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Novel potentiometric determination of torasemide for antidoping purpose, using β-cyclodextrin and calixarene

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ABSTRACT

Two novel Torasemide (TOR) sensors were investigated, using β-cyclodextrin and calix[4]arene as ionophores, where linear responses within concentration ranges of (10^{-7}-10^{-2} and 10^{-8}-10^{-2}M) and Nernstian slopes of (58.77 and 59.214 mV/decade) over the pH range of 7-12 were obtained, respectively. The selectivity coefficients of the developed sensors indicate excellent selectivity for TOR, where the second sensor shows a higher sensitivity if compared to the first one. The proposed fabricated sensors display useful analytical characteristics for the determination of TOR (for anti-doping purpose) in bulk powder, biological fluids and in pharmaceutical formulation.

KEYWORDS

Torasemide;
β-cyclodextrin;
Calix[4]arene;
Potentiometry;
Antidoping.

INTRODUCTION

Torasemide (TOR), as shown in Figure 1, 1-isopropyl-3-(4-m-toluidinepyridine-3-sulphonyl) urea[1], is the leader of sulphonylurea class of high ceiling loop diuretics, used mainly in the treatment of hypertension and edema associated with congestive heart failure. One adverse effect of loop diuretics is the induction of kaliuresis resulting from increased potassium excretion rates. TOR is a potent natri-uretic and potassium-sparing more than the most often used loop diuretic 'furosemide', consequently it is the most favorable one to be used[2,3].

Nowadays, the use of diuretics is not limited to therapeutic aims, owing to their features that make them attractive in the world of sport and fitness, for some different purposes, including fast weight reduction through water loss[(a)] masking the presence of other drugs through 'faster excretion, urine dilution and urine pH variation[(b)] and emphasizing muscles where it is essential for body building[(c)]. Due to these reasons, diuretics have

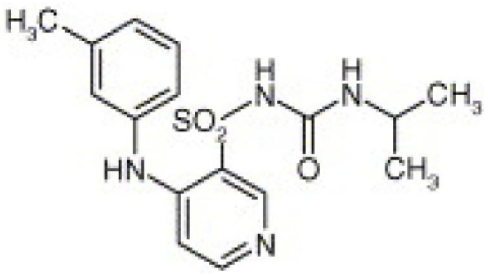

Figure 1 : Torasemide

been banned by the International Olympic Committee [IOC] since 1988 and are currently included in the list of substances prohibited in- and out-of-competition[4].

Because of its high potency, low therapeutic doses are required, where in once daily dose; TOR is effective in the treatment of hypertension without either a significant hypokalemia, elevation of blood sugar or lipid disorders if compared with those of thiazides and indapamide. TOR is well absorbed, yields bioavailability of about 80-90% and highly bound to plasma proteins 99%. It undergoes extensive hepatic metabolism 'including hydroxylation at various positions, oxidation and reduction' and only 20-25% of the parent drug is excreted unchanged in urine[5-7].

Several methods have been reported for determination of TOR in bulk powder, pharmaceutical formulations and in biological fluids for antidoping purpose, including Colorimetric methods[8], Differential pulse adsorptive stripping voltammetry[9], Capillary zone electrophoresis[10,11], Gas chromatography[12], Micellar Liquid Chromatography[13] and High Performance Liquid Chromatography either coupled to UV detector[7,14-21], or coupled to mass detector[4,22-30]. All of the last mentioned methods did not include Potentiometric techniques adopted for determination of TOR.

The present work describes two novel Potentiometric methods, using β-cyclodextrin and calix[4]arene as ionophores, as shown in Figure 2, for the determination of TOR. Cyclodextrins are known to accommodate a wide variety of organic, inorganic and biologic guest molecules to form stable host-guest inclusion complexes or nanostructure supramolecular assemblies in their hydrophobic cavity while exhibiting high molecular selectivity and enantioselectivity[31,32]. They could be applied as sensor ionophores in potentiometric determination of fluorinated surfactants[33], chiral molecules incorporating aryl rings[34], protonated amines[35] and quaternary ammonium drugs[36].

Calixarenes are cavity-shaped cyclic oligomers made up of phenol units linked via alkylidene groups. Their configurations include a number of selective factors, such as cavity-size, conformation and substituents, which facilitate the formation of typical host-guest complexes with numerous compounds, allowing a variety of applications in potentiometric techniques[37-39]. Each of β-cyclodextrin and calix[4]arene were successfully utilized for ion-selective electrode potentiometric determination of TOR in bulk powder, biological fluids (plasma and urine) and in pharmaceutical formulation.

