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Solid Contact Risperidone Potentiometric Sensors

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Solid contact potentiometric sensors based on β-cyclodextrin (β-CD) were fabricated for flow potentiometric determination of risperidone (RIS). Electrode matrices compositions were optimized referring the effect of type and content of the sensing ionophore, anionic sites and plasticizer. Silver coated wire electrodes incorporated with heptakis (2,3,6-tri-o-methyl)-β-CD as sensing ionophore, potassium tetrakis (4-florophenyl) borate (KTFPB) as anionic site and *o*-nitrophenyloctylether (*o*-NPOE) as electrode plasticizer showed the best electroanalytical performances. The fabricated electrodes worked satisfactorily in the RIS concentration range from 5×10^{-6} to 10^{-2} mol L^{-1} with Nernstian compliance of 59.9±0.7 mV $decade^{-1}$ and detection limit of 2.7×10^{-6} mol L^{-1}. The developed sensors possessed fast response (3 s) and improved selectivity towards risperidone. The sensors have been successfully applied for the potentiometric determination of RIS in pharmaceutical preparations under batch experiments and flow injection analysis (FIA).

Keywords: Screen Risperidone; Solid contact potentiometric sensor; Cyclodextrin; pharmaceutical preparations; Flow injection analysis (FIA).

1. INTRODUCTION

Risperidone (RIS), 3– [2 – [4 –(6 – Fluoro – 1,2 – benzisoxozol – 3 –yl] ethyl] -2 – methyl – 6, 7,8, 9 – tetrahydro – 4 H – pyrido [1,2 – a] pyrimidin – 4 – one is an analytical antipsychotic drug, which blocks serotonin 5 – HI_2 and dopamine D_2 receptors and is widely used in the treatment of schizophrenia [1-4].

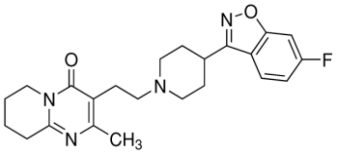

Figure 1. Chemical structure of risperidone

Several methods have been reported for the determination of RIS in pharmaceutical dosage forms and biological fluids including validated LC – MS / MS [5], sensitive and liquid chromatography and tandem mass spectrometry [6-8]. Amongst other analytical methods, capillary electrophoresis [9], FT–Raman spectroscopy [10], and dopamine D_2-receptor occupancy method [11], polarography [12], chemiluminescence [13] and colorimetry [14] have been utilized for its determination.

The widespread dosification and/or adulteration of commercially available pharmaceutical preparations demand reliable methods for drug quality control that are preferably selective, rapid and can be undertaken with simple equipment. Nevertheless, most of these methods involve several manipulation steps before the final result of the analysis, have poor selectivity or require expensive apparatus. This is in contrast to potentiometric methods using ion selective electrodes, which is now a well-established method, when applied to the analysis of pharmaceutical products, can be considered to be advantageous due to their simplicity, short measurement time, low cost, adequate precision and accuracy, wide analytical range (usually more 5 decades), the ability to measure the activity of various drugs from the formulation matrix in colored or cloudy samples as well as non-destructive measurement of the analyte sample. This makes ISE potentiometry very attractive tools for pharmaceutical analysis [15-19].

In our previous work [20], an ion pair based RIS sensor was reported. The cited electrode, based on $RISH^+$-tetraphenylborate ion pair as the electroactive component, showed near Nernstian response to $RISH^+$ over the concentration range 10^{-5} to 10^{-2} mol L^{-1}. Potentiometric sensors incorporated with ion-pair associates [21, 22] are generally plagued by limited selectivity and their applications are restricted to more challenging matrices; therefore more selective molecular recognition component is clearly required. Efforts to improve ISEs characteristics have been proposed through the use of species capable of molecular recognition [23, 24]. Different types of ionophores such as crown ethers, calixarenes, cyclodextrins (CDs) or porphyrins have been proposed; however, CDs were by far the most commonly used. CDs are naturally occurring macrocyclic oligosaccharides formed of 1,4-glucosidic bond linked D-(+)-glucopyranose oligomers of 6, 7, and 8 glucose units yielding α-, β-, and γ-CD, respectively, with toroidal three-dimensional cage configuration [25, 26]. Due to the presence of primary and secondary hydroxyl group pointing outside the cavity, the exterior surface is hydrophilic whereas the interior surface, lined with C-H groups and ether-linked oxygen atoms, is hydrophobic. CDs can form inclusion complexes with different types of guests without the formation of chemical bonds or changing their structure where the binding forces associated with the inclusion formation are attributed to number of factors, such as hydrophobic forces, hydrogen bonding, size of the cavity, shape of the guest molecule and electrostatic interaction. Such unique properties introduced CDs as sensing materials in potentiometric sensors for many pharmaceutically important drugs [27-32].

