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Novel Electroanalytical Technique for Determination of Trospium Hydrochloride

Rehab O. El-Attar* Hassan N.A. Hassan and Elmorsy Khaled

Microanalysis Laboratory, Applied Organic Chemistry Department, National Research Centre, El Bohouth st., Dokki, 12622-Giza, Egypt

Abstract

The present work describes for the first time the fabrication of a novel disposable screen printed sensor for the potentiometric determination of trospium hydrochloride (TRO). The cited sensors were based on alpha cyclodextrin as molecular recognition element and multi walled carbon nanotubes (MWCNTs) as transducer. Comprehensive and deep investigations were done on the sensing membrane components including the nature of sensing material, cavity size, additives, and plasticizers in addition to nanomaterial. The fabricated sensors were highly sensitive, selective and accurate towards TRO with Nernstian slope 60.8± 0.5 mV decade^{-1} in the concentration range from 10^{-6} to 10^{-2} mol L^{-1}. This can be attributed to the incorporation of MWCNTs to the electrode matrix which accelerates response time and improves potential reading stability. The fabricated sensors were used for the potentiometric determination of TRO under batch and flow injection technique with agreeable recoveries compared with the standard methods. Moreover, the high selectivity of the sensors towards TRO in presence of its degradation products suggests application of this method as stability indicating technique for TRO quality control.

Keywords: Trospium hydrochloride; Disposable potentiometric sensor; α-cyclodextrin; Carbon nanotubes, Pharmaceutical and biological samples.

1. Introduction

Trospium (TRO) (1, 3, 5) -3-[Hydroxydiphenyl acetyl) oxy] spiro [8-azoniabicyclo [3.2.1.] octane-8, 1-pyrolidinium] is a quaternary ammonium antimuscuranic and antispasmodic agents [**1**]. It is suggested for the treatment of overactive bladder with urge incontinence [**2**] and prevents the acetylcholine effect on muscarinic receptors in innervated organs cholinergically [**3**]. TRO is a new official pharmacopial compound which officially determined by potentiometric titrimetric against 0.1 M silver nitrate [**4**].

For TRO analysis in pharmaceutical and biological samples, few analytical methods were reported in literature including high performance liquid chromatography-tandem mass spectrometry [**5, 6],** LCMS/MS [**7, 8]**, RP-HPLC [**9].** To less extent spectrophotometric [**10, 11**], fluorimetric using RP-UFLC [**12**] and atomic absorption [**13**] techniques were found. The main drawbacks are using toxic organic solvents, numerous manipulation steps and expensive operating conditions.

Electroanalytical techniques with their advantages of adequate sensitivity with considerable operation coast and short measurement time are now well established technique for pharmaceutical analysis [**14-23**]. To the best of our knowledge, no electroanalytical studies were performed for determination of trospium, therefore, the aim of present work was to establish a novel, simple, accurate and precise potentiometric screen printed sensor using the macrocyclic/nanomaterial composite for potentiometric determination of TRO in both pharmaceutical and biological samples.

*Corresponding author e-mail: rhbattar@yahoo.com
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2. Experimental

2.1 Reagents and Chemicals

All reagents were of the analytical grade and bidistilled water were used in the experiments. Three families of sensing macromolecules were used such as; native alpha-(**I**, Sigma-Aldrich), gamma-(**II**, Sigma-Aldrich) and beta-cyclodextrins (**III**, Sigma-Aldrich), heptakis (2,6-di-O-methyl)-beta-cyclodextrin (**IV**, Sigma-Aldrich), heptakis (2,3,6-tri-O-methyl)-beta-cyclodextrin (**V**, Sigma-Aldrich), 12-crown-4 ether (**VI**, Fluka), 15-crown-5 ether (**VII**, Fluka), 18-crown-6 ether (**VIII**, Fluka), 21-crown-7 ether (**IX**, Fluka), dibenzo 24-crown-8 ether (**X**, Fluka), 30-crown-10 ether (**XI**, Fluka), calix[4]arene (**XII**, Aldrich) and calix[8] arene (**XIII**, Sigma-Aldrich).

