Anal. Bioanal. Electrochem., Vol. 6, No. 4, 2014, 688-700

Analytical & Bioanalytical Electrochemistry

www.abechem.com

Full Paper

Ion-Selective Membrane Sensors for the Determination of Tinidazole and Clarithromycin in Bulk Powder and Pharmaceutical Formulation

Safa·a M. Riad [1], Mamdouh R. Rezk [1], Kholoud Ahmed [2,*] and Hesham Salem[3]

[1]Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, 11562 Cairo, Egypt
[2]Pharmaceutical and Analytical Chemistry Department, Faculty of Pharmacy, October University for Modern Sciences and Arts, 6th of October city, Egypt
[3]Pharmaceutical and Analytical Chemistry Department, Faculty of Pharmacy, Deraya University, Egypt

* Corresponding Author, Tel.: 0122-648-3727
E-Mail: m.habashyyy@hotmail.com

Received: 23 September 2014 / Received in Revised form: 3 December 2014 / Accepted: 6 December 2014 / Published online: 31 December 2014

Abstract- The construction and electrochemical response characteristics of three selective electrodes were investigated using precipitation based technique with phosphotungstate and phosphomolybdate; respectively upon using polyvinyl chloride (PVC) matrix and dioctyl phthalate (DOP) as a plasticizer. The resultant membrane sensors were tinidazole phosphotungestate (TND-PTA) electrode (sensors 1), tinidazole phosphomolybdate (TND-PMA) electrode (sensors 2) and clarithromycin phosphotungestate (CLR-PTA) electrode (sensors 3). Linear responses of TND and CLR within the concentration ranges of 10^{-6} to 10^{-2} mol/L for sensors 1, 2 and 3 were observed. Nernstian slopes of 58.3, 57.1 and 58.8 mV/decade were observed over the pH range of 3-7 for sensors 1and 2 and over range of 3-8 for sensor 3, respectively. The selectivity coefficients of the developed sensors indicated excellent selectivity for TND and CLR. The proposed sensors displayed useful analytical characteristics for the determination of TND and CLR in bulk powder and pharmaceutical formulation (Helicure® tablets).

Keywords- Tinidazole, Clarithromycin, Preciptation based technique and pharmaceutical formulation

1. INTRODUCTION

Tinidazole (TND) is 1-[2- (ethyl sulphonyl) ethyl] -2- methyl-5-nitro-1H-imidazole, [1] (Figure 1). It is used as antiprotozoal agent. Clarithromycin (CLR), is (3R,4S,5S,6R,7R,9R,11R,12R,13S,14R)-4-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-a-L ribohexopyranosyl)oxy]-14-ethyl-12,13-dihydroxy-7-methoxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-b-D-xylo hexo pyranosyl] oxy] oxacyclotetradecane-2, 10-dione (6-O-methylerythromycin A), [1] (Figure 2). CLR is semi-synthetic macrolide antibacterial agent [1].

The literature survey reveals several analytical methods for quantitative estimation of Tinidazole in body fluids and in pharmaceutical formulations by spectrophotometry [2-7], potentiometry [7], HPLC methods [7-9], polarography [10-11] and resonance light scattering technique [12]. Clarithromycin has been reported to be estimated in body fluids and in pharmaceutical formulations by spectrophotometry [13], HPLC methods [14-19]. Omeprazole, Tinidazole and Clarithromycin were simultaneously determined by spectrophotometry [20,21].

The pharmaceutical formulation (Helicure ® tablets) contains the three drugs tinidazole, clarithromycin and omeprazole simultaneously in one tablet. The aim of this work is to develop a novel ion selective electrode technique to determine both tinidazole and clarithromycin in their combined pharmaceutical dosage form without interference from each other or any other excipients.

The developed sensors were successfully applied for the electrochemical determination of tinidazole and clarithromycin in their bulk powders and in pharmaceutical formulation, without the need of preliminary extraction or cleaning up procedures. The method has the advantages of high sensitivity, accuracy, selectivity and the possibility of direct determination of the drug in turbid and colored solutions.