For routine analysis, the symmetric PVC membrane electrode configuration was generally preferred. Conventional PVC membrane electrodes still have certain inherent limitations as they were mechanically complicated, difficult to be manufactured in small size, higher detection limit and the short lifetime due to leaching of the electroactive material into both internal and external solutions. To overcome the aforementioned limitations, new kinds of all solid-state electrodes were reported which

refer to a type of ISEs in which the internal reference elements were in direct contact with the electroactive membrane. Due mainly to the elimination of internal reference solution, these electrodes had advantages of simplicity, small size and ability to operate at higher pressure environment where the symmetric ISEs might be damaged. Examples of this sensor design include the coated-wire electrodes (CWEs) [33] and coated graphite electrodes [34]. Advantages and applications of solid contact electrodes in electrochemistry were discussed in details elsewhere [35].

In a reported work [9], Danel et al., studied the complexation of risperidone and 9-hydroxyrisperidone with seven cyclodextrins (CDs) of pharmaceutical interest by affinity capillary electrophoresis (ACE) and nuclear magnetic resonance spectroscopy (NMR). The 1:1 stoichiometry of the complexes was established by ^{1}H NMR spectroscopy using the continuous variation method developed by Job. In addition, RIS-cyclodextrin inclusion complex was prepared as potential injectable product [36]. Based on this work, cyclodextrin can be tested as a sensing ionophore for potentiometric determination of RIS. In the present work, simple potentiometric solid contact electrodes applying β -CDs as a sensing material, have been characterized and optimized for rapid, accurate and low cost quantification of RIS. The fabricated sensors were subjected to a series of tests to select sensor possessing the most favorable analytical characteristics for potentiometric sensor under batch and FIA conditions.

2. EXPERIMENTAL PART

2.1. Reagents

All reagents were of the analytical grade, and bidistilled water was used throughout the experiments. Different cyclodextrin derivatives namely: native β-CD (I, Sigma), 2-hydroxypropyl-β-CD (II, Aldrich), heptakis (2,6-di-o-methyl)-β-CD (III, Aldrich) and heptakis (2,3,6-tri-o-methyl)-β-CD (IV, Aldrich), were used as sensing ionophores. Sodium tetraphenylborate (NaTPB, Fluka), sodium tetrakis (4-fluorophenyl) borate (NaTFPB, Fluka) or potassium tetrakis (4-chlorophenyl) borate (KTCPB, Fluka) were used as anionic sites. *o*-Nitrophenyloctylether (o-NPOE, Sigma), dibutylphthalate (DBP, Sigma), dioctylphthalate (DOP, BDH) and dioctylsebacate (DOS, Avocado) were tested as membrane plasticizers. Polyvinylchloride (PVC, relative high molecular weight, Aldrich) was used for membrane fabrication.

2.2. Authentic Samples

As the RIS free base is practically insoluble in water, stock $RISH^{+}$ solution (10^{-2} mol L^{-1}) was prepared by adding few drops of 1 mol L^{-1} HCl to a stirred aqueous suspension containing 0.41 g RIS till complete dissolution of solid, the pH was then adjusted to 6.5 using 0.1 mol L^{-1} NaOH, the solution was quantitatively transferred into 100 mL measuring flask and 10 mL of phosphate buffer (pH 6.5; 0.5 M) were added and the volume was finally completed to the mark with water.

2.3. Pharmaceutical preparations

Sigmadone tablet (SIGMA Pharmaceutical Industries, claimed to contain 3 mg per tablet) and Psychodal (DELTA PHARMA S.A.E, Tenth of Ramadan city, Egypt, 1 mg per tablet). Ten tablets were weighed, grinded and dissolved in bidistilled water, filtered and completed to 50 mL with bidistilled water.

2.4. Apparatus

Potentiometric measurements were carried out using a 692-pH meter (Metrohm, Herisau, Switzerland, Art. no. 1.691.00100) with Ag/AgCl double-junction reference electrode (Metrohm, Art. no.6.0726.100) and a combined pH glass electrode (Metrohm, Art. no. 6.0202.100). Single line flow injection system was composed of four channel peristaltic pump (MCP Ismatec, Zurich, Switzerland), sample injection valve (ECOM, Ventil C, Czech Republic) with exchangeable sample loops (5–500 lL) and continuous flow cells adapted for both PVC and coated electrodes [37]. The change of electrode potential was monitored using 46-Range Digital Multimeter (Radioshack) with PC interface.

2.5. Procedures

2.5.1. Sensor construction

CWEs and CGEs were constructed using silver metal wires (1 mm diameter, 99.9% Fluka) and graphite rod (spectroscopic grade, 3 mm diameter and 10 mm long) following the procedures described in details elsewhere [38]. The polished and cleaned electrodes were dipped in matrix cocktail composed of 2.5 mg of heptakis (2,3,6-tri-o-methyl)-β-CD **(IV)**, 2.0 mg NaTFPB, 360 mg *o*-NPE, 240 mg PVC and 6 mL THF for 15 times, after each the solvent was evaporated using air gun. The coated electrodes were left to dry at room temperature and preconditioned in 10^{-3} mol L^{-1} RIS solution for 2 h before use.

For conventional PVC membrane electrode, the same electrode matrix was poured in a Petri dish (5 cm diameter). After evaporation of THF, circular pieces (2 cm diameter) of the PVC membranes were mounted on the end of the PVC tubing, and the electrodes were filled with 10^{-2} mol L^{-1} KCl and 10^{-2} molL^{-1} of RIS solution using Ag /AgCl as internal reference electrode. The fabricated electrodes were soaked in10^{-3} molL^{-1}drug solution for 24 h before use [19].