Anionic sites such as potassium tetra chlorophenyl borate (KTClPB, Fluka), sodium tetra fluoro phenyl borate (NaTFPB, Sigma), sodium tetraphenylborate (NaTPB, Fluka) were studied. Different types of plasticizers were also examined including: *o*-nitrophenyl octylether (*o*-NPOE, Sigma), 2-fluorophenyl 2-nitrophenyl ether (*f*-PNPE, Fluka), dioctylphthalate (DOP, Sigma-Aldrich), dioctylsebacate (DOS, Avocado) and tricresyl phosphate (TCP, Fluka).

Poly (vinyl chloride) (PVC, relative high molecular weight, Sigma-Aldrich), graphite powder (synthetic 1– 2 µm, Sigma-Aldrich), silver and silver chloride powders (Sigma-Aldrich) were used for preparation of the printing ink. Multi-wall carbon nanotubes (MWCNTs, Sigma-Aldrich), single-wall carbon nanotubes (SWCNTs, Sigma-Aldrich), graphene (rG, Sigma-Aldrich) were incorporated in the sensing membrane matrix.

Interferents solutions of Li^{+}, NH_4^{+}, Ca^{+2}, Mg^{+2}, Ni^{+2}, Co^{+2}, phosphate, citrate, maltose, starch, sucrose, glucose, fructose, glycine, caffeine and cysteine were prepared from analytical grade chemicals and used in measurements of the selectivity coefficient.

2.2. Authentic samples

The authentic sample of TRO ($C_{25}H_{30}ClNO_3$, 427.969 g mol^{-1}) was from the National Organization for Drug Control and Research (NODCAR), Giza, Egypt.

For preparation of 1×10^{-2} mol L^{-1} stock drug solution, dissolve the appropriate amounts of the drug in bidistilled water. Then, the dilution was made covering the concentration range from 1×10^{-7} to 1×10^{-2} mol L^{-1} were with bidistilled water.

2.3 Preparation of the degradation products

The TRO degradation products were synthesized as described before [**13**]. Briefly, TRO (100 mg) was refluxed with 50 mL of 1.0 $molL^{-1}$ NaOH solution for 2h and tested for complete degradation by TLC using acetonitrile/glacial acetic acid (5:5 v/v) as the mobile phase. The degraded solution was then cooled at room temperature, neutralized with 1.0 mol L^{-1} HCl solution to pH 7. After drying, the residue was transferred quantitatively with methanol to 50 mL volumetric flask.

2.4. Pharmaceutical preparations

Trospamexin, tablets (20 mg TRO/tablet; Cairo, Egypt) were obtained from local drug stores. Ten tablets were weighed, grinded and an accurate weight of the powder assigned to contain 20 mg TRO was dissolved in bidistilled water, filtered and completed to 50 mL with bidistilled water.

2.5. Biological samples

The biological fluid aliquots (urine or plasma, obtained from a donor healthy male) were spiked with different concentrations of TRO ,and then treated with 0.1 mL of 70% perchloric acid diluted to 10 mL and centrifuged for 10 min at 13000 rpm. The supernatants were neutralized with NaOH to the appropriate pH value and the volume was completed to 25 ml with water.

2.6. Apparatus

Potentiometric measurements were carried out using 46-Range Digital Multimeter (Radio shack, China) with a PC interface and a 692-pH meter (Metrohm, Herisau, Switzerland). FIA measurements was performed using a single line flow injection system which was composed of a four channel peristaltic pump (MCP Ismatec, Zurich, Switzerland), sample injection valve (ECOM, Ventil C, Czech Republic) and continuous flow cells adapted for screen printed electrodes [**24**].