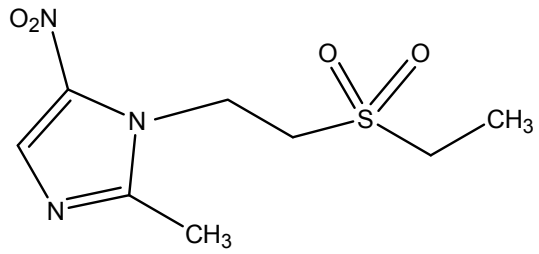

Fig. 1. The structural formula of Tinidazole (M.W. 247.3 g)

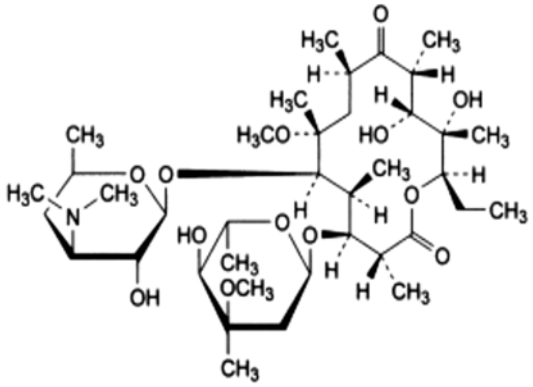

Fig. 2. The structural formula of Clarithromycin (M.W. 748 g)

2. EXPERIMANTAL

2.1. Apparatus

A Jenway digital ion analyzer model 3330 (Jenway, UK) with Ag/AgCl double junction reference electrode no Z113107-1EA (Aldrich, USA) was used for potential measurements. A Jenway pH glass electrode (Jenway, UK) was used for pH adjustments.

2.2. Chemicals and reagents

2.2.1. Pharmaceutical pure samples

Reference tinidazole (TND) was kindly donated by EGYPHAR Pharmaceuticals Co., Cairo, Egypt.

2.2.2. Pharmaceutical dosage form

Heli-cure® *tablets*: labeled to contain 20 mg of OMP,500 mg of TND and 250 mg CLR per tablet (BN 911260) supplied by EGYPHAR

2.2.3. Reagents

All chemicals and reagents used throughout this work were of analytical grade. Water used was bi-distilled. Hydrochloric acid and Sodium hydroxide scales were obtained from biochem., Cairo, Egypt. Polyvinyl chloride (PVC), Phosphomolybdic acid (PMA), Phosphotungestic acid (PTA), Dioctyl phthalate (DOP) and Tetrahydrofuran (THF) were obtained from Aldrich, USA. Potassium chloride was obtained from El-Nasr pharmaceutical chemicals, Cairo, Egypt. Starch, lactose, $CaCl_2$, NaCl, glucose, mannitol and urea were obtained from Adwic, Cairo, Egypt.

2.3. Standard solutions

2.3.1. TND standard stock solution (1×10^{-1} M)

It was freshly prepared daily by transferring 2.472 g of TND into 100-Ml volumetric flask and dissolving in distilled water by the aid of few drops of conc. HCl and tightly closed.

2.3.2. TND working solutions (1×10^{-7}-1×10^{-2}M)

They were freshly prepared by suitable dilution from their stock solution using distilled water and kept in well-closed tight container.

2.3.3. CLR standard stock solution (1×10^{-1}M)

It was freshly prepared daily by transferring 7.479 g of CLR into 100 mL volumetric flask and dissolving in acetone and complete to the mark with distilled water and tightly closed.

2.3.4. CLR working solutions (1×10^{-7}-1×10^{-2} M)

They were freshly prepared by suitable dilution from their stock solution using distilled water and kept in well-closed tight container.