2.5.2. Sensor calibration

For batch measurements, sensors were calibrated by transferring 25 mL aliquots of 10^{-6}–10^{-2} mol L^{-1} RIS solutions into measuring cell at 25 ^{o}C followed by immersing the sensor in conjugation with reference electrode in the measuring solution. The potential readings were recorded and plotted against drug concentration in logarithmic scale (log[RIS]) [39].

For FIA measurements, 500 µL of freshly prepared RIS solutions, covering the range from 10^{-6} to 10^{-3} mol L^{-1}, were injected in the flowing water stream at a flow rate of 30 mL min^{-1}. The corresponding peak heights using RIS-CWE were recorded and used to draw the calibration graphs.

2.5.3. Potentiometric determination of RIS in pharmaceutical preparations

RIS was potentiometrically determined in pure solution and in pharmaceutical preparations using the developed electrodes by potentiometric titration and FIA conditions. For potentiometric titration, aliquots of the sample solutions containing 2.05 to 20.5 mg RIS were titrated against standardized NaTPB solution [22]. The titration process was monitored using RIS sensor in conjugation with the conventional Ag/AgCl reference electrode and the potential values were plotted against the titrant volume to fix the end point.

Under FIA conditions, 200 µL of sample solutions were injected in the flowing stream and the peak heights were measured at the optimum conditions and compared to those obtained from injecting standard solutions of the same concentration.

3. RESULTS AND DISCUSSION

Chemically modified electrodes (CMEs) were suggested for improving the electroanalytical performance through application of molecular recognition species selective to the target analyte. It was reported that RIS can form inclusion complex with cyclodextrin [9, 36], therefore cyclodextrin can be tested as a sensing ionophore for the potentiometric determination of risperidone. Extensive studies were done for the optimization of the electrode matrix composition including the influence of the nature and content of CDs, ionic additives and plasticizers to select the optimal sensor processing and the proper performance.

3.1. Optimal sensor matrices compositions

The response of ionophore-based potentiometric sensors is usually governed by the molecular recognition ability between the analyte (guest) and the host molecule. Preliminary experiment showed that the sensor fabricated without incorporation of CDs (blank electrode) showed non-Nernstian response towards both drugs (slope 33.3±1.1 $mVdecade^{-1}$), while those modified with different cyclodextrin derivatives gave Nernstian responses with different slope values, demonstrating the crucial rule of the ionophore on the electrode response. Sensors modified with both α- and γ-CDs showed low Nernstian response (44–49 $mVdecade^{-1}$), which may be attributed to the incompatible cavity size for inclusion complex formation. On the contrary, electrodes incorporated with β-CDs ionophores (I–IV) showed reasonable responses (Fig. 2 a) and heptakis (2,3,6-tri-o-methyl)-β-CD (IV) was the best (Nernstian slope was 54.6±0.2 $mVdecade^{-1}$ in the concentration range from 5×10^{-6} to 10^{-2}

mol L^{-1}). Such variation in the electrode performances can be explained on the basis of the stability constants of the formed inclusion complexes and fitting of the RIS molecule within the β-CD cavity.

On constructing an ISE, the amount of the sensing material in the electrode matrix should be sufficient to obtain reasonable complexation at the electrode surface that is responsible for the electrode potential. Furthermore, β-CD (IV) content in the fabricated electrode matrices was varied from 1 to 4.0 mg (Fig. 2b). Incorporation of 2.5 mg of β-CD was sufficient to get the proper performance (slope values were 58.6 ± 1.2 $mVdecade^{-1}$).

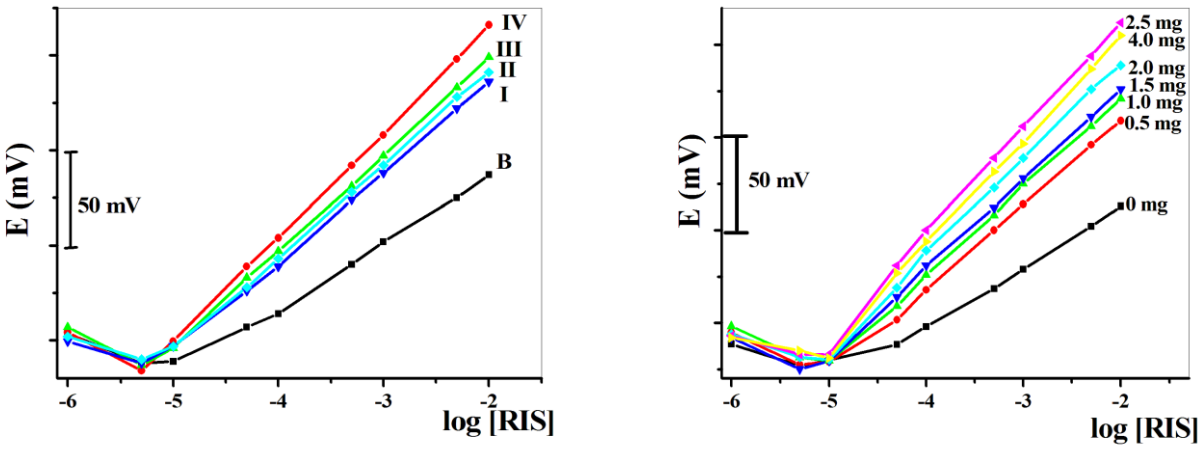

Figure 2. Effect of CD type and content of β-CD **(IV)** on RIS electrode performance.

