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Potentiometric determination of tolterodine in batch and flow injection conditions

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ABSTRACT

Two new ion-selective electrodes of the plastic membrane type for the determination of Tolterodine (Tol) were prepared. These electrodes depend on the incorporation of the ion-exchangers of the above mentioned drug with phosphotungestic acid (PTA) or Silicotungestic acid (STA) in a PVC matrix. A comparative study is made between the performance characteristics of electrodes containing ion-exchanger in batch and FIA conditions.

The usable concentration range of the electrodes was found to be (1.0×10^{-5}–1.0×10^{-2} and 5.0×10^{-5}–1.0×10^{-2} M) in batch and FIA conditions, respectively. The electrodes have nearly the same usable concentration, pH range and exhibited high selectivity toward Tol in the presence of many inorganic cations and can be used in biological fluids such as urine and plasma. The dissolution profile of the investigated drug as well as its assay in pure and pharmaceutical preparations was performed, and the results were relatively accurate and precise as indicated by the recovery values and coefficients of variation.

1. Introduction

Tolterodine tartarate (R)-N,N-diisopropyl-3-(2-hydroxy-5-methylphenyl)-3-phenylpropanamine L-hydrogen tartarate, CAS Registry Number 124937-52-6, is an antimuscarinic drug that is used to treat urinary incontinence by having a high binding affinity for the cholinergic muscarinic receptors that mediates contraction of the urinary bladder and thus enhances salivation [1].

A limited number of methods are available in literature to determine tolterodine and its metabolite 5-hydroxy-methyltolterodine, including HPLC-mass spectrometry [2], HPLC [3], capillary chromatography [4], capillary electrophoresis [5], mass spectroscopy [6] and gas chromatography [7]. In this study, two ion-selective membrane electrodes were developed for Tolterodine (Tol) based on the use of the ion-association complexes of (Tol) with phosphotungestic acid (Tol-PTA) and silicotungestic acid (Tol-STA). The prepared sensors were used for the determination of Tol in its pure state, pharmaceutical preparation, Detrusitol® capsule (4 mg/cap) and artificial urine and plasma samples both in batch and flow injection (FIA) conditions and to follow the dissolution profile of Detrusitol® capsule (4 mg/cap). This information can be helpful to set an analytical profile for the drug especially, it is not included in any of the commonly known pharmacopeias.

2. Experimental

2.1. Reagents and chemicals

All reagents used throughout the work were of analytical-reagent grade and solutions were made with doubly distilled water. Pure grade Tolterodine (Tol) and its pharmaceutical preparation Detrusitol® capsule (4 mg/cap), were provided by Pharmacia Company, Egypt under license from Pfizer®, USA. Phosphotungestic acid (PTA) and silicotungestic acid (STA), (Fluka), were used. Dioctylphthalate (DOP), diisononylphthalate (DIP), dibutylsebacate (DBS), and dibutylphthalate (DBP), (Fluka) were used as plasticizers. High relative molecular weight Polyvinyl chloride (PVC) (Fluka), was used for preparing membranes.

2.2. Apparatus

The potentiometric measurements in batch mode were carried out using an automatic titrator (Schott tritroline TA 10 plus, Germany). A circulatory thermostat was used to control the temperature of the test solutions (Lauda, Germany). Packed saturated calomel (SCE) (Sentek, UK) was used as an external reference electrode.

