RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

COMPARATIVE STUDY OF NORMAL, MICRO & NANO-SIZED IRON OXIDE EFFECT IN POTENTIOMETRIC DETERMINATION OF FLUCONAZOLE IN BIOLOGICAL FLUIDS.

Lobna A. Hussein*, Amira M. El-Kosasy, Yossra A. Trabik

Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Abbassia, 11566 Cairo, Egypt

***Corresponding author:** Dr. Lobna Abdelaziz
Associated Prof. of Analytical Chemistry, Ain Shams University Cairo, Egypt
E Mail: lobna.analytical@yahoo.com
Mobile phone: +202/01223404348 Fax No: +202/23628426

Abstract

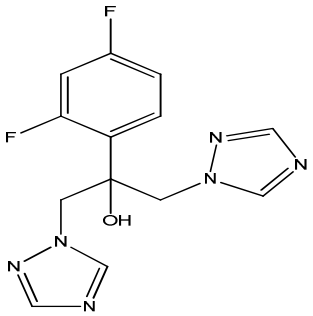
Three novel Fluconazole (FLU) selective electrodes were investigated with di-octyl phthalate as plasticizer in a polymeric matrix of polyvinyl chloride, and 2-hydroxypropyl-β-cyclodextrin as ionophore. Potentiometric strategy was based on functionalized magnetic iron oxide particles. A mixture of equal volumes of $1x10^{-2}$ M FLU & $1x10^{-2}$ M KCl was used as an internal reference solution, for sensor 1. An aqueous dispersion of magnetic micro iron oxide particles was introduced into internal reference solution for sensor 2, while an aqueous dispersion of magnetic nano iron oxide particles was introduced for sensor 3. Applying a magnetic field, the iron oxide particles could be attached to the surface of ionophore free polymeric membrane. FLU was accurately determined in spiked human plasma, spiked cow milk.

Keywords: Fluconaole, nano, micro, 2-hydroxypropyl-β-CD, potentiometry

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].



Chemical structure of fluconazole

Measuring its concentrations in the plasma of patients is of clinical relevance when pharmacokinetics is unpredictable. FLU 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 FLU from plasma needs labour intensive procedures using organic solvents and/ or solid- phase extraction [4]. However, some of these methods need expensive equipment and /or are time-consuming. Also, several spectroscopic methods have been developed for determination of FLU [5,6,7,8,9] , but they need pretreatment steps before application in biological fluids.

In recent years, there has been a growing need for constructing chemical sensor for the fast and economical monitoring of pharmaceutical compounds [10,11,12,13,14].The present work describes the use of functionalized iron oxide micro & nano particles, with 2-hydroxypropyl-β-cyclodextrin as ionophore for the development of novel sensors for the determination of FLU in bulk powder, different pharmaceutical formulations, biological fluids (plasma, urine and milk), and in presence of other co-administrated drugs as Tinidazole.

2. Experimental

2.1. Apparatus

A Jenway pH meter 3310 pH /mV /℃ meter with Orion, reference electrode (Ag/Agcl, double junction) model 63178 USA 314-771-5750, A Jenco digital ion analyzer model 6209 were used for potential measurements. Jenway pH glass electrode (UK) and Bandelin Sonorox, Rx 510 S, magnetic stirrer (Budapest, Hungary) were used for pH adjustment. Malvern Zetasizer (United Kingdom), and JEOL JEM-2100 Transmission Electron Microscope (München,Germany) were used for characterization of iron oxide particles.

2.2. Chemicals and reagents

Fluconazole, 100%, was obtained from Eipico Co. (Egypt). Treflucan ® tablets (nominally containing 150 mg of fluconazole per tablet, Eipico Co.,Cairo,Egypt) and were used in this work.

All chemicals and reagents used were of analytical reagent grade, and water was bi-distilled.

- Polyvinyl chloride carboxylate (PVC carboxylate), and 2-hydroxypropyl-β-cyclodextrin (β-CD) were obtained from Fluka Chemie Gmbh (Steinheim, Germany).
- Di-octyl phthalate (DOP) was purchased from Aldrich (Steinheim, Germany).
- Tetrahydrofuran (THF) was obtained from Merck (Dermstadt, Germany). Iron oxide NPs (5 nm diameter) were prepared by and purchased from Nanotech Egypt for photo electronics Co. (Dreamland, 6th October City, Egypt).
- Ferric chloride hexahydrate ($FeCl_3$.6 H_2O), and Ferrous sulphate ($FeSO_4$), were obtained from Aldrich (Steinheim, Germany).
- Potassium chloride , ammonium sulphate, methanol, citric acid, disodium hydrogen phosphate, sodium hydroxide, hydrochloric acid

- Zinc Sulphate ($ZnSO_4$),Magnesium sulphate ($MgSO_4$), Glycine, L-cysteine, Ethylene diamine tetra-acetic acid (EDTA), Sodium lauryl sulphate (SLS), and Cetyl trimethyl ammonium bromide (CTAB),were obtained from Prolabo (Pennsylvania, USA).
- Tinidazole 99.50% was kindly provided by Medical Union Pharmaceuticals, (Ismailia, Egypt).
- Plasma and urine were supplied by VACSERA (Giza, Egypt) and used within 24 h.
- Cow milk was purchased from the market.