Addition of lipophilic ionic sites promotes the interfacial ion-exchange kinetics and decrease the bulk resistance by providing mobile ionic sites in the electrode matrix [40-42]. β-CDs behave as neutral carrier ionophores, therefore, their ISEs functional only when anionic sites are incorporated. From different tested anionic sites (NaTPB, KTClPB, PTA or PMA), addition of NaTFPB to the electrode matrix afforded the highest slope value (59.0± 0.6 $mVdecade^{-1}$). The content of NaTFPB was changed from 0 to 4.0 mg and 2.0 mg was selected.

Sensitivity and selectivity obtained for a given ionophore based ion-selective electrode are greatly influenced by the polarity of the electrode matrix, which is defined by the dielectric constant of the electrode plasticizer [42, 43]. It should be noted that the nature of the plasticizer affects not only the polarity of the electrode phase but also the mobility of ionophore molecules and the state of the formed inclusion complexes. Herein, the influence of the plasticizer on the performance of RIS sensors containing β-CD (IV) and NaTFPB was studied using four plasticizers having different dielectric constants, namely; *o*-NPOE, DOS, DBP and DOP (ε = 24, 5.2, 4.7 and 3.8, respectively). Plasticizer selection was crucial for appropriate sensor performance, as application of the less polar plasticizers decreased the sensitivity while the proper sensitivity was observed for electrodes containing high polar plasticizer, *o*-NPOE (Nernstian slope was 59.1±1.2 $mVdecade^{-1}$).

3.2. Sensor performances

The potentiometric response characteristics of the developed RIS sensors, at the optimal matrices compositions, were evaluated according to the IUPAC recommendation [39]. The fabricated sensors displayed Nernstian cationic responses towards RIS with slope values and sensitivities depending on the nature of the electrode. Data obtained (Table 1 and Fig. 3) indicated that the developed sensors can be successfully applied for the potentiometric determination of RIS in the concentration range from 5.0×10^{-6} to 10^{-2} mol L^{-1}. Moreover, CWEs were the most sensitive as the measured LOD was 2.7×10^{-6} mol L^{-1} with Nernstian slope 60.44±0.43 mV $decade^{-1}$.

Table 1. Analytical performances [a] of various RIS/ /β-CD sensors

Sensors	CWE		CGE	PVC
	Batch	FIA		
Concentration range (mol L^{-1})	5.0×10^{-6}-10^{-2}	10^{-6}-10^{-2}	5.0×10^{-6}-10^{-2}	10^{-5}-10^{-2}
Slope (mV $decade^{-1}$)	60.44±0.43	56.2 ± 0.78	57.80±0.65	52.42±0.81
R	0.99988	0.99972	0.99981	0.99941
LOD (mol L^{-1})	2.7×10^{-6}	4.0×10^{-6}	2.7×10^{-6}	10^{-5}
Response time (s)	3		5	10
Lifetime (week)	10		12	6

[a] Results are the average of five different calibrations..

PVC membrane showed lower sensitivity and slope values (52.42±0.81 mV $decade^{-1}$, LOD was 10^{-5} mol L^{-1}) compared with solid contact electrodes, which may be attributed to the electrode configuration with the internal reference solution. It is noteworthy to mention that sensors based on β-CD as sensing material showed higher sensitivity than that based on RIS-ion pairs [20], which may be explained on the basis of encapsulation of RIS molecule into the CDs toroidal cavity (host-guest interaction).

For analytical applications, the sensor response time is of critical importance; therefore, the dynamic response times of the fabricated sensors were tested by measuring the time required to achieve a steady state potential (within ±1 mV) after sudden increase in the RIS concentration from 10^{-6} to 10^{-3} mol L^{-1} (Fig. 3). The response time of both CWE and CGE was fast as (about 3, 5s) while PVC electrodes gave a stable potential reading after 10s.