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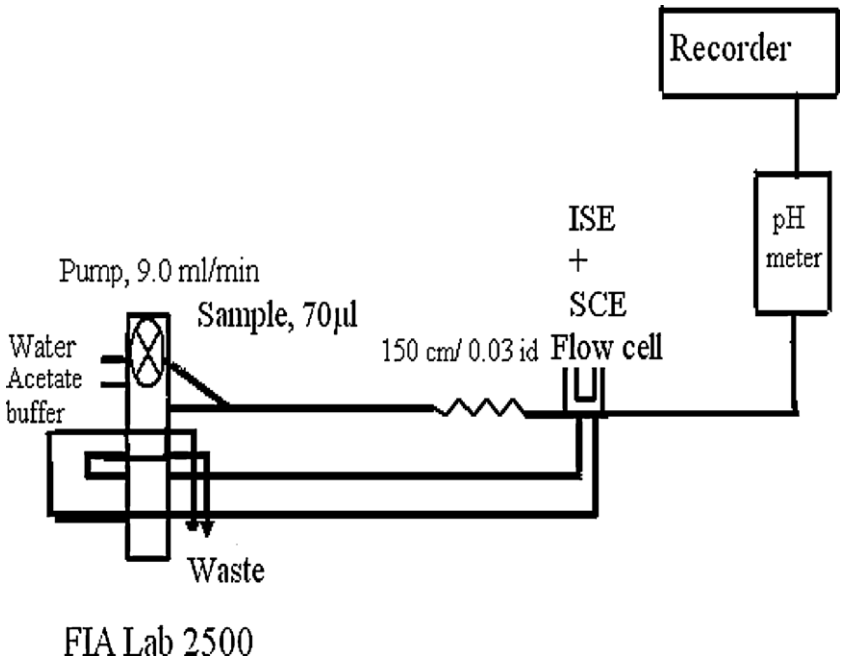

Fig. 1. Schematic diagram of the flow injection system used in the measurements.

A flow injection analysis (FIA lab 2500, USA) was used for the FIA measurements which is composed of a 4 channel peristaltic pump and a 6 port 2 way injection valve connected to a Jenway 3510 pH-meter (England); and interfaced to a strip-chart recorder model BD111 from Kipp and Zonn (Deft, Netherlands). A wall-jet-cell, giving low dead volume, fast response, good wash characteristics, ease of construction and compatibility with electrodes of various shapes and sizes, was used in flow determinations where a Perspex cup with axially positioned inlet polypropylene tubing is mounted at 0.5 mm from the sensing surface of the electrode body [8,9]. Fig. 1 represents the flow injection setup used in measurements.

Capsule dissolution measurements were made according to the method provided by the company (Pfizer®) using an Erwika dissolution device, Germany (USP-type apparatus).

2.3. Factors affecting the performance characteristics of the electrodes

The ion-exchangers tolterodine phosphotungstate, (Tol-PTA) and tolterodine silicotungestate (Tol-STA) were prepared by the addition of 150 ml of 1.0×10^{-2} M Tol solution to 50 ml of 1.0×10^{-2} M of phosphotungestic acid (PTA) or silicotungestic acid (STA). The resulting precipitates were left in contact with their mother liquor overnight to assure complete coagulation, filtered and washed with distilled water, and left to dry at room temperature for at least 48 h. Membranes of different compositions were prepared by mixing the required amounts of ion-exchangers, PVC and plasticizer (DOP, DBS, DIP or DBP) (on testing effect of plasticizers) of total weight 0.35 g in a 5.0 cm (diameter) Petri-dish containing 10.0 ml tetrahydrofuran (THF). To obtain homogeneous and uniform thickness, the amount of solvent was kept constant and the membranes were left covered to dry in air (not less than 48 h) [10,11]. The working conditions of the electrodes were optimized in terms of ionic strength, Usable pH, selection of appropriate buffer and thermal stability [12,13].

For FIA measurements optimization, many parameters were tested such as sample volume (70–230 µl), flow rate (2.0–10.0 ml/min), the carrier type and mixing coil length. The effect of pH of the test solution on the electrode response in FIA was studied by preparing a series of solutions of concentration that is 1.0×10^{-2} M of Tol with different pH values in the range from 1.00 to 14.00 adjusted using HCl and NaOH before injection in the flow stream and measuring the obtained peak heights.

The effect of interfering ions was determined by applying separate solution method in case of charged ions [14], while the matched potential method was applied for neutral molecules [15]. In case FIA, selectivity was tested by measuring the potential of 1.0×10^{-2} M solution of the interferent in comparison to the recordings of a standard series of different concentrations of the drug and the selectivity coefficient was calculated as the ratio between the potential obtained to that of the standard series that gave an equivalent potential change [16].

2.4. Assay of pharmaceutical dosage forms

In batch measurements, the standard additions method was applied [17], while for FIA measurements, series of standard drug solution and another series for capsules, spiked urine and plasma samples of the same concentrations, and the peak heights corresponding to these solutions were measured at the optimum FIA parameters related to the sensor used. The percentage recovery can be calculated as the ratio of the peak height of sample to that of an equivalent concentration of standard drug.