2.3. Procedures

2.3.1. Membrane fabrication:

In a 5-cm Petri dish, 0.04 gm 2-hydroxypropyl-β-CD was thoroughly mixed with 0.19 gm PVC and 0.35 ml DOP, the mixture was the dissolved in 5 ml THF till complete homogeneity. The petri dish was covered with filter paper and left to stand overnight to allow solvent evaporation at room temperature. A master membrane of 0.1 mm thickness was obtained.

2.3.2. Preparation and characterization of iron oxide MPs:

Deionized water was deoxygenated by bubbling N_2 gas for 10 minutes prior to the use. $FeCl_3.\ 6H_2O$ (1.28 M), $FeSO_4$ (0.64 M) stock solutions were prepared as a source of iron by dissolving the respective chemicals in deionized water under vigorous stirring. A stock solution of 1.5 M NaOH was prepared as alkali source and 0.4M HCl as acid source. A solution of 0.01M HCl was prepared for surface neutralization.

Aqueous dispersion of magnetic MPs was prepared by alkalinizing an aqueous mixture (equal volumes) of ferric and ferrous salts with NaOH. N_2 gas was blown through the reaction medium during synthesis operation in a closed system, where 25ml of iron source was added drop-wise into

250ml of alkali source under vigorous mechanical stirring (2000 rpm) for 30 min at room temperature.

The precipitated powder was isolated by applying an external magnetic field, and the supernatant was removed from the precipitate by decantation. Deoxygenated deionized water was added to wash the powder and the supernatant was decanted after centrifugation at 3500 rpm. After washing the powder 4 times, 0.01M HCl was added to neutralize the anionic charge on the particle surface. The cationic colloidal particles were separated by centrifugation and peptized by adding deoxygenated deionized water.

The obtained iron oxide MPs were characterized by measuring particle size using Malvern Zetasizer as shown in Figure 1.

Insert Figure 1

2.3.3. Characterization of iron oxide NPs:

Purchased iron oxide NPs were characterized under JEOL JEM-2100 Transmission Electron Microscope as shown in Figure 2.

Insert Figure 2

2.3.4. Functionalization of iron oxide micro and nano ferrofluids:

0.1 gm 2-hydroxypropyl- β-CD and 0.45 ml DOP were successively dissolved in 5 ml THF, to which 5 ml of the prepared iron oxide MPs magnetic fluids were added.

By means of ultrasonic treatment for 30 min, a stable magnetic fluids solution was obtained. Through the evaporation of THF from the mixed solution for 24 hrs, the final magnetic fluids were obtained. The product was collected and stored at room temperature for use in assembly of sensor 2.

The same procedure was repeated using 5 ml of 0.5 gm ml^{-1} iron oxide NPs aqueous dispersion in case of sensor 3. Diagrams 1and 2.

2.3.5.Electrode assembly:

A disk of an appropriate diameter (about 8 mm) was cut from the previously prepared master membrane using a cork borer and cemented using THF to an interchangeable PVC tip that was previously clipped into the end of an appropriate glass outer casting for the three proposed sensors. A mixture of equal volumes of $1x10^{-2}$ M FLU & $1x10^{-2}$ M KCl was used as an internal reference solution ,for sensor 1, into which Ag/AgCl wire (1 mm diameter) was immersed as an internal reference electrode .

For sensor 2 and 3, 0.2 ml of magnetic MPs and NPs ferrofluids were added to a mixture of equal volumes of $1x10^{-2}$ M FLU & $1x10^{-2}$ M KCl, respectively, and used as an internal reference solution into which Ag/AgCl wire (1 mm diameter) was immersed as an internal reference electrode.

The sensors were conditioned by soaking in 10^{-2} M aqueous FLU solution for 24 hrs, and they were stored in the same solution when not in use.

2.3.2. Sensors calibration

The conditioned sensors were calibrated by separately transferring 50 ml aliquots of solutions (10^{-11} to 10^{-2} M) of FLU into a series of 100-ml beakers. The membrane sensors, in conjunction Ag/Agcl double junction reference electrode, were immersed in the above test solutions and allowed to equilibrate while stirring. The potential was recorded after stabilizing to ±1 mV and the electromotive force was plotted as a function of the negative logarithm of FLU concentration. The sensors were washed in distilled water between measurements

2.3.3. Effect of pH

The effect of pH on the response of the investigated electrodes was studied using 10^{-4} and 10^{-5} M solutions of FLU with pH ranging from 3 to 8 (while adjusting pH using citro-phosphate buffer).

2.3.4. Sensors selectivity

The potentiometric selectivity coefficients (KpotA.B) of the proposed sensors towards different substances were determined by a separate solution method using the following equation [15]:

$-\log (\text{KpotA.B}) = E1 - E2/ (2.303\ RT/Z_AF) + (1- Z_A/Z_B) \log \alpha A$

where KpotA.B is the potentiometric selectivity coefficient, E1 is the potential measured in 10^{-3} M FLU solution, E2 is the potential measured in 10^{-4} M interferent solution, ZA and ZB are the charges of FLU and interfering ion, respectively, αA is the activity of the drug and 2.303RT/ZAF represents the slope of the investigated sensors (mV/concentration decade).

