Preparation of a Fluconazole Potentiometric Sensor and its Application to Pharmaceutical Analysis and to Drug Recovery from Biological Fluids

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A novel fluconazole ion-selective electrode is prepared, characterized and used in pharmaceutical analysis. The diflucan complex with different ion-pairing agent was obtained in situ by soaking the PVC membranes in a 1×10^{-2} M diflucan solution. Among four different solvent mediators tested, dibutyl phthalate (DBP) exhibited a proper behavior including Nernstian slope of the calibration curve, fast response time and good reproducibility of the emf values. The electrode exhibits a near Nernstian slope of 57.0 ± 1 mV decade^{-1} for fluconazole in the concentration range $5.0 \times 10^{-5} - 5.0 \times 10^{-2}$ M with a limit of detection of 4.0×10^{-5} M. The electrode displays a good selectivity for diflucan with respect to a number of common foreign inorganic and organic species. It can be used in a pH range 4.5-6.0. The membrane sensor was successfully applied to the determination of fluconazole in its capsules as well as in its recovery from blood serum and urine samples.

1. INTRODUCTION

Fluconazole,2-(2,4,-difluorophenyl)-1,3-bis(1H1,2,4,-triazol-1-yl)propan-2-ol] (Diflucan), is a first line antifungal drug, which is used in the treatment of supercritical and systemic candidiasis and in the treatment of cryptococcal in patients with the acquired immunodeficiency syndrome (AIDS). It acts by blocking the synthesis of ergosterol, an essential component of the fungal cell membrane [1].
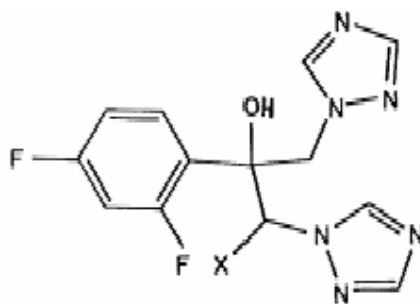
Scheme 1. Chemical structure of tested compounds (X=H)

Measuring its concentrations in the plasma of patients is of clinical relevance when pharmacokinetics are unpredictable. Fluconazole (FLC) concentrations in plasma can be measured rather by HPLC or by bioassay. However, because of its robustness, HPLC remains the reference method and is required to validate the development of any bioassay [2-3]. Prior to HPLC analysis, careful sample preparation to extract FLC from plasma needs labour intensive procedures using organic solvents and/ or solid- phase extraction [4-5]. Due to the vital importance of the assay of diflucan in pharmaceutical and in biological fluids, several analytical methods including bioassay [6], gas chromatography [7], have been reported for the determination of the drug in its pure and dosage forms. However, some of these methods need expensive equipment and /or are time-consuming.

In recent years, there has been a growing need or desire for constructing chemical sensor for the fast and economical monitoring of pharmaceutical compounds [8-11]. In this work , we report a simple potentiometric PVC-membrane sensor for the determination of diflucan in pharmaceutical preparations. The membrane electrode proposed in this was made from plasticized-PVC using a water–insoluble sodiumtetraphenyl borate – fluconazole ion pair complex as an ion-exchanger.

2. EXPERIMENTAL PART

2.1. Reagents

Analytical-reagent grade dibutyl phthalate (DBP), acetophenone (AP), o-nitrophenyl octyl ether (NPOE), sodiumtetraphenyl borate (NaTPB), phosphotangstate (PT), phosphomolybdate (PM), high relative molecular weight PVC and tetrahydrofuran (THF) were purchased from Merck and used as revived. The diflucan sodium (from Merck), β-cyclodextrins (from Fluka) and nitrate salts of the cations used (all from Merck) were of the highest purity available and used without any further purification expect for vacuum drying. Doubly distilled deionized water was used throughout.

2.2. Preparation of electrodes

The general procedure to prepare the membrane electrodes was to mix thoroughly 32 mg of powdered PVC and 2 mg of different ion pairing agents, with 66 mg of DBP as solvent mediator in the

minimum amount of THF. The resulting mixture was transferred into a glass dish of 2 cm diameter. The solvent was evaporated slowly until an oily concentrated mixture was obtained. A polyethylene tube of 5 mm i.d. was dipped into the mixture for about 10 s so that a transparent membrane of about 0.3 mm thickness was formed. After removing the tube from the mixture it was kept at room temperature for about 2 h. The tube was filled with internal filling solution (1.0×10^{-2} M fluconazole). The electrode was finally conditioned for 2 h by soaking in 1.0×10^{-2} M solution of fluconazole. A silver/silver chloride electrode was used as an internal reference electrode. The working fluconazole solutions were prepared by appropriate dilution of a 0.01 M stock standard fluconazole solution.