For analysis of Detrusitol® capsules (4 mg/cap), 120 capsules were ground and appropriate weights were taken as samples and dissolved in 5 ml 0.1 M HCl and distilled water up to 50 ml. The solution was then filtered and used to prepare different concentrations by appropriate dilution.

The performance of the Tol-PTA and the Tol-STA electrodes in artificial urine and plasma samples was tested in batch and FIA conditions. Artificial urine is composed of 6.16 g sodium chloride, 5.03 g sodium dihydrogen phosphate, 0.94 g trisodium citrate, 0.94 g magnesium sulfate, 2.40 g sodium sulfate, 4.74 g potassium chloride, 0.84 g calcium chloride, 2.43 g ammonium chloride, 18.01 g urea and 7.48 g creatinine then dissolved in distilled water in a 1 L volumetric flask and kept in the refrigerator [18]. While artificial plasma

is prepared by mixing the following ions in specified weights: 7.13 g sodium chloride, 0.35 g potassium chloride, 0.36 g calcium chloride, 0.22 g magnesium chloride, 1.68 g sodium bicarbonate, 2.20 g glucose, 2.38 g Heppes buffer and 5.00 g Bovine serum albumin then dissolved in distilled water in a 1 L volumetric flask and the pH was adjusted to 7.35 using 0.1 N NaOH. The prepared plasma should be kept in the refrigerator for 2 h before use [19]. For the analysis of biological samples, (artificial urine and plasma), different amounts of Tol (1.189–23.780 mg) were taken and the volume was completed with artificial urine or plasma to the mark in a 100 ml volumetric flask.

2.5. Dissolution test

Capsule dissolution measurements were made using Type I basket, Erwika dissolution device, Germany, (USP-type apparatus). One capsule of Detrusitol® (4 mg/capsule) was placed in the dissolution medium, analyzed using USP Type 1 apparatus in 900 ml of pH 6.8 phosphate buffer, which was maintained at $37.0 \pm 0.5\,^{\circ}C$. The basket was rotated at 100 rpm and comprised the tolterodine selective membrane electrodes (Tol-PTA or Tol-STA) in conjunction with a standard Calomel reference electrode dipped in the solution. At time intervals (30 min), the potential values were recorded until the potential reached the plateau then the dissolution profiles were constructed.

A calibration curve, to be used for translating the measured potential into Tol concentration and % dissolved was constructing by adding, appropriate weights of the standard drug (2.56–4.20 mg) to the dissolution medium and the potential developed using both electrodes was plotted against the negative logarithmic value of the drug concentration (p drug). The data for this plot were obtained under the same experimental conditions as the dissolution curves. The only difference is that instead of the Tol capsules, subsequent Tol standard additions were performed yielding a 4.20 mg/900 ml end concentration in the dissolution medium. After each addition, the potential was recorded when it reaches a plateau [20].

3. Results and discussion

3.1. Optimization of the sensors response in batch conditions

In this work, the influence of the plasticizer type and ratio on the characteristics of the Tol-electrodes was investigated by using the four plasticizers with different physical properties namely, DOP, DBS, DIP and DBP in two plasticizer: PVC ratios that are 1:1 and 3:2 [10,21]. The increase in the amount of plasticizer improves to a large extent the adhesive properties of the membranes but on the other hand, helps their deterioration and this process depends on the properties of both ion-exchanger and the matrix. Each preparation was repeated four times to determine the optimum composition exhibiting the best performance characteristics (slope, reproducibility, more extensive usable concentration range and the lower detection time) [22].

All the electrodes have very short response time (less than 15 s) and the composition with DOP:PVC ratio 1:1, and 7.0% Tol-PTA and 5.0% Tol-STA electrodes exhibited the best Nernstian behavior, with slopes of 58.1 and 59.7 mV/concentration decade (after 1 h of soaking in 1.0×10^{-3} M Tol) in the working range (1.0×10^{-5}–1.0×10^{-2} M) for Tol-PTA and Tol-STA electrodes, respectively. The membranes with ration 3:2 were not able to maintain Nernstian slope for more than 1 h, which can be attributed to the increase of elasticity of the membranes leading to ease of leaching of ion-exchanger from the matrix.