2.7. Procedures

*2.7.1. Sensor construction***.**

The potentiometric bielectrode strips (5×35 mm) were printed on a PVC sheet using silver-silver chloride and graphite-based inks for reference and working electrodes, respectively [**25**]. The sensing matrix cocktail was prepared by dissolving 1.0 mg α-CD (**I**), 1.0 mg NaTFPB and 360 mg *f*-PNPE in 6 ml tetrahydro furan followed by addition of 240 mg PVC and 10.0 mg MWCNTs with sonication for 30 min. The matrix was casted on the surface of the graphite/PVC conducting track and left to dry at 50ºC for 24 h. Before potentiometric measurements, the fabricated electrodes were soaked in 10^{-3} mol L^{-1} of TRO solution for 20 min.

2.7.2. Sensor calibration.

The bielectrode strips were immersed in different TRO solutions covering the concentration range from 10^{-7} to 10^{-2} mol L^{-1} at 25 ºC where the potential readings were recorded and plotted against drug concentration in logarithmic scale [**26**]. For FIA measurements, the injection of the prepared drug solutions (50 µL) were carried in the flowing stream from 10^{-6} to 10^{-2} mol L^{-1} with flow rate of 12.6 ml min^{-1}. The calibration graphs were drawn by recording of the corresponding peak heights.

2.7.3. Potentiometric determination of TRO in pharmaceutical preparations and biological samples.

The developed sensors were applied for potentiometric determination of trospium chloride in pharmaceutical preparations and biological samples. For standard addition method, known increments of 1×10^{-2} mol L^{-1} TRO standard solution were added to the sample solution and the electrode potentials for each increment were used to calculate TRO concentration in the sample solution [**27**]**.**

For the potentiometric titration, Aliquots of the sample solutions containing 0.392 to 19.6 mg of TRO were titrated against standardized NaTPB solution using the fabricated sensor as indicator electrode [**28**]. The potential readings were plotted against volume added, and the equivalence points were estimated from the first derivative of the sigmoid-shape titration curves. Under FIA conditions, the injection of 50 µL of the sample solutions was carried in the flowing stream and the peak heights were compared to those obtained from injecting standard solutions of the same concentration. The obtained recoveries were compared with the official method [**4**].

3. Result and Discussion

The formation of inclusion complex between molecular recognition and drug shows one of the promising approaches for improvement of the electroanalytical procedures [**29-32**]. The interactions forces between the predominant attractive host -guest are the $\pi - \pi$ interaction, dipole –dipole, van der Waals forces or hydrogen bonding [**33-35**]. Cyclodextrins (CD) are composed of three types according to the glucose units numbers forming the corresponding alpha, beta and gamma cyclodextrin [**36**]. The interior CD cavity was lined with skeletal carbon and ether oxygen atoms of the glucopyranose units which offer a microenvironment for fitting the nonpolar part of the gust molecule and formation of the inclusion complex. The formation of such inclusion complex depends on the size of both analyte and CD and spatial structure of the guest function groups. Sensors modified such by sensing materials usually showed improved sensitivity and selectivity [**30, 31**]**.**

3.1. Optimization of the sensor compositions

3.1.1. Effect of sensing ionophores

Different macrocyclic (**I-XIII**) including cyclodextrins, crown ethers and calixarenes were incorporated in the electrode matrix as molecular recognition element. Sensors prepared without addition of the sensing element have sub Nernstian compliance (44±0.5 mV $decade^{-1}$), while those containing cyclic ionophore showed different performance based on the ring size and height for fitting the piperidin ring of the trospium molecule.