2.3.5. Determination of FLU in its pharmaceutical formulation:

The content of one capsule of Treflucan® capsules was weighed. An accurately weighed amount of powder equivalent to 0.015 gm FLU was transferred into 100 ml volumetric flask and filled to the mark with water to prepare $4.89x10^{-4}$ M stock solution. Then a suitable dilution was made from the prepared stock to obtain $4.89x10^{-5}$ and $4.89x10^{-6}$ M samples of Treflucan®. The potentiometric measurements of the prepared samples were performed using the proposed sensors in conjunction with Aldrich reference electrode, and the potential readings were recorded. Concentrations of FLU in the prepared solutions were calculated from the regression equation of each of the three electrodes.

2.3.6.Direct potentiometric determination of FLU in spiked plasma samples:

Half milliliter of each of $5x10^{-3}$, $5x10^{-4}$ and $5x10^{-5}$ M standard drug solution were added separately into three 25-ml stoppered shaking tubes each containing 1ml of plasma to prepare $1 x 10^{-4}$, $1x10^{-5}$ and $1x10^{-6}$ M FLU plasma solutions. The tubes were shaken for 1 min. The membrane sensors were immersed in conjunction with the reference electrode in the previously prepared spiked plasma

solutions and then washed with water between measurements. The emf produced for each solution was measured by the proposed sensors, and the concentration of FLU was obtained from the corresponding regression equation.

2.3.7. Determination of FLU in spiked plasma samples after treatment:

Half milliliter of each of $5x10^{-3}$, $5x10^{-4}$ and $5x10^{-5}$ M standard drug solution were added separately into three 20-ml stoppered shaking tubes each containing 1 ml of plasma and 2 ml methanol. The tubes were shaken for 1 min, centrifuged for 50 min at 8000 rpm. Then the supernatant was transferred into three 25-ml volumetric flasks and completed to the mark with water to prepare $1x10^{-4}$, $1x10^{-5}$ and $1x10^{-6}$ M FLU plasma solutions. The membrane sensors were immersed in conjunction with the reference electrode in the previously prepared spiked plasma solutions and then washed with water between measurements. The emf produced for each solution was measured by the proposed sensors, and the concentration of FLU was obtained from the corresponding regression equation.

2.3.8.Direct potentiometric determination of FLU in spiked urine samples:

One milliliter of each of $1 x10^{-3}$, $1x10^{-4}$ and $1 x10^{-5}$ M standard drug solution were added separately into three 20-ml stoppered shaking tubes each containing 9 ml of urine to prepare $1 x 10^{-4}$, $1x10^{-5}$ and $1 x 10^{-6}$ M FLU urine solutions. The tubes were shaken for 1 min. The membrane sensors were immersed in conjunction with the reference electrode in the previously prepared spiked urine solutions and then washed with water between measurements. The emf produced for each solution was measured by the proposed sensors, and the concentration of FLU was obtained from the corresponding regression equation.

2.3.9.Direct potentiometric determination of FLU in spiked milk samples:

One milliliter of each of 1 x10^{-3}, 1x10^{-4} and 1 x10^{-5} M standard drug solution were added separately into three 20-ml stoppered shaking tubes each containing 9ml of milk to prepare 1 x 10^{-4}, 1x10^{-5} and 1x 10^{-6} M FLU milk solutions. The tubes were shaken for 1 min. The membrane sensors were immersed in conjunction with the reference electrode in the previously prepared spiked urine solutions and then washed with water between measurements. The emf produced for each solution was measured by the proposed sensors, and the concentration of FLU was obtained from the corresponding regression equation.

3. Results and discussion

The molecular recognition and inclusion complexation are of current interest in host–guest and supramolecular chemistry and offer a promising approach to chemical sensing [16,17]. The use of selective inclusion complexation and complementary ionic or hydrogen bonding are two main strategies for preparing synthetichost molecules, which recognize the structure of guest molecules [18].

Modified cyclodextrins (CDs), either natural or synthetic, are viewed as molecular receptors, as is shown in Figure 3(a). In the case of natural CD, cooperative binding with certain guest molecules was mostly attributed to intermolecular hydrogen bonding between the CD molecules, while intermolecular interactions between the host and guest molecules (hydrogen bonds, hydrophobic interactions and Van der Waals forces) contributed to cooperative binding processes when synthetic CDs were used [19]. Although the size and geometry of the guest mainly govern the binding strength, it is possible to influence the host–guest interactions by modifying then three hydroxyl groups on each glucose unit. Indeed, the use of 2-hydroxy propyl β-cyclodextrin enhanced the interaction properties between host and guest molecules [20].

Sensor 1 was based on electroactive membrane consisting of HP β-CD as an ionophore. The principle of the determination lies in the ability to selectively form inclusion complexes between the host (HP-β-CD) and the guest (FLU).