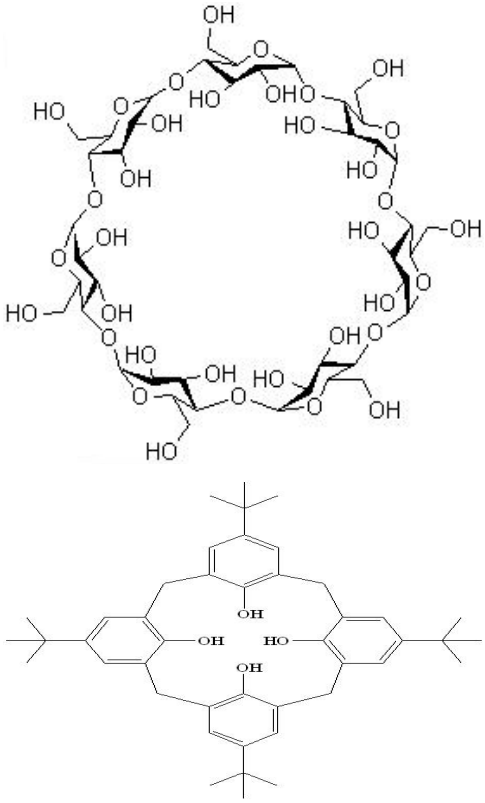

Figure 2 : Chemical structure of β-cyclodextrin (a) and calixa[4]rene

EXPERIMENTAL

Apparatus

Jenco digital ion analyzer model 6209 with Orion reference electrode (Ag/Agcl, double junction) model 63178 USA 314-771-5750 was used for potential measurements. Jenway 3310 pH meter with combined glass electrode and Bandelin Sonorox, Rx 510 S, magnetic stirrer (Hungarian) were used for pH adjustments.

Materials and reagents

TOR, 99.69% was kindly provided by Multi-Apex pharmaceutical Co. (Cairo, Egypt).

Furosemide (99.55%), anhydrous Caffeine

(99.56%), Chlorpheneramine maleate (99.58%) and Indapamide (99.55%) were kindly provided by The Arab Drug Company (ADCO), (Cairo, Egypt).

Propanolol (99.6%), Atenolol (99.65%), Bisoprolol (99.65%), Hydrochlorothiazide (99.66%), Amiloride (99.63%) and Spironolactone (99.67%) were kindly provided by Kahira Pharmaceutical Co., (Cairo, Egypt). Triametrene hydrochloride (99.68%) and Salmeterol (99.69%) were kindly provided by El-nasr Co. for Pharmaceutical and Chemical Industries, (Cairo, Egypt).

Nalbuphine hydrochloride (99.7%) and Meloxicam (99.65%) were kindly supplied from Amoun Pharmaceutical Co. (Cairo, Egypt).

Pseudoephedrine hydrochloride (99.65%) was kindly supplied from Sigma Co. (Cairo, Egypt).

Tramadol hydrochloride (99.6%) was kindly supplied from Egyptian Co. for chemicals and pharmaceuticals (10th Ramadan city, Egypt).

Aminophylline (99.65%) and Testosterone (99.6%) were kindly supplied from Cid Co. (Cairo, Egypt).

Piroxicam (99.69%) and Phenylephrine hydrochloride (99.69%) were kindly obtained from Pfizer Co.(Cairo, Egypt).

Pethidine hydrochloride was kindly provided by Misr Company and certified to contain 99.60%.

Examide® tablets used (Batch No.MT0140111), was manufactured by Multipharma (Cairo, Egypt). Each tablet contains 20 mg of TOR.

Polyvinylchloride (PVC), β-Cyclodextrin (β–CD), Calix[4]arene [Fluka], Di-octyl phthalate (DOP), methanol [Aldrich, Germany], Tetrahydrofuran (THF) (Merck, Darmstadt, Germany), Hydrochloric acid and Sodium hydroxide (BDH) each aqueous 0.1M. Potassium chloride, Nickel chloride, Tri-sodium citrate, Ammonium chloride, Barium chloride, Citric acid and Salicylic acid (Prolabo).

Plasma was supplied from VACSERA (Giza, Egypt) and urine was collected from healthy volunteers who are not subjected to any drugs.