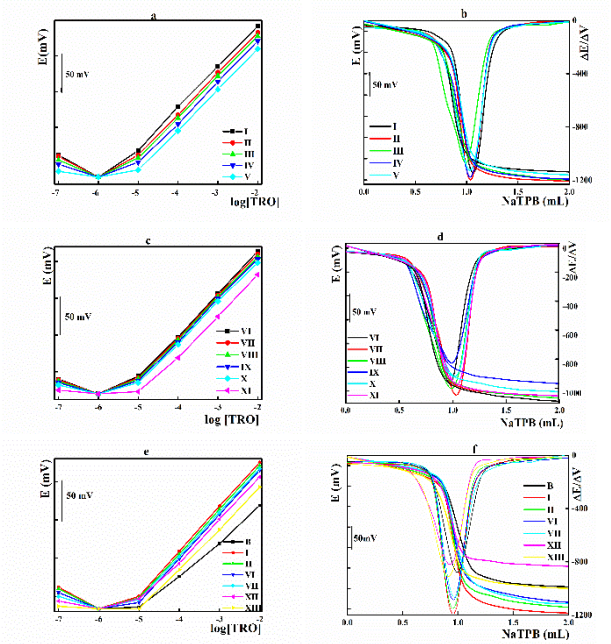

Fig. 1: a, c, e) Effect of the ionophores on TRO sensor performance, b, d , f) potentiometric titration of 1 ml of 10^{-2} mol L^{-1} TRO with 10^{-2} mol L^{-1} NaTPB solution.

From different cyclodextrin derivatives, α- and β-CD (**I, II**) gave the higher electrode sensitivity (Nernstian responses values were 51.0±0.9 and 57.6±0.6mV $decade^{-1}$, respectively) in the TRO concentration range 1×10^{-5} to 1×10^{-2} mol L^{-1} (**Fig. 1a**).Similar trend of sensitivity was observed under potentiometric titration of TRO against NaTFPB applying sensors modified with different CDs derivatives(**Fig. 1b**).

Crown ethers (macrocyclic polyethers) have the ability of trapping the guest molecule via coordination with a lone pair of electrons on the oxygen atoms [**37**]. The size of the ion relative to the ring cavity is the main factor goffering the stability of these complexes [**38, 39].**Crown ethers are able to form stable complexes with ammonium cation and protonated amines such as piperidin [**40**]. Herein,

crown ether macromolecules with different rings size (1.2 to 4.6 °A for **VI** to **XI** CE) were tested as sensing elements for potentiometric determination of TRO via formation of CE-TRO inclusion complexes. Small ring size of 12-crown-4 ether (**VI**) and 15-crown-5 ether (**VII**) were capable for fitting the TRO^{+} within the crown ether cavity with higher stability and Nernstian slope values (56.9±1.2 mV $decade^{-1}$)compared with other CEs(**Fig.1 c& d**).

Next, the performance of sensors incorporated with the best CDs (**I, II**) and CEs (**VI, VII**) were compared with those containing calixarene as molecular recognition **(Fig. 1e & f).** The recorded data confirm the priority of α-CD (**I**) over other tested sensing materials indicated by the higher Nernstian slope value and potential jump under potentiometric titration conditions.

3.1.2. Effect of ionic sites

Ionic sites are usually added to the electrode matrix to improve the membrane conductivity and the exchange of the ions at the membrane surface which in turn enhance the sensor selectivity and sensitivity [**41-43]**. Moreover, sensor based on neutral ionophores such as cyclodextrins perform only in presences of ionic sites with opposite charge to the target analyte [**43]**.

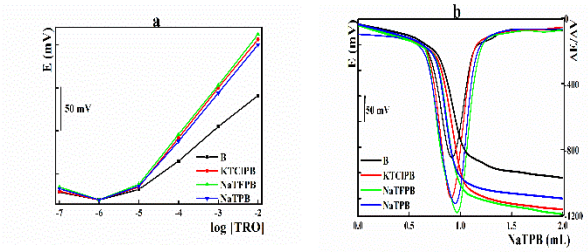

Fig.2. Effect of the ionic sites on: a) TRO sensor performance; b) potentiometric titration of 1 ml of 10^{-2} mol L^{-1} TRO with 10^{-2} mol L^{-1} NaTPB solution.