Inclusion complex of FLU with β-CDs were investigated by applying NMR and molecular modelling methods. The 1:1 stoichiometry of FLU:β-CD complex was determined by continuous variation (Job's plot) method. The shielding of cavity protons of β-CD and deshielding of aromatic protons of FLU in various 1H-NMR experiments showed complexation between β-CD and FLU. Based on spectral data obtained from 2D ROESY, a reasonable geometry for the complex could be proposed implicating the insertion of the *m*-difluorophenyl ring of FLU into the wide end of the torus cavity of β-CD. Indeed the best docked complex in terms of binding free energy supports the model proposed from NMR experiments and the *m*-difluorophenyl ring of FLU is observed to enter into the torus cavity of β-CD from the wider end as shown in Figure 3(b) [21].

Insert Figure 3

In sensor 2 and 3.The ionophore was adsorbed on the magnetic nanoparticles. By adding the resulting magnetic fluids into the sample solution, the ionophore-functionalized magnetic nanoparticles could be dispersed in the solution swiftly and symmetrically [22,23]. Upon the application of the magnetic field, the magnetic nanoparticles were aggregated to the inner side of the polymeric membrane and the ionophore adsorbed on the nanoparticles could be dissolved on the surface of the membrane, which yielded a significant potentiometric response.

The present work evaluates the influence of magnetic iron oxide particles on the response of the three proposed sensors, the effect of its particle size and the relation between their presence and size to increasing the sensitivity of the proposed sensors.

3.1. Performance characteristics of FLU sensors

Table 1 shows Electrochemical response characteristics of the three investigated FLU sensors.

Insert Table 1

Typical calibration plots are shown in Figure 4. The slopes of the calibration plots are 58.9,59.143 and 60.417 mV/concentration decade for sensors 1,2 and 3, respectively. Deviation from the ideal Nernstian slope (60 mV) is due to the electrodes responding to the activity of the drug cation rather than its concentration. The sensors displayed constant potential readings for day to day measurements, and the calibration slopes did not change by more than ±2 mV/decade over a period of 51 , 55 and 48 days for sensors 1,2 and 3 respectively. The detection limits of the three sensors were estimated according to the IUPAC definition [15] .Table 1 shows that sensor 3 could detect FLU in very dilute solutions down to 9.14 ×10−11 M, sensor 2 could detect FLU down to 9.63 x 10-10 M, while sensor 1 could only detect FLU down to 9.60 ×10−8 M. As shown in Table 2, the selectivity of the proposed sensors in the presence of related substances which may be present in the dosage form with FLU such as $MgSO_4$, CTAB, SLS and $ZnSO_4$, and in the presence of other anti-fungal drug such as Tinidazole is excellent.

Insert Figure 4

Insert Table 2

To examine the validity of the proposed sensors, the obtained results were compared to those of the reported method [24] and no significant difference was observed as shown in Table 3.

Insert Table 3

3.2. Potentiometric determination of FLU in pharmaceutical formulation

The results obtained for the determination of FLU in pharmaceutical formulation show that a wide concentration range of the drug could be determined by the investigated sensors with high precision and accuracy. The results presented in Table 4 showed that sensors 2 and 3 are more sensitive than sensor 1.

Insert Table 4

3.3. Potentiometric determination of FLU in plasma, urine and milk

The results obtained for the determination of FLU in spiked human plasma show that a wide concentration range of the drug could be determined by the investigated sensors with high precision and accuracy. The results presented in Table 5 show that sensors 2 and 3 are more sensitive than sensor 1 in plasma samples.

Insert Table 5

For the application to urine and milk, it was found that the three sensors are reliable and give stable results with good accuracy and high percentage recovery, as shown in tables 5 and 6.

Insert Tables 5&6

The response times of the proposed sensors are rapid (within seconds), so the sensors are rapidly transferred back and forth between the biological samples and the bi-distilled water between measurements to protect the sensing component from adhering to the surface of some matrix components. It is concluded that the proposed sensors could be successfully applied to in vitro studies and for clinical use without the need for any pretreatment or preliminary extraction procedures from biological fluids. There was no significant difference between the results of FLU determinations in either pretreated or untreated plasma samples as shown in tables 7 and 8.

Insert Tables 7&8

4.Conclusion

The described sensors are sufficiently simple and selective for the quantitative determination of FLU in pure form, pharmaceutical formulations, milk, plasma and urine. The proposed sensors offer advantages of fast response and elimination of drug pre-treatment or separation steps. They could therefore be used for routine analysis of FLU in quality-control laboratories. It is also noticed that

magnetic iron oxide particles play a role in increasing the sensitivity of sensor 2 than 1, and of sensor 3 than 2. Thus decreasing the particle size of magnetic iron oxide particles offers this advantage of increasing sensitivity by dramatically reducing the mass-transfer distance.

References

[1] F. X. Mathy, B.Vroman, D. Ntivunwa, A.j.d.Winne, R. K.Verbeek, V. Preat. *J. Chromathography B.,* 2003, **787**, 323.

[2] R.N. Brogden, R.C. Heel, G.E. Pakes, T.M. Speight, G.S. Avery. *Drugs*, *1980*, **20**, 24.

[3] C.S.Sastry, A.R.M Rao, T.N.V. Prasad, *Anal. Lett.*, 1987, **20**, 349.

[4] J. C. Botello, G. Perez-caballero, *Talanta*, 1995, **42(1)**, 105.