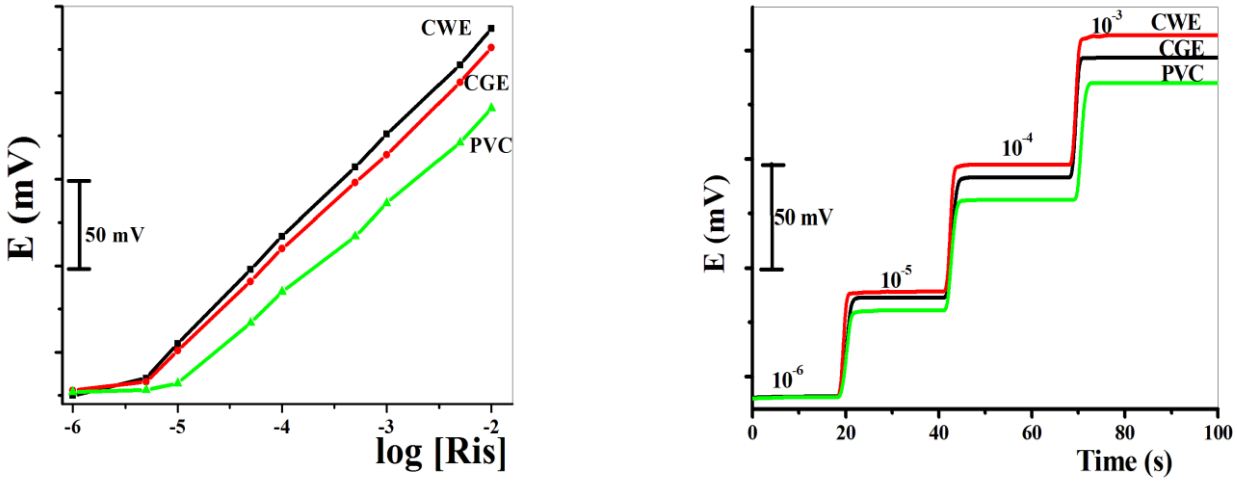

Figure 3. Electrochemical performances and response times of different RIS sensors

The lifetimes of the fabricated electrodes were tested by performing day-to-day calibration (Table 1). Different RIS sensors including CWE, CGE and PVC showed useful lifetime of 10, 12 and 6 weeks during which the Nernstian slopes did not change significantly (±2 mV/decade), while the detection limit was shifted by one magnitude at the end of this period. The relatively short lifetime of the PVC membrane may be due to leaching of the sensing ionophore into both internal reference solution and external bathing solution.

The influence of pH on the response of the fabricated electrodes was studied by recording the electrode potential readings at different pH values (pH 2–10). The electrodes responses (Fig. 4) were found to be pH independent in the range 3.0–8.0.

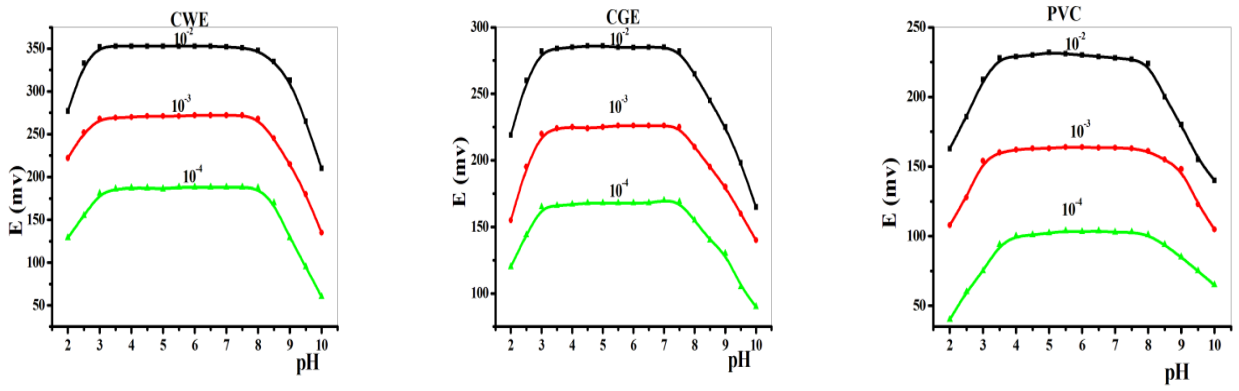

Figure 4. Effect of pH on different RIS sensors

SPEs were also fabricated using commercial carbon ink (Gwent C2010517D4) modified with CTS (0.75 g) and applied for DPASV determination of Pb^{2+}. The obtained results showed higher base

line than that of the homemade ink, which raise the detection limit of the method to 60 ng mL^{-1}. The final homemade ink composition was as follows: 18 % CTS, 72.2 % carbon and 9.8 % binder. The homemade ink showed shelf life similar to the commercial one with the advantages of easier preparation and modification.

The selectivity of the prepared RIS-CWE sensors towards different species was tested applying the separate solutions method (SSM) [44, 45] while the selectivity coefficients in case of neutral and organic species were determined by the matched potential method (MPM) [45]. Results (Table 2) revealed a high selectivity towards RIS in the presence of other interferents, additives and fillers commonly introduced in pharmaceutical formulations as well as inorganic cations.

Table 2. Potentiometric selectivity coefficients for RIS/β-CD sensors under batch and FIA conditions.

Interferent	-log $K_{A,B}$				
	Batch	FIA		Batch	FIA
NH_4^+	4.1	4.3	Glucose	4.72	
K^+	3.2	3.5	Fructose	4.88	
Na^+	3.32	3.40	Maltose	5.15	
Li^+	3.46	3.70	Lactose	5.22	
Ca^{2+}	3.37	3.52	Starch	5.35	
Mg^{2+}	3.00	3.42	Citric acid	5.05	
Mn^{2+}	3.52	3.70	Ascorbic acid	5.01	
Cu^{2+}	2.95	3.30	Glycine	4.46	
Cd^{2+}	2.75	3.15	Alanine	4.68	