Because of the importance of the presence of ionic sites in the electrode matrix, different types of lipophilic tetraphenylborate derivatives were studied. In absence of ionic sites, sensor incorporated with *α*-CD only showed sub Nernstian slope values of 36.7±0.4 mV $decade^{-1}$. Contrary, incorporation of tetraphenylborate derivatives to the matrix improved the sensor efficiency (57.0±0.9, 57.1±0.7 and 54.8±1.2mV $decade^{-1}$ for KTFPB, NaTFPB and NaTPB, respectively (**Fig. 2a**). From different matrix compositions, sensors incorporated with sodium tetrafluorophenyl borate (NaTFPB) recorded potential jump and potential break at the end point (**Fig. 2b**).

3.1.3. Effect of membrane plasticizers

The polarity of membrane plasticizer defined by their dielectric constant affects mobility of the sensing ionophore and stability of the formed inclusion complex [**43-45**]. Plasticizers having different dielectric constants were applied as membrane mediator (**Fig. 3 a & b**) including DOP, DOS, TCP, *o*-NPOE and *f*-PNPE with different dielectric constants (***ε*** = 3.8, 5.2, 17.6, 24 and 50, respectively) [**46**].The obtained results declared that highly polar plasticizers the electrode performance (Nernstian response58.5±0.9 and 57.9 ± 0.7 mV $decade^{-1}$for *o*-NPOE and *f*-PNPE, respectively) from the concentration range 1×10^{-6} to 1×10^{-2} mol L^{-1}.

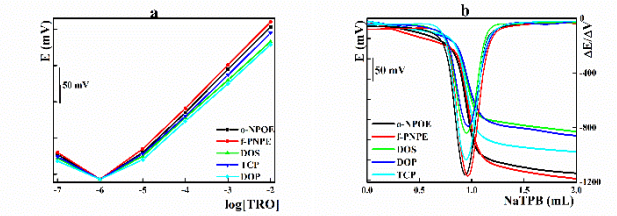

Fig. 3. Effect of the membrane plasticizer on: a) TR sensor performance; b) potentiometric titration of 1 ml of 10^{-2} mol L^{-1} TR with 10^{-2} mol L^{-1} NaTPB solution.

The potentiometric titration of TRO applying sensors contained different types of plasticizers confirmed the selection of *o*-NPOE or *f*-PNPE as solvent mediators indicated by the higher total potential jump (376 and 390 mV) compared with low dielectric constant plasticizers (**Fig.3b**).

3.1.4. Effect of nanomaterials

Nanomaterials with their unique properties and high mass/volume ratio accelerate the transduction of the chemical signal to the electrical signal within the sensor matrix that enhances the sensor performance [**47, 48**]. Single-wall carbon nanotubes, Multi-wall carbon nanotubes, graphene nanosheet and graphite sheets (1–2 mm) were incorporated in the electrode matrix.

Both SWCNTs and MWCNTs gave a higher Nernstian response (slope values were 59.6±1.2 and 60.5±0.53 mV$decade^{-1}$, respectively) compared with graphene and graphite sheets (**Fig. 4a**).In the potentiometric titration, MWCNTs gave the highest potential jump compared with the other tested nanomaterials and PVC membrane (**Fig. 4b**).

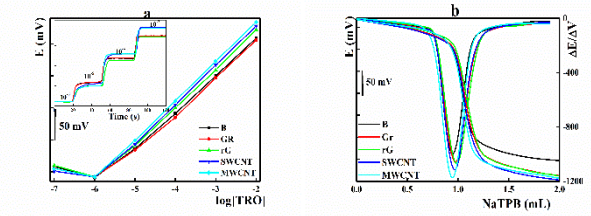

Fig.4. Effect of the different nanomaterials on a) TRO sensor performance; b) potentiometric titration of 1 ml of 10^{-2} mol L^{-1} TRO with 10^{-2} mol L^{-1} NaTPB solution

3.2. Sensors performances

Following the IUPAC recommendations [**26**], the performance of the fabricated trospium sensors incorporated with alpha cyclodextrin as sensing ionophore and MWCNTs as nanomaterials as transducer was evaluated (**Table 1**).The proposed sensors showed Nernstian slope of 60.8±0.5 mVdecade^{-1}in the linear concentration range from 10^{-6} to 10^{-2} mol L^{-1} with lower detection limit value 8.0×10^{-7} mol L^{-1}. Sensor fabrication was high reproducible with average Nernstian slope value 59.8±1.3 mV decade^{-1} and standard potential of (E^o) 412 ± 2.0 mV for 10 printed sensors within the same batch.