2.4. Procedures

2.4.1. Precipitation based technique for the preparation of PVC-membrane sensor

In two different beakers, few drops of conc. HCl were mixed with a volume of 100 mL of 1.0×10^{-2} M aqueous TND solution to form TND hydrochloride solution and with a volume of 100 mL of 1.0×10^{-2} M aqueous CLR solution to form CLR hydrochloride solution, separately. In four different beakers, a volume of 50 mL of TND hydrochloride solution was mixed with 50 mL of aqueous 1.0×10^{-2} M aqueous phosphotungestic acid solution (beaker 1), a volume of 50 mL of TND hydrochloride solution was mixed with 50 mL of aqueous 1.0×10^{-2} M aqueous phosphomolybdic acid solution (beaker 2) and a volume of 50 mL of CLR hydrochloride solution was mixed with 50 mL of aqueous 1.0×10^{-2} M aqueous phosphotungestic acid solution (beaker 3). In (beaker 4), a volume of 50 mL of CLR hydrochloride solution was mixed with 50 mL of aqueous 1.0×10^{-2} M aqueous phosphomolybdic acid solution, the resultant (in beaker 4) was a slight turbidity but it does not form a heavy precipitate to be collected, dried and weighed to form membrane as occurring in (beakers 1, 2 and 3).

The resultant precipitates in (beakers 1, 2 and 3) were filtered using Whatman no.42

paper, washed with cold water, dried at room temperature (about 20 °C) and grinded to fine powder. Elemental analyses of the formed complexes were carried out.

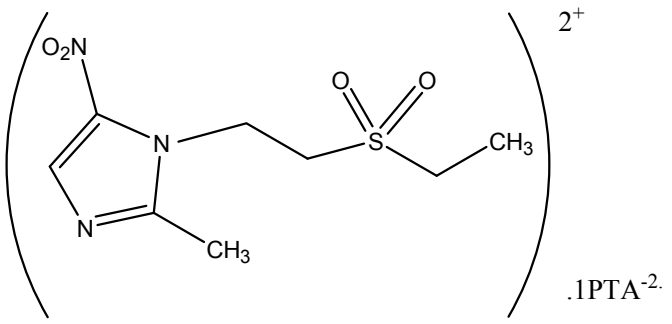

Fig. 3. Suggested structural formula of ion association complexes of TND with phosphotungestic acid

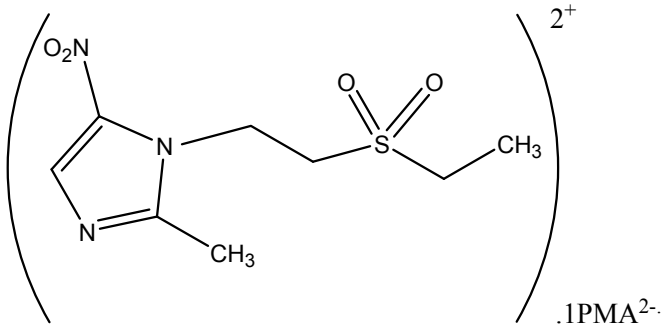

Fig. 4. Suggested structural formula of ion association complexes of TND with phosphomolybdic acid

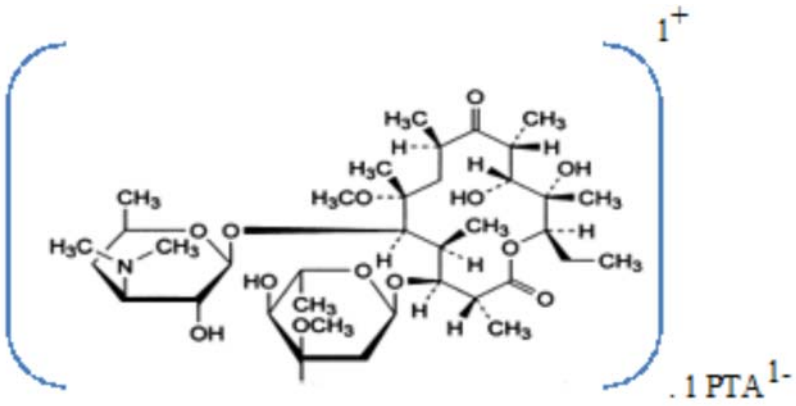

Fig. 5. Suggested structural formula of ion association complexes of CLR with phosphtungestic acid

2.4.2. Fabrication of PVC based membrane sensors

In three glasses Petri dishes (5 cm diameter), 10 mg of the three ion exchangers were separately mixed with 0.35 mL of DOP and 190 mg of PVC. The mixtures were dissolved in 5 mL of THF.