[a] Average of five measurements

3.3. Potentiometric titration

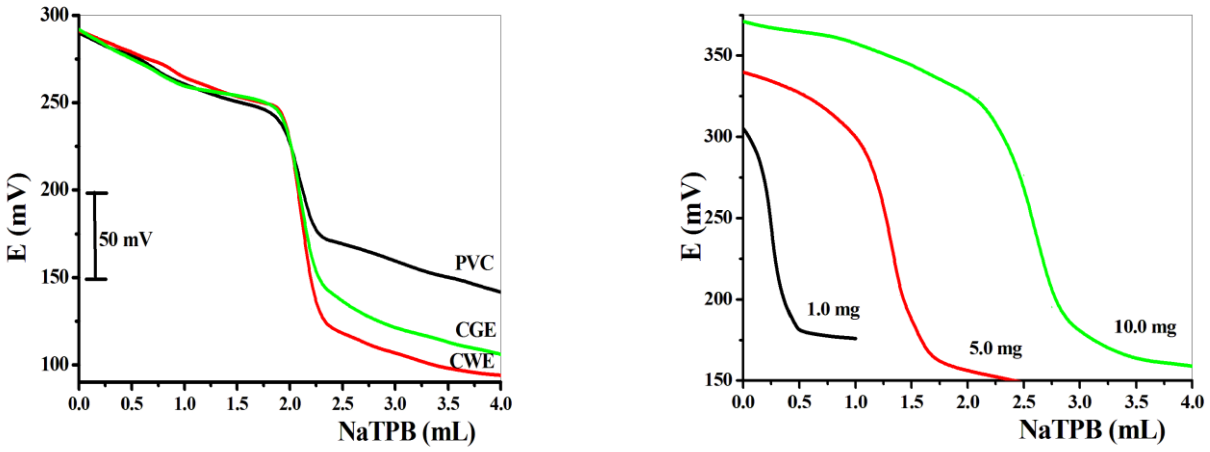

Figure 5. Potentiometric titration of: (a) 8 mg RIS using different RIS-sensors electrodes, and (b) different RIS concentrations with RIS- CWE.

In contrast to direct potentiometric measurements requiring careful calibrations of measuring cells, the potentiometric titration techniques offers the advantage of high accuracy and precision; although the cost of increased time and increased consumption of reagents used as titrants. In the potentiometric titration of RIS with NaTPB, different RIS sensors were used as indicator electrodes and both the total potential change (ΔE) and the potential break at the end point were recorded (Fig .5).

It can be concluded that, CWE showed the best titration curve compared with CGE and PVC electrodes indicated by the highest total potential change (ΔE values were 197, 186 and 150 mV for the tested electrodes in the same order). The potential break at the end point (dE/dV, first derivative of the titration curve) were also in the same sequence (317, 252 and 180 mV/mL of titrant) indicating the sensitivity of the titration process. In addition β-CD based sensors presented in this work showed better titration curve compared to those modified with RIS-TPB ion pair [20] regarding the total potential change and the inflection at the end point. Under the optimum conditions, titration curves were symmetrical (Fig. 5b) with well-defined potential jumps (DE ranged from 100 to 200 mV) allowing the determination of 2.0 mg RIS.

3.4. Electrode response under FIA conditions

Incorporation of ISEs in flow injection systems has the advantages of automation with high sampling frequency [46,47]. Fig. 6 showed peaks from the CWE electrode system when 200 µL of RIS solutions at various concentrations were injected in the water flowing stream (30 mL min^{-1}). Calibration graphs were linear in the concentration range from 10^{-5} to 10^{-2} mol L^{-1} with Nernstian slopes of 56.2 ± 0.78 mV $decade^{-1}$ and sampling output of 90 sample h^{-1}. Reproducibility was evaluated from repeated 10 injections of 200 µL of 10^{-4} mol L^{-1} RIS solution; the average peak heights were found to be 267.1 ± 2.0 mV.

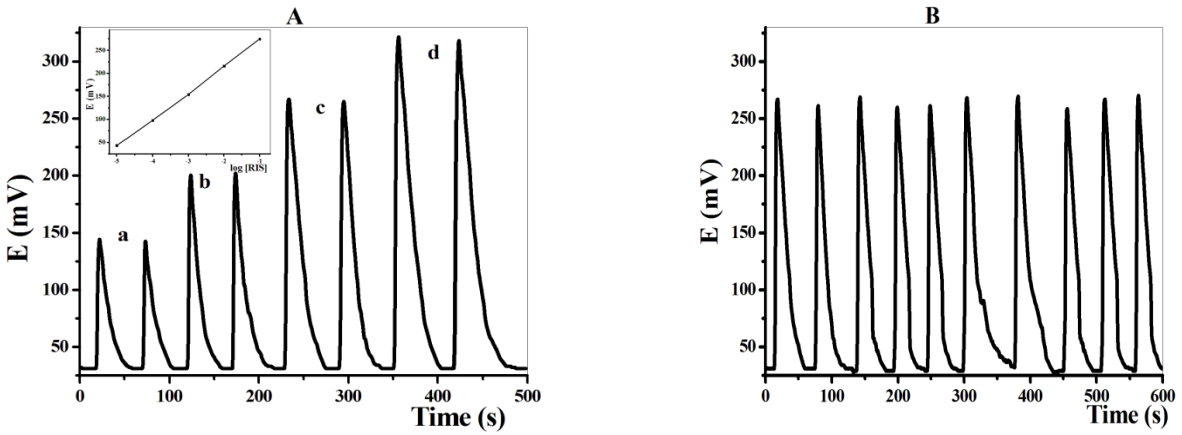

Figure 6. A) FIA potentiometric determination of RIS using β-CD-CWE: (a) 10^{-5}, (b) 10^{-4}, (c) 10^{-3} and (d) 10^{-2} mol L^{-1}. B) reproducibility of injection of 200 µL of 10^{-3} mol L^{-1} RIS under the optimum FIA conditions.