The fabricated sensors show a long lifetime (20 weeks)due to the absence of internal reference solution with reproducible Nernstian slopes values. Moreover, the same electrode can operate contentiously up to more than 10 days without diminishing of its performance.

Table 1: Analytical performances of different TRO screen printed sensors

Sensors	SPE	SPE/MWCNTs/α-CD	
		Batch	FIA
Concentration range (mol L^{-1})	10^{-6}-10^{-2}	10^{-6}-10^{-2}	10^{-5}-10^{-2}
Slope (mV decade^{-1})	58.5±0.9	60.8± 0.5	58.4±0.8
R	0.9992	0.99989	0.995
LOD (mol L^{-1})	10^{-6}	8.0×10^{-7}	5.0×10^{-6}
Response time (s)	8	<4	
Preconditioning time (min)	60	<20	
Shelf life time (week)	12	20	

The electrode response time was measured by recording the time needed to record a steady state potential after tenfold increase in the TRO concentration [**26**]. Modification with MWCNTs showed fast response (4s) due to the synergistic effect between MWCNTs nanoparticles and cyclodextrin within the sensing membrane matrix (**Fig. 4a**). The preconditioning time (time needed to get a stable potential reading for a newly used sensor) are limiting factors for application of a newly fabricated sensor. PVC and carbon paste electrodes usually soaked in the bathing solution over night to attain stable and reproducible potential reading. Solid contact electrodes such as coated wire electrodes suffer from the potential drift and the poor adhesion between the sensing membrane and metal substrate [**49, 50**]. Following the sensor fabrication protocol, co-polymerization process takes place between the conducting carbon track and the sensing membrane matrix. This co-polymerization will hinder the formation of such undefined water layer and improve the potential reading stability. Moreover, nanomaterials enhance of the hydrophobicity of the sensing membrane, which reflected to the more stable potential reading [**51**]. The present sensor showed high potential stability and need only for 20 min to achieve steady potential compared with 2 h for coated graphite electrodes and 24h for the corresponding PVC and carbon paste electrodes [**52, 53**]**.**

3.2.1. Effect of pH

The working pH range is a limiting operating factor for application of ion selective electrodes. The optimal pH range was studied by measuring the electrode potentials in TRO solutions (from 10^{-4} to 10^{-2} mol L^{-1}) at various pH values from 2 to 9 by adding very small increments of HCl and /or NaOH solutions to the drug solution. We noted that **(Fig. 5)**the potentials readings of the electrodes is relatively stable (±2.0 mV) in pH range 2 to 6, then decreased at higher pH due to the formation of the TRO deprotonated species (pKa is 4.5)**.**

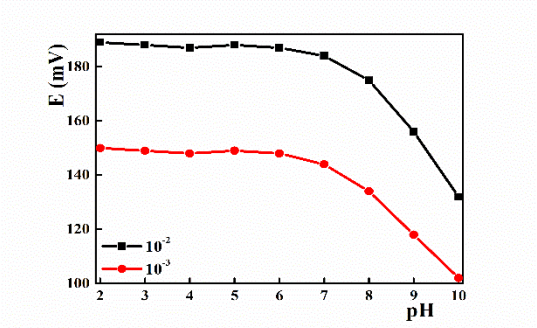

Fig. 5 : Effect of the pH on TRO sensor potential reading

3.2.2. Selectivity Coefficient

Sensor selectivity reflects their ability to respond to the target analyte in the presence of interfering ions [**54**]. The presence of excipients in pharmaceutical formulation requires more selectivity of the sensor for accurate analysis. Matched potential method (MPM) was recommended for measuring the selectivity of the ion selective electrodes in case of species with different charged or neutral compounds [**55, 56**]. By incorporation of alpha cyclodextrin to the electrode matrix, the selectivity toward TRO was improved in the presence of the interferents due to capability of α-CD to form a stable inclusion complex with TRO molecule **(Table 2)**.