The Petri dishes were covered with filter papers and left to stand overnight to allow solvent evaporation at room temperature. Master membranes with a thickness of 0.1 mm were obtained. Disks (≈8 mm diameter) were cut using a cork borer and pasted using THF to interchangeable PVC tips that was clipped into the end of the electrode glass body. Equal volumes of 10^{-2} M aqueous drug solution (TND and CLR) and 10^{-2} M KCl were mixed and the solutions were used as internal reference solutions. Ag/AgCl wire (1 mm diameter) was immersed in the internal reference solutions as an internal reference electrode. The sensors were left for equilibration in 10^{-2} M aqueous drug solution for 24 h.

2.4.3. Electrode calibration

The prepared electrodes were immersed in conjunction with the double junction Ag/AgCl reference electrode in aqueous drug solutions (TND or CLR) in the range of 1×10^{-7} to 1×10^{-2} M. They were allowed to equilibrate while stirring and recording the e.m.f. readings within ±1 mV. The membrane sensors were stored in 1×10^{-2} M drug solution (TND or CLR) and wash thoroughly with deionized bi distilled water between measurements. The e.m.f was recorded as a function of drug concentration then calibration graphs of the recorded potentials *vs.* –log drug concentrations were plotted. This calibration plots or the computed regression equations (of the linear part of the curves) were used for subsequent measurements of unknown concentrations of drugs (TND or CLR).

2.5. Study of the experimental conditions

2.5.1. Identification of the slope, response time and operative life of the studied electrode

The electrochemical performance of the proposed sensors was evaluated according to the IUPAC recommendations data. The dynamic response time of the electrodes for the concentrations of 1×10^{-7}-1×10^{-2} M drug solutions (TND or CLR) was tested. The sequence of measurements was from low to high concentrations. The time required for the electrode to reach within ±1 mV from the final equilibrium potential after increasing the drug concentration level ten folds was measured. During this period, the electrode was stored and conditioned in 1×10^{-2} M dug solution sand washed thoroughly with water between measurements.

2.5.2. Effect of pH

The effect of pH on the potential values of the proposed electrodes was studied by immersing the electrodes in 10^{-3}& 10^{-4} M drug solutions (TND or CLR) in pH ranging from 3 to 10 using 0.1 M NaOH and 0.1 M HCl.

2.5.3. Effect of interfering compounds on the electrode selectivity

The potential response of the studied electrodes was examined in the presence of a number of related substances. The potentiometric selectivity coefficient, -log(K^{Pot} Primary ion, interferent) was used; to evaluate the extent to which a foreign ion would interfere with the response of the electrode to its primary ion. The selectivity coefficients were calculated using the separate solutions method. The potentials were measured for 10^{-3} M aqueous drug solution and then for 10^{-3} M aqueous interferent solution, separately, then potentiometric selectivity coefficients were calculated using the following equation.

$$\text{Log } \textbf{\textit{Pot. K}}_{1,2} = [(E_1 - E_2) / (2.303\ RT / ZAF)\ ] + [1 - (ZA / ZB)] \log [TND]$$

Where E_1 is the potential measured in 10^{-3} M drug solution (TND or CLR), E_2 the potential measured in 10^{-3} M interferent solution, Z_A and Z_B are the charges of drug and interfering ion, respectively, a_A is the activity of drug and 2.303RT/ ZAF represents the slope of the investigated sensor (mV/concentration decade).