3.5. Analytical applications

The proposed sensors were successfully employed for RIS assaying in authentic samples and pharmaceutical formulations under FIA and potentiometric titration methods. Results clearly indicated satisfactory agreement between the RIS contents in different samples determined by the developed sensor and official HPLC method [48, 49] (Table 3). The time required for sample analysis time was short in case of FIA (about 1 min) compared with about 10 min for the potentiometric titration method. As reported in the previous work, relatively low recovery values were obtained in both the HPLC and the proposed methods when extracting the risperidone from tablets by dilute HCl in aqueous medium followed by the adjustment of the pH to 6.5 even stirring for extended period of time. This may be attributed to the incomplete release of the drug from the excipients materials or the partial physical adsorption of the drug on the insoluble excipients. Acetonitrile was used to enhance the dissolution and the extraction of RIS from Psychodal tablet and 5% acetonitrile was found to be adequate and compromise to have satisfactory results without affecting the electrode performance.

4. CONCLUSION

The present work demonstrates the fabrication of novel cyclodextrin-based risperidone potentiometric sensors. The proposed sensors showed Nernstian slopes in the concentration range 5×10^{-6}–10^{-2} mol L^{-1} with fast response time (3s) and long operational lifetime. The electrode performance was improved by comparing with those based on RIS-ion pairs as sensing material. The fabricated electrodes were successfully applied for the potentiometric determination of RIS in pure and pharmaceutical forms under FIA and potentiometric titration conditions. Compared with the already existing procedures for the determination of RIS which require special instrumentation, prior separation; FIA allows high sampling output with the possibility for incorporation in routine analysis for drug quality control.

Table 3. Determination of RIS in pharmaceutical preparations

Method	Pharmaceutical Preparation											
	Sigmdone (3 mg/tablet)						Psychodal (1 mg/tablet)					
	Found[a]	Recovery[b]	SD	RSD	F-Test[c]	t-Test[d]	Found[a]	Recovery[b]	SD	RSD	F-Test[c]	t-Test[d]
Potentiometric titration	2.920	97.3	0. 034	0.80	8.7	4.6	1.02	102.1	0.65	1.35	6.4	3.1
FIA	3.09	103.0	0.055	1.20	1.30	2.02	0.99	99.0	0.45	1.15	6.1	2.8
HPLC Method	3.12	104.0	0.006	1.04			0.984	98.4	0.056	0.75		

[a] values expressed as mg/tablet.
[b] % of the nominal values, average of three determinations.
[c] The tabulated *F*-value at 95% confidence level, F_c (0.05,3,3) = 9.28.
[d] The tabulated t- value at 95% confidence level, t_c(0.05, 4) = 2.776.