Table 2: Potentiometric selectivity coefficients of TRO with screen printed sensors under batch and FIA conditions.

Interferent	$-\log K_{A,B}$				
	Batch [a]	FIA [b]		Batch	
Li^+	3.70	3.90	Maltose	3.72	-----

NH_4^+	3.10	3.40	Starch	3.53	-----
Ca^{2+}	2.90	3.20	Sucrose	3.19	-----
Mg^{2+}	3.14	3.35	Glucose	3.31	-----
Ni^{2+}	3.40	3.65	Fructose	3.25	-----
Co^{2+}	3.33	3.63	Glycine	2.90	-----
Phosphate	3.21	3.44	Caffeine	3.40	-----
Citrate	2.96	3.30	Cysteine	2.70	-----

[a] Upper and lower concentrations of TRO, 10^{-5} and 10^{-2} mol L^{-1} respectively.
[b] A separate solution method (SSM) was used for selectivity measurements.

3.3. Analytical Applications

3.3.1 Potentiometric Titration

For more analysis accuracy and precision, potentiometric titration of TRO against NaTPB using the fabricated sensor as indicator electrode can be suggested [**28**]. Titration graphs showed relatively high potential jumps (290 to 456 mV) for 0.392-19.6 mg TRO **(Fig. 6 a, b).** Highly reproducibility of the titration process was achieved; by performing titration process of 3.92 mg TRO, the potential jump was 385.0±1.8 mV with average recovery 100.20 ± 2.15% (**Fig. 6c**).

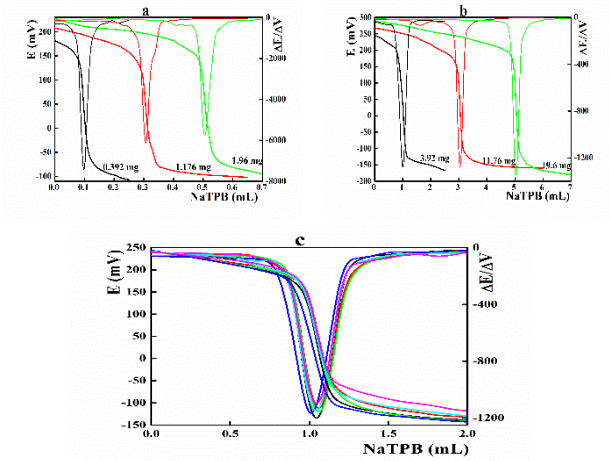

Fig. 6 : a, b) Potentiometric titration of different TRO concentrations against10^{-2} mol L^{-1} NaTPB, c) reproducibility of titration for 1 ml of 10^{-2} mol L^{-1} TRO solution with 10^{-2} mol L^{-1} NaTPB solution.

3.3.2 Flow Injection Analysis

Seeking for automatization and improvement of the analysis frequency, potentiometric sensors can be incorporated in flow injection systems [**57, 58**]. Both the sensors sensitivity and response time are the two main factors governing the performance of the electrochemical flow injection systems [**59**]. In the present work, the fabricated TRO sensors with their stable potential readings, fast response time (4s) with improved sensitivity were incorporated in FIA system. The flow injection peaks recorded via injection of 50 µL of TRO solutions were shown in **(Fig.7**). Nernstian slope value was 58.4±0.8 mVdecade^{-1}with sampling output 60 samples h^{-1}.