All chemicals and reagents used through this work are of analytical grade. Bi-distilled water is used throughout the whole work and is indicated by the word "water".

Standard solutions

Standard solution of TOR

A stock standard solution of TOR having concentration of 10^{-2}M was prepared in methanol, where the prepared solutions were further diluted with 0.1N NaOH to prepare working standard solutions in a concentration range of $10^{-9} – 10^{-2}$M.

Standard solutions of interfering substances

Stock standard solutions of Furosemide, anhydrous Caffeine, Pethidine hydrochloride, Potassium chloride, nickel chloride, tri-sodium citrate, ammonium chloride, barium chloride, citric acid and salicylic acid, having concentration of 10^{-3}M, were prepared in sodium hydroxide, where they were used as a working standard solutions.

Procedure

Fabrication of membrane sensors

PVC (0.19 gm), DOP (0.35 ml) and β-CD or calix[4]arene (0.04 gm), were thoroughly mixed to prepare sensor 1 and 2, respectively. The resultant mixtures were then dissolved in 6 ml THF in 5-cm Petri-dishes and homogenized thoroughly. Each Petri-dish was covered with filter paper and left to stand overnight at room temperature to allow solvent evaporation.

The coated graphite electrodes were constructed using graphite bars (2.5 cm length, 3mm diameter).one end of the bar was used for connection, while the other, was dipped in the electro active membrane mixtures. The process was repeated several times until a layer of proper thickness was formed covering the terminal of the graphite bar. The electrode was left standing at room temperature to dry. The uncoated end of the graphite rod was sealed in a poly tetra ethylene tube, the tube was filled with metallic mercury into which a copper wire was dipped. The 2-fabricated sensors were conditioned by soaking in 10^{-2} M methanolic TOR solution for 24 hours & stored in the same solution when not in use.

Sensors calibration

The conditioned sensors were calibrated by immersing them, separately in 50 ml of working standard solutions, respectively, in conjunction with Aldrich Ag/Agcl reference electrode, allowed to equilibrate with constant stirring and washed with distilled water between measurements. The electrode's potentials for each sensor were recorded after stabilizing to ±1 mV, plotted versus each negative logarithmic concentration of TOR

and the calibration plot was then constructed to be used for subsequent measurement of unknown samples.

Effect of pH

The effect of pH on the response of the proposed electrodes was studied, using 10^{-3} and 10^{-4} M solution of TOR in 0.1 N NaOH with pH range 7-12. The pHs of the alkaline solutions were altered using 0.01M Hcl.

Sensors selectivity

The potentiometric selectivity coefficients ($K^{pot}_{A.B}$) of the proposed sensors towards different substances were determined by using separate solution method [40].

$$-\log(K^{pot}_{A.B}) = \frac{E_1 - E_2}{2.303\,RT/Z_A F} + \left(1 - \frac{Z_A}{A_B}\right) \log a_A$$

Where ($K^{pot}_{A.B}$) is the potentiometric selectivity coefficient, E_1 is the potential measured in 10^{-3} M TOR solution, E_2 is the potential measured in 10^{-3} M interfering solution, Z_A and Z_B are the charges of TOR and interfering ion, respectively, a_A is the activity of the drug and $2.303RT/Z_AF$ represents the slope of investigated sensors (mV/ concentration decade).

Determination of TOR in pharmaceutical formulation

Ten tablets of TOR (Examide® 20 mg) were finely ground. A portion equivalent to 0.087 gm of TOR, was weighed, transferred into 25 ml volumetric flask and completed to volume with 0.1 N NaOH to obtain a solution of 10^{-2} M. Further dilutions with 0.1 N NaOH were adopted to obtain a concentration range of 10^{-5} - 10^{-3}M. The proposed electrode system was immersed in each solution with constant stirring in conjunction with Aldrich Ag/Agcl reference electrode. The obtained potential readings were compared with those of standard one.

Determination of TOR in spiked plasma samples

One milliliter of 10^{-4}, 10^{-5} and 10^{-6}M working standard solutions was added separately into three 20 ml stoppered shaking tubes containing 9 ml plasma, and the tubes were shaken for 1 min. The membrane sensors were immersed in conjunction with the reference electrode in these solutions and then washed with water between the measurements. The produced emf for each solution was measured by the proposed sensors and the concentration of TOR was determined from the corresponding regression equation.