References

1. J.E. Leysen, W. Gommeren, A. Eens, D.C. Courcelles, J.C. Stoof, P.A.J. Janssen, *J. Pharmacol. Exp. Ther.* 247 (1988) 661.
2. G. Chouinard, W. Arnott, *Can. J. Psychiatry* 38 (1993) S89.
3. G. Chouinard, B. Jones, G. Remington, D. Bloom, D. Addington, W. MacEwan,A. Labelle, L. Beauclair, W. Arnott, *J. Clin. Psychopharmacol*. 13 (1993) 25.
4. A. Schotte, P.F.M. Janssen, A.A.H.P. Megens, J.E. Leysen, *Brain Res.* 631(1993) 191.
5. M. D. Meulder, B.M.M. Remmerie, R. Vries, L. L.A.Sips, S. Boom, E.W.J. Hooijschuur, N. C. V. Merbel, M. M.Philip, B.L. Timmerman, *J. Chromatography* B, 870 (2008) 8.
6. J.P. L. Moing, S. Edouard, J. C. Levron, *J. Chromatography B: Biomedical Sciences and applications*, 614 (1993) 333.
7. B. Doherty, F. O. Donnell, W. F. Smyth, J. C. Leslie, E.O. Kane, S. M. Clean, *Talanta* 72 (2007) 755.
8. I. Locatelli, A. Mrhar, I. Grabnar *J. Pharm. and Biomedical Anal*. 50 (2009) 905.
9. C. Danel, P. Chaminade, P. Odou, C. Bartélémy, D. Azarzar, J.P. Bonte, C. Vaccher, *Electrophoresis* 28 (2007) 2683.
10. M.G. Orkoula, C.G. Kontoyannisühe, *J. Pharm. Biomed. Anal*. 47 (2008) 631.
11. D. V. Naiker, S. V. Catts, V. S. Catts, K. S. Bedi, L. J. Bryan, *European J. Pharmacology*, 540 (2006) 87.
12. C. Jeyaseelan, R. Jugade, A.P. Joshia, *Croat. Chem. Acta* 79 (2006) 541.
13. X.F. Xie, X.D. Shao, Z.H. Song, *Chem. Pap*. 60 (2006) 288.
14. W. E. Hassan, *Chem. Pharm. Bull*. 56 (2008) 1092.
15. V.V. Cosofret, R.P. Buck: Pharmaceutical Applications of Membrane Sensors, CRC Press, Boca Raton, FL, 1992.
16. K. Vytras; in: Encyclopedia of Pharmaceutical Technology, J. Swarbrick, J.C. Boylan (Eds.); pp. 347–388, Marcel Dekker, New York, 1995.
17. E.G. Kulapina, O.V. Barinova: *Pharm. Chem. J*. 31 (1997) 667.
18. R.I. Stefan, G.E. Baiulescu, H.Y. Aboul-Enein: *Crit. Rev. Anal. Chem*. 27 (1997) 307.
19. T.S. Ma, S.S.M. Hassan, Organic Analysis Using Ion Selective Electrodes, Academic Press, London, 1982.
20. M.E.M. Hassouna, S.A.A. Elsuccary, G.A. Zaki, *International J. Chem*. 35 (2014) 1538.
21. K. Toei, *Anal. Sci*. 3 (1987) 479.
22. K. Vytras, *Ion Select. Electrode Rev*. 7 (1985) 77.
23. J.A. Cox, M.E. Tess, T.E. Cummings, *Rev. Anal. Chem*. 15 (1996) 173.
24. J. Zen, A.S. Kumar, D. Tasi, *Electroanalysis* 15 (2003) 1073.
25. M.I. Bender, M. Komiyama, *Cyclodextrin Chemistry, Springer*, NY, 1978.
26. G. Astray, C.G. Barreiro, J.C. Mejuto, R.R. Otero, J.S. Gara, *Food Hydrocolloid*. 23 (2009) 1630.
27. J. Mosinger, V. Tomankova, I. Nemcova, J. Zyka, *Anal. Lett.* 34 (2001) 1979.
28. J. Szejtli, Cyclodextrins and their Inclusion Complexes, Akademia Kiado, Budapest, 1982.
29. P. Shahgaldian, U. Pieles, *Sensors* 6 (2006) 593.
30. T. Ogoshi, A. Harada, *Sensors*, 8 (2008) 4961.
31. A. Radi, S. Eissa, *Open Chem. Biomed. Methods J*., 3 (2010) 74.
32. E. Khaled, M.S. Kamel, H.N. Hassan, S.H. Abd El-Alim, H.Y. Aboul-Enein, *Analyst* 137 (2012) 5680.
33. R.W. Cattrall, H. Freiser, *Anal. Chem*. 43 (1971) 1905.
34. A. Ansaldi, S.I. Epstein, *Anal. Chem*. 45 (1973) 595.
35. B. Bengi, S. Ozkan, *Combinatorial Chemistry & High Throughput Screening* 10 (2007) 495.
36. D Shukla, S Chakraborty, S Singh, B Mishra, *DARU J. Pharm. Sci*. 17 (2009) 226.
37. M.N. Abbas, A.A. Radwan, *Talanta* 74 (2008) 1113.

38. E. Khaled, K. Vytras, H.N.A. Hassan, B.N. Barsoum, *Sci. Pap. Univ. Pardubice, Ser* A 7 (2001) 33.
39. R.P. Buck, E. Lindner, *Pure Appl. Chem*. 66 (1994) 2527.
40. E. Bakker, E. Pretsch, *Anal. Chim. Acta* 309 (1995) 7.
41. M. Telting-Diaz, E. Bakker, *Anal. Chem*. 73 (2001) 5582.
42. E. Bakker, P. Buhlmann, E. Pretsch, *Electroanalysis* 11 (1999) 915.
43. W. E. Morf, The Principles of Ion-Selective Electrodes and Membrane Transport, Elsevier, New York, 1981.
44. G.G. Guibault, R.A. Durst, M.S. Frant, H. Freiser, E.H. Hansen, T.S.Light, E. Pungor, G.A. Rechnitz, N.M. Rice, T.J. Rohm,W. Simon, J.D.R.Thomas, *Pure Appl. Chem*. 48 (1976) 127.
45. Y. Umezawa, P. Buhlmann, K. Umezawa, K. Tohda, S. Amemiyai, *Pure Appl. Chem*. 72 (2000) 1851.
46. A. Danet, L.L. Zamora, J.M. Calatayud, *J. Flow Injection Anal*. 15 (1998) 168.
47. M. Trojanowicz, M. Szewcznska, M. Wcislo, *Electroanalysis* 15 (2003) 347.
48. T. Nagasaki, T. Ohkubo, K. Sugawara, N. Yasui, H. Furukori, S. Kaneko, *J. Pharm. Biomed. Anal*. 19 (1999) 595.
49. P.C. Meier, R.E. Zünd, Statistical Methods in Analytical Chemistry, 2nd ed., John Wiley & Sons Inc., New York, 2000.