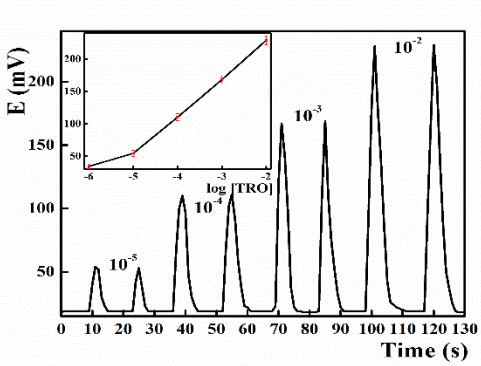

Fig. 7: FIA potentiometric determination of TRO using α-CD/MWCNTs screen printed sensors.

3.3.3. Determination of trospium in presence of its degradation product

According to Ramadan et al [**13**], alkaline degradation of trospium take place under mild condition producing benzilic acid and (1R, 3r, 5S)-3-hydroyspiro [8-azoniabicyclo [3.2.1] octane-8, 1'-pyrrolidinium]. The expected interference of both degradation products was tested under potentiometric titration conditions.

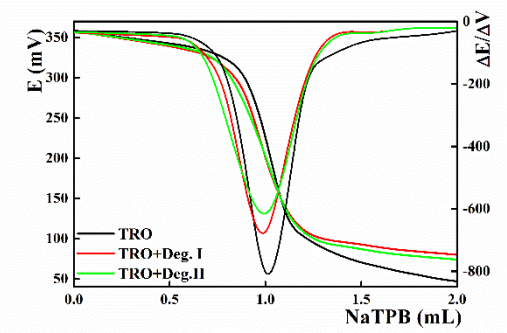

Fig. 8: Potentiometric titration of 3.92 mg TRO in presence of its degradation products against 10^{-2} mol L^{-1} NaTPB.

Preliminary experiments did not show the formation ion pair between NaTPB and either (**Deg. I**) or (**Deg. II**). Performing the potentiometric titration of 3.92 mg TRO in presence of both degradation products showed no interference of both compounds **(Fig. 8)** and therefore the method can be used for the determination of the drug in presence of its degradation product.

3.3.4. Sample Analysis

The achieved high sensitivity and selectivity of the suggested sensors towards TRO suggests their application as an effective tool for trospium analysis

in pharmaceutical formulations and biological fluids with high accuracy and precision (**Table 3**).

4. Conclusions

This work described a new sensitive, selective and accurate method for determination of trospium chloride using screen printed sensors. The sensor performance was improved via incorporation of alpha cyclodextrin as sensing material and MWCNTs as transducer. The fabricated sensors showed Nernstian slope 60.8± 0.5 mVdecade^{-1} within the concentration range from 10^{-6} to 10^{-2} mol L^{-1}. TRO sensors showed a long operational lifetime with a fast response time (<4s). The fabricated electrode was used for potentiometric determination of TRO with high accuracy and precision. No interference was detected from the TRO degradation product suggesting the application of this method as stability indicating technique for TRO quality control.

5. Conflicts of interest

"There are no conflicts to declare".

6. Formatting of funding sources

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7. Acknowledgment

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Table 3: Potentiometric determination of TRO in pharmaceutical preparations and biological fluids using the fabricated trospium screen printed sensor

	Taken (µg)	Found					
		Trospamexin ®		Spiked Urine		Spiked Plasma	
		Recovery [a]	RSD [a]	Recovery	RSD	Recovery	RSD
Standard	**3.92**	**97.2**	**2.1**	**94.2**	**2.8**	**94.0**	**1.8**
addition	**39.2**	**98.4**	**2.9**	**96.0**	**3.1**	**97.2**	**2.6**
	392	**101.6**	**3.2**	**98.4**	**3.9**	**98.0**	**2.9**
Titration	**392**	**98.1**	**2.0**				
	1176	**99.4**	**1.8**				
	1960	**101.0**	**1.6**				
FIA [b]	**1.96**	**99.9**	**1.1**	**96.4**	**2.2**	**95.9**	**2.4**
	19.6	**101.6**	**1.4**	**97.6**	**1.6**	**98.2**	**2.2**
	196	**102.2**	**1.0**	**101.1**	**1.2**	**99.9**	**1.7**

[a] Mean recovery and relative standard deviations of five determinations

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