[5] P. Sadasivudu, N.Shastri, M. Sadanandam, *International Journal of ChemTech Research*, 2009, **1(4),** 1131.

[6] R. P.Gondalia, D. P. Patel, P. J. Savaliya, *International Journal of Pharmacy &Technology,* 2010, **2(4)**, 938.

[7] A.Elezović, A. Elezović, S. Hadžović. *American Journal of Analytical Chemistry,* 2011, **2** 109.

[8] B. N.Basha , K. Prakasam, D.Goli. *Int. J. Drug Dev. & Res.,* 2011, **3(4)**, 109.

[9] J. C. R. Corrêa, C. Reichman, H. R. N. Salgado. *Quim. Nova*, 2012, **35(3)**, 530.

[10] M.Shamsipur, F. Jalali, S. Haghgoo. *J. Pharm. Biomed. Anal.,* 2002, ***27***, 867-872.

[11]M. Shamsipur, F. Jalali. *Anal. Sci.*, 2000, **16(5)** , 549.

[12] M. Shamsipur, F. Jalali, Preparation of A Clotrimazole. *Anal. Lett.*, 2002, **35(1)**, 53.

[13] M.Shamsipur, F.Jalali , S. Ershad. *J. Pharm. Biomed. Anal* , 2005, **37**, 943.

[14] S.Ershad, S.Kangari . *Int. J. Electrochem. Sci.*, 2009, *4*, 1100.

[15] Y.Umezawa, P. Bühlmann, K.Umezawa, K.Tohda, S. Amemiya. *Pure Appl. Chem*, 2000, **72 (10)** , 1851.

[16] S.K.Mittal, A. Kumar, N.Gupta, S.Kaur, and S. Kuma. *Anal. Chim. Acta.* , 2007, 585(1), 161.

[17] A.R. Zanganeh, M.K. Amini. *Sens. Actuators B: Chem*., 2008, **135**, 358.

[18] L. Gorski, A.Matusevich, P.Parzuchowski, I.Łuciuk, E. Malinowska, *Anal. Chim.Acta*, 2010, **665 (1)**, 39.

[19] E.E. Sideris, G. N. Valsami , M. A.Koupparis, P. E. Macheras. *Eur. J. Pharm. Sci.*, 1999, **7(4)**, 271.

[20] S. Flink, F.C.J.M. Van Veggel, D.N. Reinhoudt, *Adv. Mater*., 2000, **12(18)**, 1315.

[21] S. K.Upadhyay , G. Kumar, *Chemistry Central Journal*, 2009, **3(9)**,1.

[22] E.Bakker, M.E. Meyerhoff. *Anal. Chim. Acta*, 2000, **416 (2)**, 121.

[23] H. Zhang, M.E. Meyerhoff. *Anal. Chem.,* 2006, ***78***, 609.

[24] N. Basha , K. Prakasam, D. Goli. *Int. J. Drug Dev. & Res.*, 2011, **3 (4)**, 109.

Figure Captions

Fig (1). Determination of synthesized iron oxide particles' size by Malvern zeta sizer.

Fig(2). Nano iron oxide particles under JEOL JEM-2100 Transmission Electron Microscope.

Fig(3). (a) Structure of HP- β-CD, (b) FLZ docked with β-CD (top-view)

Fig (4). Profile of the potential in mv versus - log concentration of fluconazole in M obtained by using the sensors 1, 2 & 3.

Table 1: Electrochemical response characteristics of the three investigated FLU sensors

Parameter	**Sensor1**	**Sensor2**	**Sensor3**
Slope (mV/decade) a	58.90	59.14	60.42
Intercept (mV)	582.5	709.71	844.33
LOD (M) b	9.60×10^{-8}	9.63×10^{-10}	9.14×10^{-11}
Response time (sec.)	60	40	25
Working pH range	4-6	4-6	4-6
Concentration Range (M)	$1x10^{-7}$ to $1x10^{-3}$	$1x10^{-9}$ to $1x10^{-3}$	$1x10^{-10}$ to $1x10^{-3}$
Stability (days)	51	55	48
Correlation Coefficient	0.9998	0.9999	0.9999
Average recovery(%)± S.D	99.52 ± 0.52	99.26 ± 1.05	99.53± 0.86
Repeatability (SD r)	0.72	0.65	0.41
Intermediate precision (SD int)	0.91	0.87	0.65

[a] Average of three determinations

[b] Limit of Detection (measured by intersection of the extrapolated arms of calibration curve)

Table 2: Potentiometric selectivity coefficients (Kpot.I) of the two proposed sensors using the separate solutions method (SSM)

Interferent [b]	**Selectivity coefficient** [a]		
	Sensor1	Sensor2	Sensor3
$MgSO_4$	2.43×10^{-3}	3.28×10^{-4}	1.05×10^{-6}
Tinidazole	9.47×10^{-5}	7.79×10^{-5}	1.11×10^{-6}
Glycine	2.93×10^{-5}	2.62×10^{-5}	2.64×10^{-6}
L-Cysteine	8.79×10^{-4}	1.50×10^{-3}	2.07×10^{-5}
EDTA	6.69×10^{-4}	1.51×10^{-5}	8.95×10^{-6}
CTAB	3.73×10^{-3}	5.25×10^{-4}	3.27×10^{-5}
SLS	3.70×10^{-4}	1.97×10^{-3}	1.06×10^{-6}
$ZnSO_4$	6.69×10^{-4}	2.62×10^{-5}	4.86×10^{-6}