2.3. Emf measurements

All emf measurements were carried out with the following cell assembly:

Ag-AgCl | KCl (3 M) | internal solution, 1.0×10^{-2} M fluconazole | PVC membrane | test solution | Hg-Hg$_2$Cl$_2$, KCl (sat'd)

A Metrohm ion analyzer pH/mV meter was used for potential measurements at $25.0 \pm 0.1\ ^0$C. All measurements were carried out in a thermostaeted 50 ml double-walled glass cell, with constant magnetic stirring of the test solution

3. RESULTS AND DISCUSSION

3.1. Membrane material and composition

The membranes with different ion-pairing agents such as Dif-PM, Dif-PT and Dif- TPB were obtained in situ by soaking the PVC membranes in a 0.01 M diflucan solution for 2 h. Fluconazole reacts with different ion-pairing agents in acidic media to form a stable water-insoluble ion-pair complex.
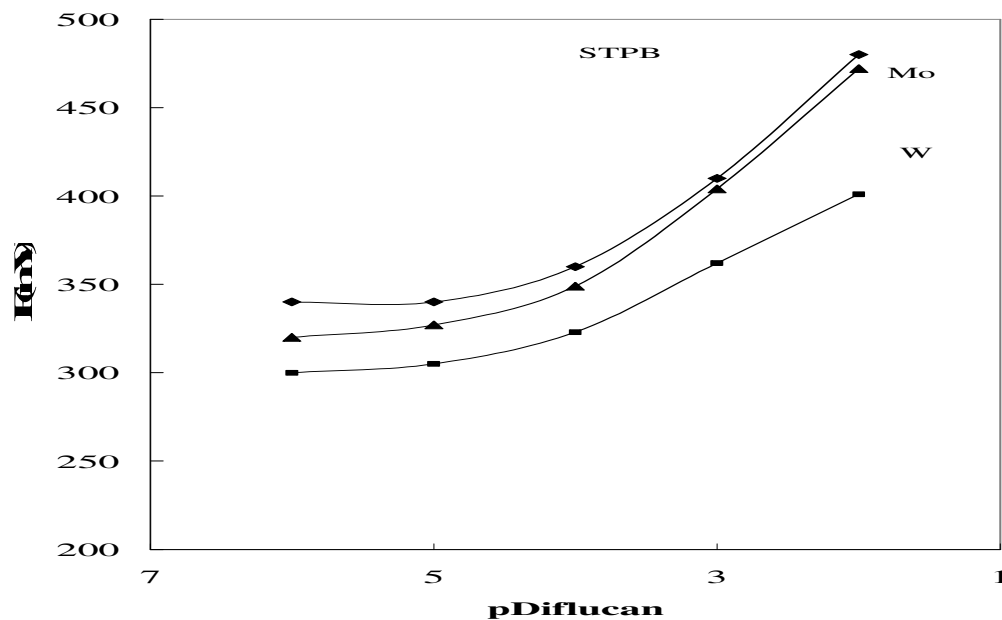

Figure 1. Effect of ion-paring agent on the response of sensor

The optimized ion-pairing agent demonstrated Nernstian response (Fig. 1). The potentiometric response of the prepared electrodes was examined in the concentration range 1.0×10^{-6} - 1.0×10^{-2} M fluconazole solutionsIt is well known that sensitivity and selectivity of ion-selective electrodes depend not only on the nature of ion-pair complex was used, but also significantly on the membrane composition and the properties of the plasticizer employed. A study of the influence of solvent mediators on the potentiometric response characteristics of the diflucan ion-selective electrode based on drug- sodium tetraphenyl borate (NaTPB) ion-pair complex were investigated and the results are summarized in Table 1 and the corresponding emf responses are shown in Fig. 1. As seen, among the four different plasticizer used, the use of 66% DBP in the presence 2% NaTPB (no. 2, Table 1) results in the best selectivity (with a Nernstian slope of 56.7 mV decade^{-1}) and the widest linear range.

Table 1. Effect of solvent mediator on the response of diflucan electrode.