2.5.4. Application of the proposed method for determination of tinidazole and clarithromycin in Helicure® tablets

The content of 10 tablets was weighed and powdered and two amounts of the powdered tablets equivalent to 0.2472 g TND and 0.7479 g CLR, respectively, were separately transferred to two 100-mL volumetric flasks and completed to the mark with water to prepare a 10^{-2} M aqueous solution of TND and CLR, respectively, then 10^{-3}& 10^{-4} M drug solutions were prepared by suitable dilution using distilled water. The e.m.f. produced was recorded by immersing the prepared electrode in conjunction with the double junction Ag/AgCl reference electrode in the prepared solutions then the concentration of drug (TND and CLR) was determined from the calibration curve of the corresponding electrode.

3. RESULTS AND DISCUSSION

The present work evaluated the possibility of quantitative determination of TND and CLR by using selective membrane sensors with ion exchanger PMA and PTA in its composition using PVC as a polymeric matrix to immobilize the sensors and to attain the formation of

highly stable complexes. It was found that the three ionic exchangers have low solubility product and suitable grain size. TND was found to form 1:1 ion association complex with PTA and with PMA, and CLR was found to form 1:1 ion association complex with PTA, as proven by elemental analysis and the obtained nernstian slopes Table 1. The proposed sensors were used for the determination of TND and CLR in bulk powder and pharmaceutical formulations (Helicure® tablets.)

Table 1. Elemental analysis of TND and phosphtungestic acid as aninonic complexing reagent

Parameters	**TND-PTA ***			**TND-PMA ***			**CLR-PTA ***		
	C	H	N	C	H	N	C	H	N
Calculated %	3.07	0.51	1.34	4.63	0.77	2.02	12.57	2.00	0.38
Found %	3.12	0.52	1.35	4.62	0.79	2.27	12.65	2.01	0.39

***** Calculated according to 1:1 ratio

3.1. Performance characteristics of TND and CLR sensors

The electrochemical performance characteristics of the investigated were evaluated according to the IUPAC recommendation data [22]. It is summarized in Table 2.

Table 2. Electrochemical response characteristics of the investigated electrodes

Parameter	**TND-PTA**	**TND-PMA**	**CLR-PTA**
Slope (mV/decade)	58.3	57.1	58.8
Intercept (mV)	435.2	430.8	456.8
LOD (mol. L^{-1}) **	2.5×10^{-6}	2.5×10^{-6}	2.5×10^{-6}
Response time (s)	15	15	15
Working pH range	3-7	3-7	3-8
Concentration range (M)	10^{-6}-10^{-2}	10^{-6}-10^{-2}	10^{-6}-10^{-2}
Stability (days)	30	30	30
Accuracy (Mean±S.D.)*	100.17±1.367	99.92±1.146	99.99±0.377
Correlation coefficient (r)	0.9985	0.9989	0.9999

***** Average of 5 determinations

****** Limit of detection measured by interception of the extrapolated arms.

It has been reported that PVC matrix is a regular support and reproducible trap for ion association complexes in membrane sensors. In the present study, dioctyl phthalate (DOP) has been used in the fabrication of the proposed membrane sensors. It adjusted the permittivity of the final organic membranes and mobility of the ion exchanger sites. The membranes constituents were dissolved in THF that was slowly evaporated at room temperature leading to membrane formation. The three sensors showed best sensitivity, where linearity was obtained in the range of (10^{-2} –10^{-6} M). Sensors 1, 2 and 3 had good slope 58.3, 57.1 and 58.8 mV/decade, respectively. Typical calibration plots are shown in Figure 6. Deviation from the ideal Nernstian slope (60 mV) is due to the electrodes responding to the activity of the drug cations rather than its concentration. The potential displayed by the proposed electrodes for constructive measurements of standard drug solution in the same day and from day to day did not vary by more than ±2 mV using the proposed sensors. Calibration slope didn't change by more than ±2 mV per decade concentration. The detection limits of the three sensors were estimated according to the IUPAC definition [23]. The slopes of the calibration plot did not change significantly but show a gradual decrease in sensitivity.

