 ± 0.01
Injection (5.0 mg per mL)	5.04 ± 0.02	5.0 ± 0.01

applicability of the sensor to measure metronidazole in a synthetic serum sample. The composition of the synthetic serum is given in Table 3. The concentration of each component was chosen to match its normal level in human serum. Recovery studies were conducted with samples containing various amounts of metronidazole. The result of recovery studies with the proposed sensor and HPLC are given in Table 4.

The electrode was also used for direct determination of metronidazole in oral synthetic antiprotozoal as an antibacterial agent, metronidazole tablets, sterile metronidazole (Injection) and metronidazole gel. One gram of each sample was dissolved in water in a 50-mL volumetric flask. To these solutions 5 mL of buffer sodium acetate-acetic acid (pH of 6.6) was added and diluted to the mark (50 mL) with water. Then, the proposed sensor, using the calibration method, determined the metronidazole contents of the resulting solutions. The results obtained by the sensor together with the declared amounts are summarized in Table 5. As can be seen, there is a satisfactory agreement between the results obtained by the proposed metronidazole sensor and the declared amounts.

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