Determination of TOR in spiked urine samples

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

RESULT AND DISCUSSION

The molecular recognition and inclusion complexation are of current interest in 'host-guest and supramolecular chemistry' and offer a promising approach in chemical sensing[41,42]. The use of selective inclusion complexation and complementary ionic or hydrogen bonding are two main strategies for preparing synthetic host molecules, which recognize the structure of guest molecules[43].

Modified cyclodextrins (CDs) either natural or synthetic, are viewed like molecular receptors, as shown in Figure 3.

In 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 host and guest molecules (hydrogen bonds, hydrophobic interactions and Van der Waals forces) contributed to cooperative binding process when synthetic CDs were used[44].

Calixarenes are well-known as selective ligands for various ions through dipole-dipole interactions, as shown in Figure 3. They can form complexes with a large variety of cation substrates to form stable host-guest inclusion complexes. This property of calixarenes has been largely exploited for the development of a number of cation selective electrodes[45-47].

Performance characteristics of TOR sensors

The electrochemical performance characteristics of the proposed sensors were systemically evaluated

according to IUPAC standards[40], where typical calibration plots were obtained as shown in Figure 4.

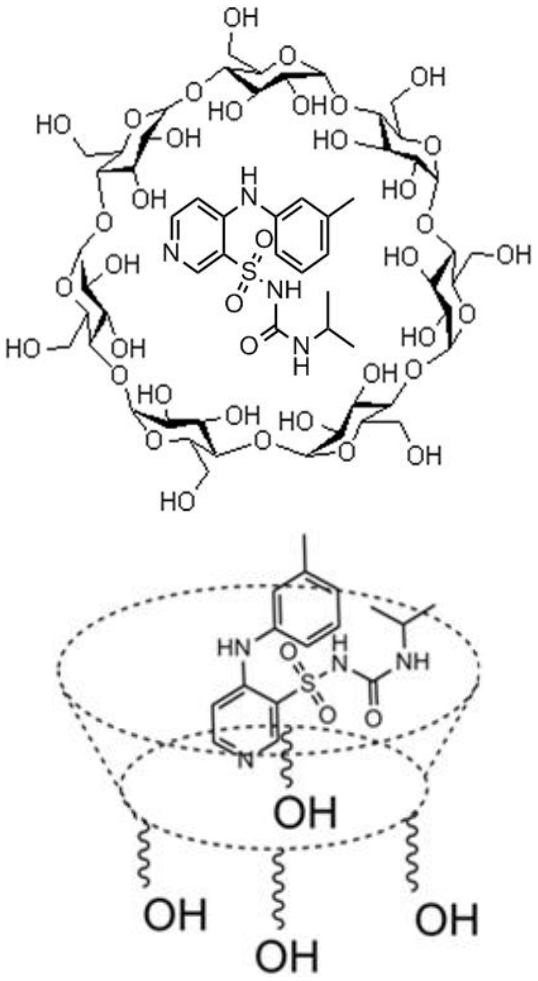

Figure 3 : Inclusion complex of β-cyclodextrin (a) and calixa[4]rene (b)

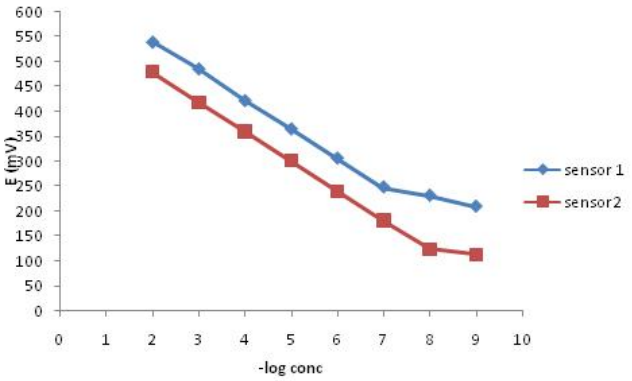

Figure 4 : Profile of the potential mV versus –log concentrations of TOR in M obtained with sensors 1 and 2.

The slopes of the calibration plots are 58.77and 59.214 mV/concentration decades, the limit of detection are1.4 × 10^{-7} and 1.127 × 10^{-8} for sensor 1 and 2, respectively. Deviation from the ideal Nernstian slope (60 mV) is due to 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 do not change by more than ± 2mV/decade over a period of 40 and 55 days for the utilized sensors, respectively. All the results reported in TABLE 1 are estimated according to the IUPAC definition[40].