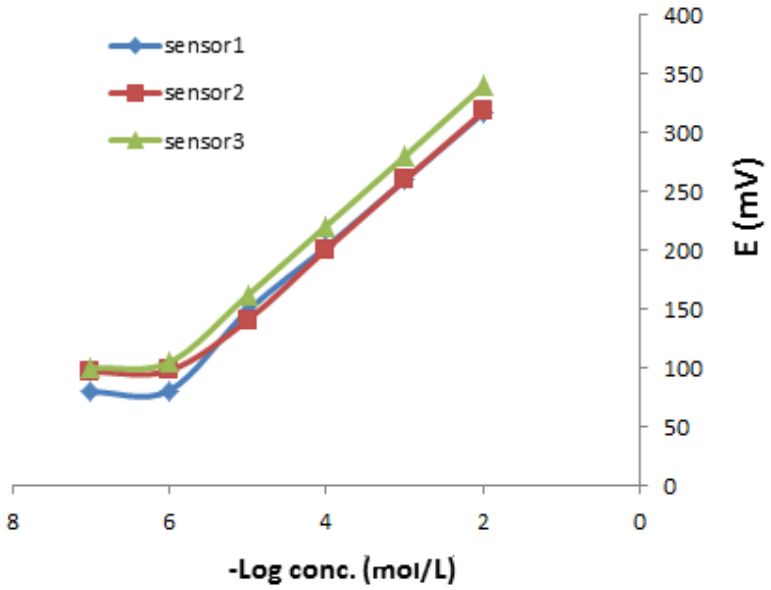

Fig. 6. Profile of the potential in mV versus –log concentration of TND and CLR in M obtained by using the three proposed electrodes

3.2. Dynamic response time

Dynamic response time is an important factor for analytical applications of ion-selective sensors. In this study, practical response time was recorded by increasing drug concentration

by up to 10 fold. The required time for the sensors to reach values within ±1 mV of the final equilibrium potential was 15 sec. for the sensors. The response time increases with increasing the concentrations.

3.3. Effect of pH

For quantitative measurements with ion selective electrodes, studies were carried out to reach the optimum experimental conditions. The potential pH profile, Figure 7, indicated that the responses of the sensors 1and 2 were fairly constant over the pH range 3-7. While for sensor 3, the constant working pH is 3-8. Therefore, pH 5 was chosen as optimum pH for working by the proposed sensors.

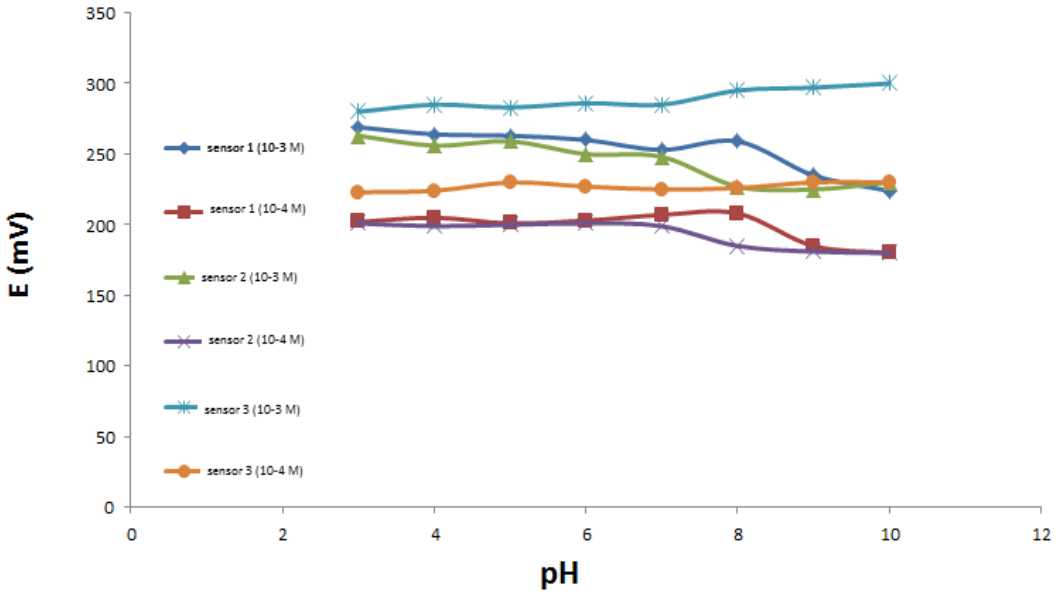

Fig. 7. Effect of pH on the response of the three sensors at 10^{-3} M and 10^{-4} M