[a] *Each value is the average of three determinations*

[b] *All interferents are in the form of 1 x 10^{-4} M solution*

Table 3: Satistical comparison for the results obtained the proposed electrodes and the official method **(Basha 2011)** for the analysis of fluconazole in pure powder form

Item	Sensor1	Sensor2	Sensor3	Reported method [b]
Mean [a]	99.52	99.26	99.53	100.38
S.D [a]	0.52	1.05	0.86	0.94
Variance	0.27	1.11	0.75	0.88
N	5	7	8	5
Student's t-test [c]	1.80(2.45)	1.94(2.62)	1.65(2.31)	--
F value [c]	3.26(6.39)	1.26(6.16)	1.17(4.12)	--

[a] *Average of three determinations*

[b] *Spectrophotmetric measurement at 260 nm*

[c] *The values in parentheses are the corresponding theoretical values for t and F at P=0.05*

Table 4: Determination of Fluconazole in pharmaceutical formulation by the three proposed sensors

Sensor 1			**Sensor 2**			**Sensor 3**		
Taken (M)	Found (M)	%Recovery[a]	Taken (M)	Found (M)	%Recovery[a]	Taken (M)	Found (M)	%Recovery[a]
$4.89x10^{-4}$	$4.92 x 10^{-4}$	100.61	$4.89x10^{-4}$	$4.91 x 10^{-4}$	100.41	$4.89x10^{-4}$	$4.83 x 10^{-4}$	98.77
$4.89x10^{-5}$	$4.90 x 10^{-5}$	100.20	$4.89x10^{-5}$	$4.90 x 10^{-5}$	100.2	$4.89x10^{-5}$	$4.86 x 10^{-5}$	99.39
$4.89x10^{-6}$	$4.86 x 10^{-6}$	99.39	$4.89x10^{-6}$	$4.92 x 10^{-6}$	100.61	$4.89x10^{-6}$	$4.86 x 10^{-6}$	99.39
Mean ± S.D		100.01± 0.62	**Mean ± S.D**		100.41± 0.21	**Mean ± S.D**		99.18± 0.36

[a] *Average of three determinations*

Table 5: Determination of Fluconazole in spiked human plasma (pretreated) by the proposed sensors

Added (M)	**%Recovery ± S.D[a]**		
	Sensor 1	Sensor 2	Sensor 3
10^{-4}	99.33±1.70	98.80 ± 1.31	99.80 ± 1.31
10^{-5}	98.47±1.55	98.83 ± 1.77	99.20 ± 1.72
10^{-6}	98.63± 0.95	98.10 ±1.15	99.83 ± 1.11

[a]Average of three determinations

Table 6: Determination of Fluconazole in spiked human urine by the proposed sensors

Added (M)	**%Recovery ± S.D**[a]		
	Sensor 1	Sensor 2	Sensor 3
10^{-4}	98.63 ± 1.87	99.67 ± 1.53	99.8 ± 1.31
10^{-5}	97.60 ± 1.61	98.67 ± 1.46	99.10 ± 0.30
10^{-6}	98.30 ± 1.47	98.87 ± 1.33	99.83 ± 1.11

[a]Average of three determinations

Table 7: Determination of Fluconazole in spiked cow milk by the proposed sensors

Added (M)	**%Recovery ± S.Da**		
	Sensor 1	Sensor 2	Sensor 3
10^{-4}	98.90 ± 1.01	99.00 ± 1.32	99.47 ± 0.92
10^{-5}	98.63 ± 1.58	98.40 ± 1.60	99.10 ± 1.90
10^{-6}	99.10 ± 1.23	99.33 ± 0.83	99.83 ± 1.11

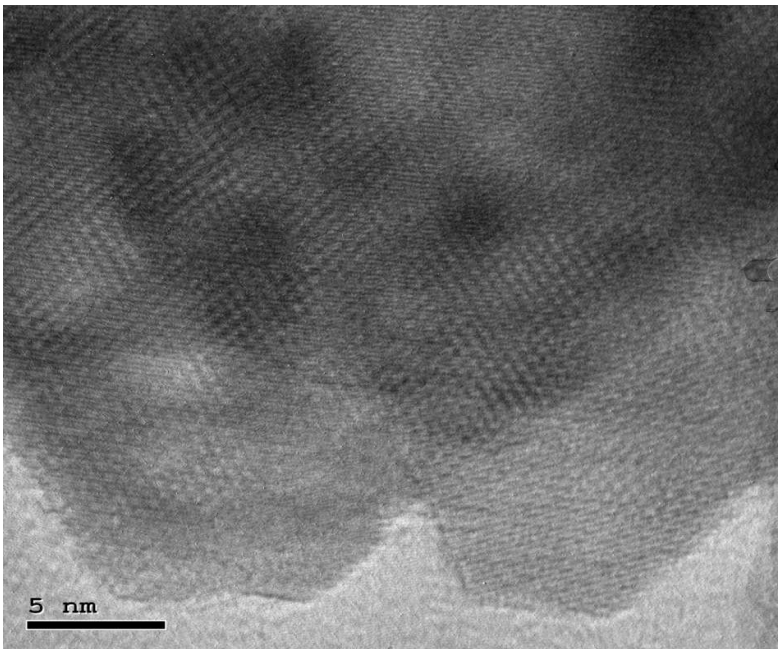
[a]Average of three determination

Table 8: Determination of Fluconazole in spiked human plasma (untreated) by the proposed sensors