Ionic strength effect was tested and the slopes obtained in 0.01 M, 0.1 M and 1 M NaCl solutions were found to be 58.0, 58.3 and 58.0 mV concentration decade and 59.6, 59.1 and 59.2 mV/concentration decade, for Tol-PTA and Tol-STA, respectively which does not differ significantly from those on using water so no ionic strength adjustor was used for the measurements.

The effect of using two different buffers on performance was tested, acetate buffer of pH 4.75 and phosphate buffer of pH 6.75, the slopes obtained for Tol-PTA electrode were 57.9 and 44.3 mV/concentration decade, respectively and for Tol-STA electrode 59.1 and 45.2 mV/concentration decade, respectively. Therefore, acetate buffer was used for pH adjustment in all the consequent measurements.

The effect of continuous soaking on the electrode response was tested. A slope of was 58.1 mV/concentration decade was obtained for Tol-PTA after 1 h of soaking and decreased gradually reaching 55.9 mV/concentration decade after 48 h and 50.40 mV/concentration decade after 30 days of continuous soaking. While in case of Tol-STA, the slope was 59.7 mV/concentration decade after 1 h of soaking, decreasing to 53.8 mV/concentration decade after 48 h, reaching 49.6 mV/concentration decade after 30 days of continuous soaking.

It is noteworthy to mention that electrodes, when not is used, were kept dry in the refrigerator at $4\,^{\circ}C$ and they were found to be able to maintain their Nernstian slope for intervals of time reaching 6 months.

3.2. Optimization of the flow injection analysis parameters

A two line FIA system is used, with water as carrier in one line and 0.1 M acetate buffer of pH 4.50 buffer in the other, which maintained more stable base line within the working range of the electrodes compared to using only water or buffer in both lines [24–28].

Samples of various ranges (70–230 μl) were injected into the flow stream, the peak heights were found to increase with sample volume until a plateau is reached at sample volume 140 μl. A sample volume of 70 μl was used-throughout this work which gives about 95% of the maximum peak height obtained using 140 μl loop, but requires shorter time necessary to recover the base line and lower consumption of reagents [23]. Different flow rates (2.0–10.0 ml/min) were tested and a flow rate 9.0 ml/min was used throughout the work, which has faster achievement of the base line especially in high concentrations.

A (150 cm/0.03 mm id) mixing coil was used throughout this work which provided reasonable peak heights and more stable base line compared to the (50 cm/0.03 mm id) coil as it offered enough time of contact and washing between sample and electrode surface. The dispersion coefficient was found to be 1.01, this value is affected by all the above studied parameters, i.e., sample volume, flow rate and carrier composition, etc. which were kept constant throughout all the work [21,29].

The slope of the calibration graph increases in FIA condition to 71.9 mV/concentration decade, average of four calibrations, compared to 58.1 mV/concentration decade in batch conditions for Tol-PTA electrode, while for Tol-STA electrode, the slope was found to be 67.0 mV/concentration decade compared to 59.7 mV/concentration decade in batch conditions. The super Nernstian slopes can be explained by different rates of increase of the signal at low and high concentrations. The short residence time of the sample solution at the sensing surface of the electrode, results in the observation of different proportions of the steady state for each concentration [28].

The usable concentration range in FIA for the electrodes is 5.0×10^{-5}–1.0×10^{-2} M as the concentrations lower than this range in FIA gave very small peaks to be detected, compared to 1.0×10^{-5}–1.0×10^{-2} M for Tol-PTA and Tol-STA electrodes in batch conditions, respectively. This small decrease in the