TABLE 1 : Shows the results obtained over a period of 2 months for two different assemblies of each sensor. Typical calibration plots are shown in Figure 3

Parameter	Sensor1	Sensor2
Slope(mV/decade)[a]	58.77	59.214
Intercept (mV)	658.97	597.36
LOD (M)[b]	1.4 × 10^{-7}	1.127 × 10^{-8}
Response time (s)	15	10
Working pH range	7-12	7-12
Concentration range(M)	10^{-7}-10^{-2}	10^{-8}-10^{-2}
Stability(days)	40	55
Average recovery (%)±S.D.[a]	99.55±0.7266	99.75±0.603
Correlation coefficient	0.9998	0.9999
Ruggedness[c]	99.2	99.6

[a] Average of five determinations.

[b] Limit of detection (measured by interception of the extrapolated arms of Figure 3

[c] Average recovery percent of determining 10^{-3} and 10^{-4} M TOR for the studied electrodes using Jenco digital ion analyzer model 6209 instead of the Jenway.

Dynamic response time

Dynamic response time is an important factor for analytical application of ion-selective electrodes. In this study, practical response time was recorded by increasing TOR concentration up to 10-fold. The required time for the adopted sensors to reach values within ±1mV of the final equilibrium potential was 15 and 10 seconds, respectively.

Effect of pH and temperature

For quantitative measurements with ion-selective electrodes, studies were carried out to reach the optimum experimental conditions. The potential pH profile obtained indicates that the responses of the two sensor are fairly constant over the pH range 7-12. As the pH decrease below 7, a noisy response occurs then a sharp decrease which may be attributed to precipitation of

the primary cation.

The obtained results, suggest that the electrodes exhibit a slight increase in their potential as the temperature rises in the range of 20-35°C, where the calibration plots obtained at different temperature are parallel, the limits of detection, slope and response time do not significantly vary with temperature, indicating a reasonable thermal stability of suggested sensors up to 35°C.

Sensors selectivity

TABLE 2 shows the potentiometric selectivity coefficients of the proposed sensors in the presence of other doping agents including diuretics, stimulants, narcotics, $\beta_2$agonists, β blocker, anabolics, citric acid, salicylic acid and some other inorganic cations that are usually found in biological fluids. The results reveal that the proposed membrane sensors show high selectivity.

Potentiometric determination of TOR in pharmaceutical formulation

The proposed sensors were applied for the analysis of TOR pharmaceutical formulations in alkaline solutions. The results are shown in TABLE 3

Potentiometric determination of TOR in plasma and urine

The results obtained for the determination of TOR in spiked human plasma and urine show that a wide concentration range of the drug can be determined by the investigated sensors with high precision and accuracy. As shown in TABLE 4 and 5, sensor 2 is more sensitive than sensor 1 in urine samples. The response time of the proposed sensors are instant (within 15s), so the sensors are rapidly transferred back and forth between the biological samples and the deionized 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 can be successfully applied to *in vitro* studies and for clinical use.

To examine the validity of the proposed sensors, the obtained results were compared to those of the reference published method[21] and no significant difference was observed. Moreover the proposed sensors do not require prior extraction as described in the USP method as shown in TABLE 6.

TABLE 2 : Potentiometric selectivity coefficients ($K^{pot}_{A,B}$) of the two proposed sensors using the separate solutions method (SSM)[40]