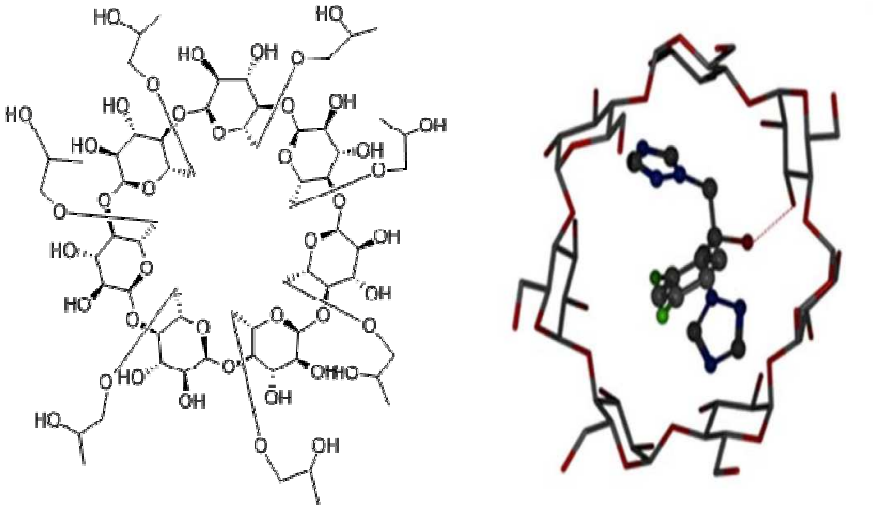
Added (M)	**%Recovery ± S.D***		
	Sensor 1	Sensor 2	Sensor 3
10^{-4}	96.49±1.27	97.53 ± 1.29	99.60 ± 1.31
10^{-5}	98.10±1.90	98.87 ± 1.27	99.10 ± 1.90
10^{-6}	98.00±1.97	99.40 ± 0.72	99.33 ± 1.60

[a]Average of three determinations

Fig (1): Determination of synthesized iron oxide particles' size by Malvern zeta sizer



Fig(2): Nano iron oxide particles under JEOL JEM-2100 Transmission Electron Microscope



(a) ***(b)***

Fig. (3): (a) Structure of HP- β-CD, (b) FLZ docked with β-CD (top-view)

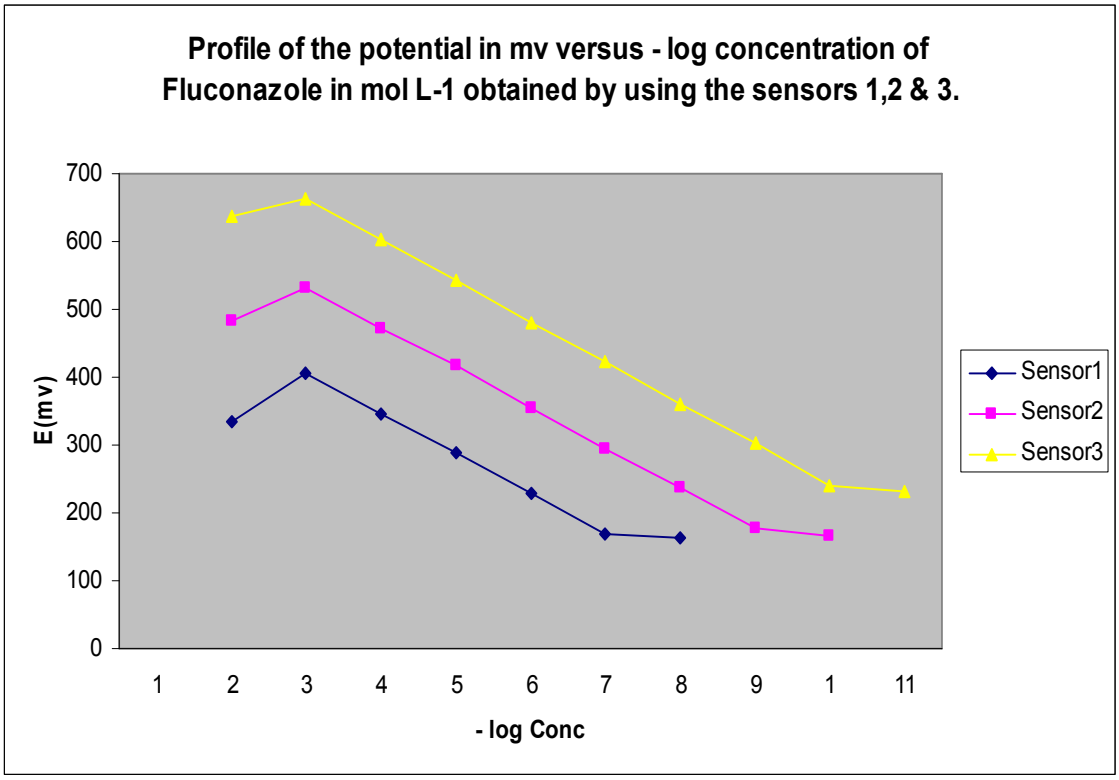


Fig. (4): Profile of the potential in mv versus - log concentration of fluconazole in M obtained by using the sensors 1, 2 & 3.