(%) Composition					
Membrane Number	PVC	Plasticizer	HDPB	Slope (mV decade^{-1})	Linear range (M)
1	32	NPOE, 66	2	52.5	1.0×10^{-5}-3.0×10^{-2}
2	32	DBP, 66	2	59.0	1.0×10^{-5}-1.2×10^{-2}
3	32	DOS, 66	2	57.0	2.0×10^{-5}-1.0×10^{-2}
4	32	AP, 66	2	31.0	1.0×10^{-5}-1.0×10^{-2}

The influence is due to the polarity of the plasticizer, which can be estimated from the interaction of charged species with a continuum of given dielectric constant [12,13]. As seen, the use of AP reduces both the sensitivity and linear range of the electrode because it produces a glassy fragile membrane with a high ohmic resistance.

The proposed selective electrode was also examined at different concentrations of the inner reference solution. The concentration of the internal solution of diflucan in the electrode was changed from 1.0×10^{-2} to 1.0×10^{-4} M and the potential response of the ion-selective electrode was measured. It was found that variation of the concentration of internal solution does not cause any significant difference in the potential response of the electrode. A 1.0×10^{-3} M concentration of internal solution is quite appropriate for proper functioning of the electrode.

The optimum conditioning time for the membrane electrode is 2 h. It then generates stable potentials when placed in contact with diflucan solutions.

3.2. Calibration curve, response time and life time

The emf response of the membrane at varying concentration of fluconazole (Fig.2.) indicates a rectilinear range from 1.0×10^{-5} to 1.0×10^{-1} M. The slope of the calibration curve was 57.0 ± 1.0 mV decade^{-1} of diflucan concentration.
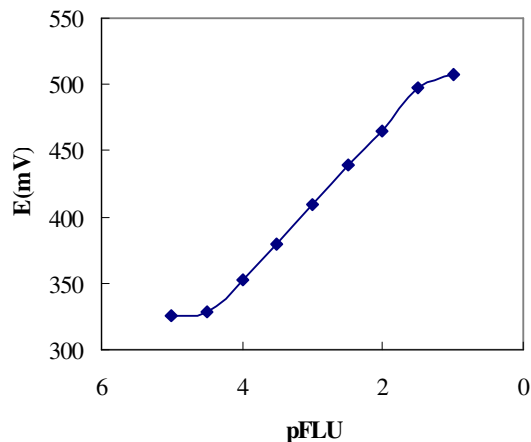

Figure 2. Calibration curve for respected electrode

The limit of detection, as determined from the intersection of the two linear segments of the calibration graph, was 4.0×10^{-6} M (1.3 ppm). The membrane electrode prepared was found to have a very fast potential response. The static response time obtained for the electrode is only about 10 s, over the entire concentration range. The membrane electrode prepared could be used for at least 3 months without any measurable divergence in potential.

3.3. Effect of pH

The influence of pH of the test solution on the potential response of the membrane (for a 1.0×10^{-3} M diflucan solution) was tested in a pH range 3.0-11.0 (adjusted with either HNO_3 or NaOH) and the results are shown in Fig. 3.
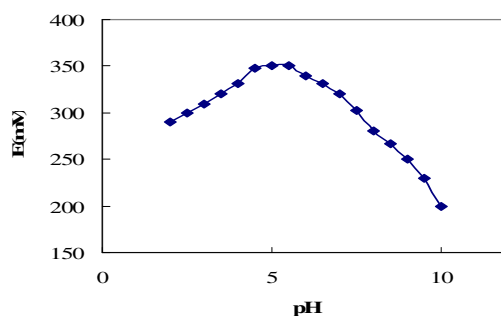

Figure 3. Effect of pH on the response of electrode (0.0005 fluconazole)

As seen, potentials remain constant from pH 4.5-6.0 beyond which the potential changes considerably. Thus, the solution pH was modified using pH = 5.0 acetate buffer solution.

3.4. Interference studies

In order to investigate the selectivity of the proposed membrane ion selective electrode toward diflucan with respect to various interfering ions, we used the fixed interference method [14,15]. The potentiometric selectivity coefficients (K_{flu}^{pot}) were calculated graphically using the expression log K = $a_k/(a_i)^{1/z}$, where a_k is the activity of diflucan and a_i that of interfering ion (1.0 x10^{-2} M), and z is the charge of interfering ion. The resulting selectivity coefficients are summarized in Table 2. As is obvious from Table 2 none of the interfering species tested does significantly influence the potentiometric response of the proposed PVC-membrane electrode toward diflucan ion.

Table 2. Selectivity coefficient of various interfering ions for diflucan electrode.