3.4. Sensors selectivity

Sensors 1, 2 and 3 showed high selectivity coefficient values that correspond with more attack by interfering cations on the electrode membrane. The higher the selectivity coefficient value, the more the electrode membrane is attacked by the interfering cations. Diluents and excipients normally used in tablet formulations, such as magnesium carbonate, potassium chloride, sodium chloride, calcium chloride, sucrose, mannitol, lactose, glucose, urea did not show any interference. Thus, analysis was carried out without prior treatment or extraction.

Table 3 shows the potentiometric selectivity coefficients of the proposed sensors in the presence of tablet excipients, some organic and inorganic related substance and the results revealed that the proposed membrane sensors displayed high selectivity and that no

significant interference was observed from interfering species except for OMP because it is structurally related to TND.

Table 3. Potentiometric selectivity coefficients of the proposed electrodes

Interferent	**Selectivity coefficient**		
	TND-PTA	**TND-PMA**	**CLR-PTA**
Mannitol	5.8×10^{-3}	1.1×10^{-2}	8.7×10^{-3}
Urea	7.4×10^{-3}	3.2×10^{-3}	8.9×10^{-3}
KCl	8.8×10^{-3}	3.0×10^{-4}	1.3×10^{-2}
NaCl	9.1×10^{-3}	6.1×10^{-4}	1.1×10^{-2}
$CaCl_2$	1.2×10^{-2}	1.2×10^{-3}	1.7×10^{-2}
$MgCO_3.6H_2O$	1.4×10^{-3}	6.1×10^{-4}	1.7×10^{-2}
Sucrose	1.5×10^{-2}	3.7×10^{-3}	1.2×10^{-4}
Lactose	1.4×10^{-2}	3.4×10^{-3}	8.9×10^{-3}
Glucose	1.4×10^{-2}	3.9×10^{-3}	8.7×10^{-3}
Omeprazole	1.6×10^{-1}	1.2×10^{-1}	1.1×10^{-2}
Clarithromycin for (TND sensors) Omeprazole for (CLR sensor)	1.4×10^{-2}	1.4×10^{-2}	1.4×10^{-2}

* Average of 3 determinations.

** All interferents are in the form of 1×10^{-3} M solution

3.5. Potentiometric determination in pharmaceutical formulations (Helicure® tablets.)

The proposed sensors were applied for the analysis of TND and CLR in Helicure® tablets. The results obtained prove the applicability of the method as in Table 4. As demonstrated by the accurate and precise percentage recovery, it can be used for determination of TND in presence of OMP in their pharmaceutical dosage form without interference due to the presence of OMP in very low ratio compared to TND (1:25) in pharmaceutical dosage form. Other excipients such as glucose, sucrose, lactose and other organic and inorganic additives do not show interference with the measured drugs. Results obtained by the proposed procedure for the determination of pure samples of the drug were statistically compared to those obtained by the official B.P. method; no significant differences between the results were obtained as shown in Table 5.