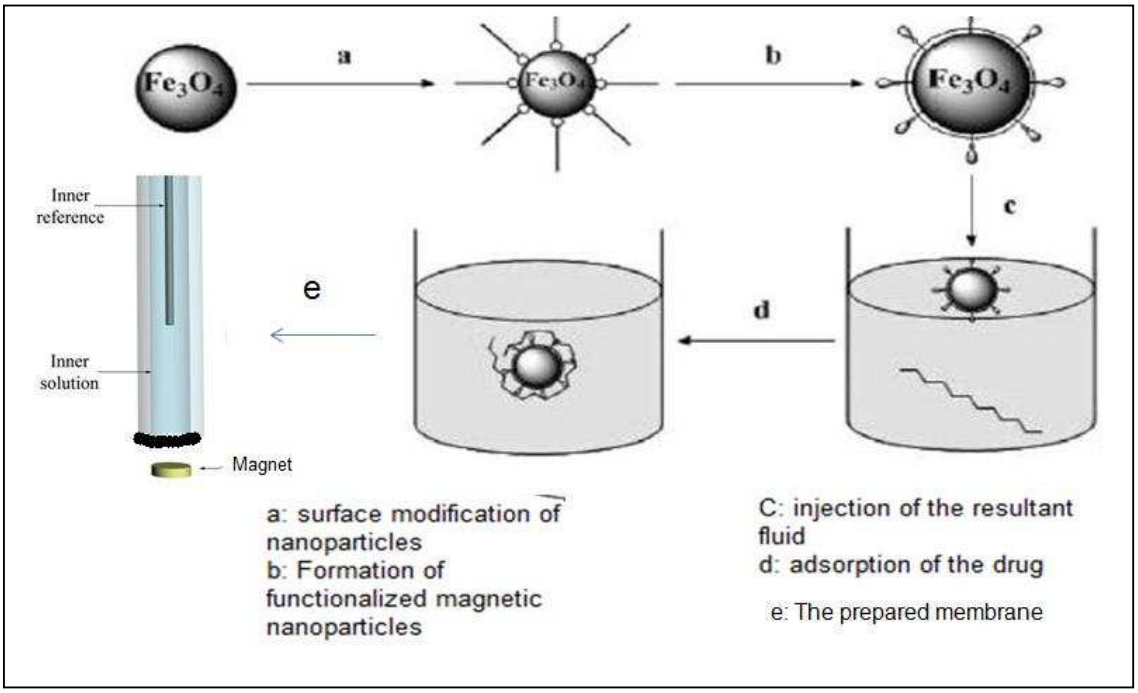


Diagram1: Schematic representation for the steps of preparation of the membrane

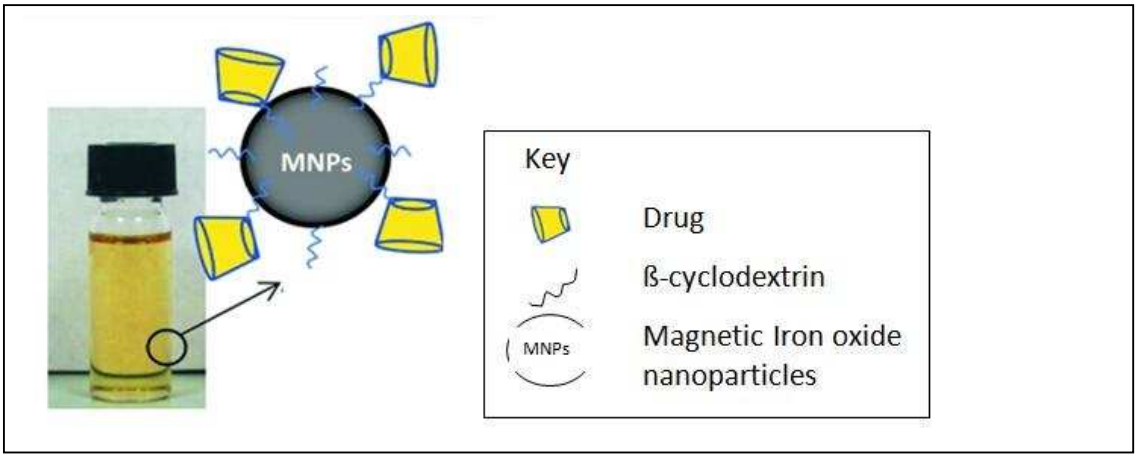


Diagram2: Schematic representation for the functionalization of iron oxide nano particles.