Interference	K^{pot}
Cl^-	2.5×10^{-3}
Br^-	5.1×10^{-4}
NO_3^-	5.1×10^{-3}
Ca^{2+}	7.9×10^{-4}
Mg^{2+}	6.0×10^{-4}
Na^+	5.0×10^{-2}
K^+	1.0×10^{-3}
Glycine	2.5×10^{-3}
L-Histidine	5.0×10^{-3}
Glucose	1.47×10^{-3}
Miconazole	1.2×10^{-4}

3.5. Applications

It is known that cyclodextrins (CDs) have the property of forming inclusion complexes with guest molecules with suitable characteristics of polarity and dimension [16-17]. This ability has been widely used in studies of general inclusion phenomena and enzyme-substrate interactions, applied to food and pharmaceutical studies.

In this work, the proposed electrode was applied to evaluate the equilibrium constant of the β-cyclodextrin- diflucan inclusion complex:

$$\text{Fluconazole} + \beta\text{-Cyclodextrin} \quad \xrightleftharpoons{K_s} \quad \text{Inclusion Compound} \qquad (1)$$
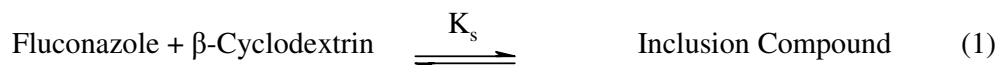
 Figure. 4 shows the emf response of the diflucan -selective electrode in the absence and presence of β-cyclodextrin. It should be noted that, after each titration of the cyclodextrin solution, an experiment was performed in the absence of cyclodextrin to confirm the reproducible response of the proposed ion-selective electrode. The data were analyzed by first assuming that equilibrium (1) between diflucan and the cyclodextrin involves a 1:1 complexation. In this case, the equilibrium constant, K_s, for each solution can be evaluated from the emf data using the classical Scatchard equation in the following form [17]:

$$\upsilon / m_1 = K - K\upsilon \qquad (2)$$

where υ is the concentration of diflucan complexed with cyclodextrin over the total concentration of β-cyclodextrin and m_1 is the drug monomer concentration. A plot of υ/m_1 vs. υ for the data involving diflucan binding to β-cyclodextrin was found to be quite linear. The linearity of this plot confirms the 1:1 stoichiometry of the complex. The binding constant obtained for the fluconazole complex with β-cyclodextrin, obtained from equation (2), was found to be 3100.

The proposed sensor was successfully used as an indicator electrode in the potentiometric titration of 10.0 ml of 0.001 fluconazole with 0.005 M NaTPB. Figure 5 shows the resulting titration curve. As is obvious, the concentration of fluconazole can be accurately determined by the proposed electrode.
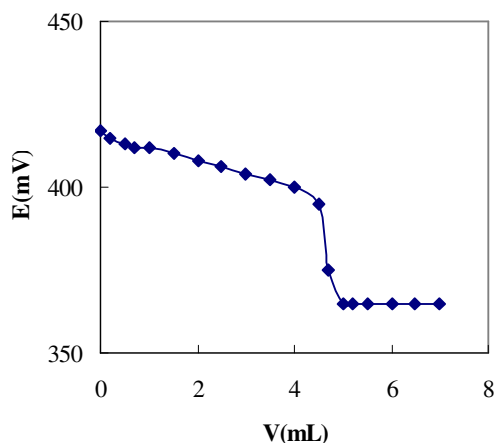

Figure. 4. Titration curve for 25.0 mL of 0.001 fluconazole with 0.005 M STPB (pH=5.0, acetate buffer)

In order to investigate the applicability of the new sensor to the determination of the drug in the biological fluids, it was applied to the recovery of diflucan from urine and blood serum samples. A 2.5

ml portion of 0.001 M fluconazole solution was transferred into a 10 ml volumetric flask After addition of a 2.5 ml portion of urine or serum samples, the solution was diluted to the mark with water. The diflucan content of the solution was then determined by the proposed electrode, using the calibration method. The recovery from three replicate measurements was found to be 101 % and 103 %, respectively.

4. CONCLUSIONS

The new sensor was also used for assay of fluconazole content in capsules with two dosages. The results for the determination of fluconazole amount in capsules are shown in Table 3. As it is seen, the results are in satisfactory agreement with the labeled amounts.

Table. 3. Potentiometric determination of Fluconazole in some pharmaceutical formulations

Application sample	Labeled amount (mg/cap.)	Found (mg/cap.)
Fluconazole Capsules, Pars Darou, Tehran, Iran	50.0	48.0 ± 0.5
Fluconazole Capsules, Pars Darou, Tehran, Iran	100.0	98.0 ± 2.0

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