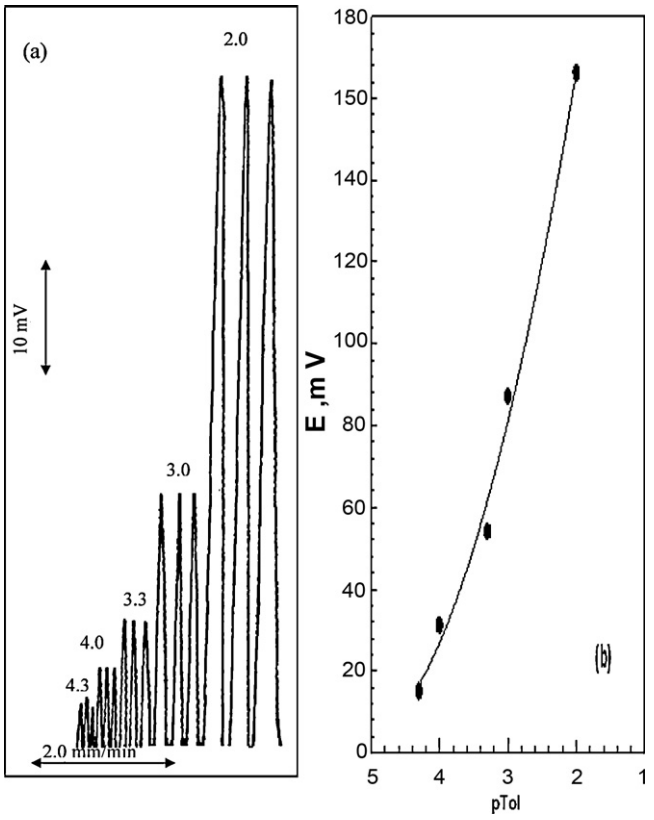

Fig. 2. The recordings (a) and their corresponding calibration curve (b) on using Tol-STA electrode at optimum FIA conditions.

concentration range, and accordingly the limits of detection and quantification in FIA can mainly be attributed to the difference in dispersion coefficient compared to batch conditions.

Fig. 2 is a representative for the recordings (a) and their corresponding calibration graphs (b) obtained for Tol-STA electrode at optimum FIA conditions, flow rate 9.0 ml/min, 75 μl loop, two lines manifold, water plus acetate buffer as carrier and 150 cm/0.03 mm id, mixing coil.

3.3. Effect of pH on the electrode response

The results revealed that, the change in pH does not affect the potential readings in batch conditions within the range 2.00–6.00 and 2.00–6.50 for Tol-PTA and Tol-STA electrodes, respectively. While in FIA, the peak heights representing the pH are almost stable in the range 2.00–5.50 for and 2.00–6.00 Tol-PTA and Tol-STA electrodes, so the electrodes can be used safely in these ranges with the use of acetate buffer regulation especially on testing solutions of pharmaceutical preparations that are usually dissolved in acids and have pH value around 2.00 compared to the pure drug (pH = 4.57). The potential decrease above these ranges in both conditions can be attributed to the release of free base in the solution, leading to a decrease in the concentration of the detected tolterodine cation [10,11].

3.4. Selectivity of the electrodes

The response of the electrode toward different substances and ionic species such as inorganic cations, urea and creatinine present in artificial urine and plasma was checked both in batch and FIA conditions and the values of selectivity coefficients, shown in Table 1, were used to evaluate their degree of interference.

A considerably high concentration of the interferent ion is used (1.0×10^{-2} M) to ensure that there will be no interference if lower concentrations than this are present [14,30,31]. The selectivity coefficients ranged from 2.58 to 4.61 and 2.64 to 4.59 for Tol-PTA and Tol-STA electrodes, respectively.

In FIA, where the sample remains in contact with the electrode for a short period of time, the apparent selectivity coefficient is expected to be different from that found at batch conditions. The selectivity coefficient was calculated using the matched potential method, as a ratio between the concentration of drug that gave a similar response to a solution that is 1.0×10^{-2} M of the interfering ion. The peak heights of 1.0×10^{-2} M were compared to calibration graph obtained from a standard series 5.0×10^{-5}–1.0×10^{-2} M of the pure drug. For some of the interfering ions, this concentration of drug could not be exactly determined as it was out of the detection limit of the electrode and since the peaks of those interferants were shorter than the peak of 5.0×10^{-5} M solution of the drug which is the minimum concentration detected by the electrode such as in case of NaCl, $MgCl_2$, $NaHPO_4$, glucose and urea.

The mechanism of selectivity is mainly based on the stereospecificity and electrostatic environment and it is dependent on how much fitting is present between the locations of the lipophilicity sites in the two competing species in the bathing solution side and those present in the receptor of the ion-exchanger. The selectivity coefficients ranged from 1.60 to >2.30 and 1.49 to >2.30 for Tol-PTA and Tol-STA electrodes, respectively as given in Table 1.