Table 4. Determination of TND and CLR in Helicure® tablets by the proposed electrodes

Helicure®Tablets	Recovery % ± S.D.*		
	TND-PTA	TND-PMA	CLR-PTA
B.N.: 911260	100.47±1.389	99.62±0.802	100.54±0.931

* Average of 3 determinations for 3 different dilutions

Table 5. Statistical comparison for the results obtained by the proposed electrodes and the official method for the analysis of TND in pure powder form

Parameter	TND-PTA	TND-PMA	Official method*	CLR-PTA	Official method*
Mean	100.17	99.92	100.13	99.99	100.16
SD	1.367	1.146	0.722	0.377	0.531
N	5	5	6	5	6
Variance	1.869	1.313	0.521	0.142	0.282
Student's t-test	0.058 (2.26)**	0.36 (2.26)**		0.62 (2.26)**	
F value	3.56 (5.05)**	2.52 (5.05)*		1.98 (5.05)**	

***** B.P. method

** The values in parentheses are the corresponding tabulated t- & F values at P=0.05

4. CONCLUSION

The studied electrodes are sufficiently simple and selective for the quantitative determination of TND and CLR in pure form and in pharmaceutical formulation. The use of the proposed sensor offers the advantage of fast response, elimination of drug pre-treatment or separation steps, wide pH and concentration range and direct determination of the drug in turbid solution. They can therefore be used for the routine analysis of the drug in quality control laboratories.

REFERENCES

[1] British pharmacopoeia I & II (2009) 3952**.**
[2] L. Singh, J. Pharm. Res. 3 (2010) 1211.
[3] L. O. Okunrobo, World J. Chem. 2 (2007) 63.
[4] C. V. N. Prasad, V. Sripriya, R. N. Saha, and P. Parimoo, J. Pharm. Biomed. Anal. 21 (1999) 961.
[5] O. A. Adegoke, and O. E. Umoh, Acta Pharm. 59 (2009) 407.
[6] C. V. N. Prasad, C. Parihar, K. Sunil and K. Parimoo, JPP 3 (1997) 337.
[7] K. Basavaiah, P. Nagegowda, and U. Chandrashekar, IJCT 12 (2005) 273.
[8] M. X. Feng, H. Gao, and F. Y. Yu, Indian J. Pharm. Sci. 17 (1997) 247.
[9] P. N. S. Pai, G. K. Rao, B. Srinivas, and S. Puranik, Indian J. Pharm. Sci. 70 (2008) 670.
[10] A. Z. Abu Zuhri, S. Al-Khalil, R. M. Shubietah, and I. El-Hroub, J. Pharm. Biomed. Anal. 21 (1990) 881.
[11] P. V. Rege, P. A. Sathe, V. S. Salvi, and S. T. Trivedi, IJPRD 3 (2011) 15.
[12] X. Y. Jiang, X. Q. Chen, Z. Dong, and M. Xu, J. Autom Method. Manag. Chem. 2007 (2007) 86857.
[13] J. Shah, J. R. Jan, and S. Manzoor, Biomed. Chrom. 15 (2001) 507.
[14] I. Niopas, and A. C. Daftsios, Biomed. Chrom.15 (2001) 507.
[15] F. De Velde ., J. W. Alffenaar, A. M. Wessels, B. Greijdanus, and D. R. Uges, J. Chromatogr. B 877 (2009) 1771.
[16] H. Amini, and A. Ahmadiani, J. Chromatogr. B 817 (2005) 193.
[17] Y. Jiang, J. Wang, H. Li, Y.Wang, and J. Gu, J. Pharm. Biomed. Anal. 43 (2007) 1460.
[18] S. Gurule, P. R. P. Verma, T. Monif , A. Khuroo, and P. Partani, J. Liquid Chromatogr. Related Technol. 31 (2008) 2955.
[19] S. Oswald , J. Peters , M. Venner, and W. Siegmund, J. Pharm. Biomed. Anal. 55 (2011) 194.
[20] H. M. Lotfy, Bull. Fac. Pharm. Cairo. Univ. 44 (2006) 27.
[21] H. M. Lotfy, and M. A. Hegazy, Spectrochim. Acta A 96C (2012) 259.
[22] IUPAC, Analytical Chemistry Division, Commission on Analytical Nomenclature, Pure. Appl. Chem. 72 (2000) 1851.
[23] A. Ber, G. Moody, and J. Thomas, Analyst 10 (1976) 1179.