Interferent*	Selectivity coefficient	
	Sensor 1	Sensor 2
Caffeine[a]	7.05×10^{-4}	1.9×10^{-4}
Bumetanide[b]	5.9×10^{-3}	9.5×10^{-4}
Triametrene[a]	3.9×10^{-3}	5.2×10^{-4}
Salmeterol[a]	6.6×10^{-4}	5.4×10^{-4}
Pethidine HCL[a]	2.7×10^{-4}	4.75×10^{-5}
Tramadol[b]	1.8×10^{-4}	3.2×10^{-4}
Nalbuphine[a]	8.6×10^{-4}	2.4×10^{-4}
Propanolol[a]	6.8×10^{-4}	9.3×10^{-4}
Chlorpheneramine maleate[a]	4.9×10^{-3}	3.5×10^{-3}
Meloxicam[c]	7.1×10^{-4}	7.2×10^{-4}
Pseudoephidrine[a]	5.7×10^{-4}	8.4×10^{-4}
Aminophylline[a]	7.4×10^{-3}	2.8×10^{-3}
Piroxicam[b]	4.4×10^{-4}	2.4×10^{-4}
Bisoprolol[b]	5.6×10^{-3}	1.3×10^{-4}
Indapamide[b]	7.1×10^{-3}	5.8×10^{-4}
Amiloride[a]	2.6×10^{-3}	6.5×10^{-4}
Furosemide[b]	9.7×10^{-4}	3.1×10^{-4}
Phenylephrine[a]	2.5×10^{-4}	6.9×10^{-4}
Testosterone[b]	1.3×10^{-4}	5.5×10^{-4}
Hydrochlorothiazide[b]	3.9×10^{-3}	1.4×10^{-4}
Spironolactone[b]	3.8×10^{-3}	8.3×10^{-4}
Morphine[a]	3.6×10^{-4}	6.2×10^{-4}
Atenolol[b]	2.5×10^{-4}	6.4×10^{-4}
Salicylic acid[a]	1.89×10^{-3}	2.86×10^{-5}
Trisodium citrate[a]	1.138×10^{-3}	1.32×10^{-5}
$Nicl_2$ hexahydrate[a]	4.31×10^{-3}	1.2×10^{-4}
KCl[a]	6.84×10^{-4}	3.87×10^{-4}
NH4Cla	8.3 × 10-4	1.49 × 10-4
$Bacl_2$[a]	5.84×10^{-4}	9.9×10^{-5}
Citric acid[a]	2.2×10^{-3}	9.9×10^{-5}

*** Average of three determinations**

[a] Aqueous solutions of 1×10^{-3} M were used.

[b] The drug is dissolved in a least amount of methanol and completed to volume with water.

[c] The drug is dissolved in 0.1N NaOH.

TABLE 3 : Determination of TOR in examide® 20 mg tablets by the two proposed sensors.

Pharmaceutical formulation Examide® tablets (20mg/tab)	Average recovery ± SD[a]	
	Sensor 1	Sensor 2
10^{-5}M	99.83 ± 0.57735	100.333 ± 0.72
10^{-4} M	99.8 ± 0.69282	99.96 ± 1.02632
10^{-3} M	99.13 ± 0.75055	99.533 ± 0.4509

[a]Average of five determinations.

TABLE 4 : Determination of TOR in spiked human plasma by the proposed sensors

Added (µg/ml)	average recovery ± S.D.[a]	
	Sensor 1	Sensor 2
10^{-5}	99.16 ± 0.577	98.566 ± 0.577
10^{-6}	98.566 ± 0.55	99.36 ± 0.57
10^{-7}	98.32 ± 0.49	99.5 ± 0.95

[a] average of three determinations.

TABLE 5 : Determination of TOR in spiked human urine by the proposed sensors

Added (µg/ml)	Recovery (%) ± S.D.[a]	
	Sensor 1	Sensor 2
10^{-6}	98.83 ± 0.404	99.13 ± 0.98
10^{-7}	98.66 ± 1.001	98.7 ± 0.754
10^{-8}	----------	99.13 ± 0.98

[a] average of three determinations.

TABLE 6 : Statistical comparison between the results obtained by applying the proposed potentiometric method and the reference method of analysis of TOR

Item	Sensor 1	Sensor 2	Reference method[20]
Mean*	99.55	99.78	100.64
SD	0.799	0.6	0.81
V (variance)	0.64	0.35	0.65
t -test[a]	-2.24 (2.262157)	-2.03 (2.36)	
f -test[a]	1.02 (5.19)	1.87 (4.53)	

*n= 6 , 7, 5 for sensors1,2 and the reference method respectively.

[a] The values in parentheses are the corresponding theoretical values for *t* and *f* at $p = 0.05$

CONCLUSION

The described sensors are sufficiently simple and selective for the quantitative determination of TOR in pure form, pharmaceutical formulation, plasma and urine. The proposed sensors offer the advantages of fast response and elimination of the drug pretreatment or separation steps. Both sensors show high sensitivity for TOR (below its Minimum Required Performance Limit given by WADA which is 250 ng/ml). However, sensor 2 showed higher sensitivity and selectivity than sensor 1, also more stable, accurate and precise. They can therefore be used for routine analysis of TOR in quality control laboratories.

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