3.5. Analytical applications

In this work, artificial biological samples were tested in order to assure the consistency of the sample and avoid possible contamination or infections on using real human samples. It is well known that urine is a variable fluid, both between individuals and in the same individual over time. It is more concentrated in the morning, and is affected by fluid intake, exercise, ambient temperature and diet. Artificial plasma samples, which are commonly used now in research labs, are found to be safe and reliable as far as they comprise the major components of real plasma samples. The interference of the components of these samples was tested in the selectivity part, thus any results obtained on applying the electrodes in these matrixes indicate the electrode response for Tol as given in Table 2.

3.5.1. Potentiometric determination using standard additions method in batch conditions

The standard additions method was applied by adding small portions of 1.0×10^{-2} M standard Tol solution to 50 ml distilled water containing 1.189–23.780 mg Tol The results of the determination of Tolterodine in the pure substance and the pharmaceutical preparation Detrusitol® capsules 4 mg, spiked artificial urine and plasma samples by standard additions method are given in Table 2. The mean recovery values for determining Tol ranged from 99.7 to 100.4% and 97.4 to 99.5% with coefficient of variation ranging from 0.5 to 1.0 and 0.3 to 0.9 for Tol-PTA and Tol-STA electrodes, respectively for pure drug solutions.

The mean recovery values for determining tolterodine using Tol-PTA electrode ranged from 97.0 to 99.5% with coefficient of variation 0.2–0.8 for Detrusitol® capsules, 98.2–100.5% with coefficient of variation 0.3–0.8 for spiked artificial urine samples and 97.4–99.5% with coefficient of variation 0.4–0.9 for spiked artificial plasma samples. The mean recovery values for determining Tolterodine using Tol-STA electrode 98.2–102.5% with coefficient of variation 0.5–0.9, for Detrusitol® capsules 97.0–98.8% with coefficient of variation 0.3–0.6 for spiked artificial urine samples and 98.1–100.5% with coefficient of variation ranged from 0.4 to 0.7 for spiked artificial plasma samples.

Table 1
Selectivity coefficients and tolerance values for Tol-PTA and Tol-STA electrodes.

Interferent	$-\text{Log } K^{pot}_{\text{Tol},J^{Z+}}$							
	Tol-PTA				Tol-STA			
	Batch		FIA		Batch		FIA	
	[a]SSM	[b]MPM	SSM	[b]MPM	[a]SSM	[b]MPM	SSM	[b]MPM
NaCl	2.78	–	1.93	>2.30	3.55	–	1.70	2.10
KCl	3.88	–	1.90	2.10	4.31	–	1.85	>2.30
$CaCl_2$	4.00	–	1.76	2.05	3.61	–	1.62	1.85
$MgCl_2$	3.66	–	1.80	>2.30	3.39	–	1.79	2.13
$NaHCO_3$	2.86	–	1.65	2.00	2.98	–	1.55	1.75
Na_2HPO_4	2.93	–	1.97	>2.30	2.89	–	1.67	2.10
Na-Citrate	2.70	–	1.87	2.10	2.76	–	1.89	>2.30
$MgSO_4$	3.66	–	1.62	1.92	3.47	–	1.91	>2.30
Na_2SO_4	2.99	–	2.01	>2.30	3.05	–	1.52	1.70
NH_4Cl	3.45	–	1.90	2.90	4.21	–	1.77	>2.30
Glucose	–	2.58	1.86	>2.30	–	2.64	1.70	2.10
Hepes	–	3.46	1.78	2.06	–	3.25	1.67	1.84
BSA	–	4.30	1.78	2.05	–	4.59	1.85	>2.30
Urea	–	4.61	1.98	>2.30	–	4.37	1.49	1.60
Creatinine	–	3.11	1.60	1.90	–	3.28	1.65	2.10

[a] SSM: Separate Solution Method.
[b] MPM: Matched Potential Method.

These values are very close to the taken amounts as indicated in the drug pamphlets or spiked urine and plasma samples which reflect the high accuracy and precision of the studied electrodes as sensors for their respective drug.

3.5.2. Potentiometric determination using peak height comparison in FIA conditions

In FIA conditions, the peak heights of series of solutions of different concentrations from the capsules, or spiked artificial urine or plasma samples were measured at flow rate 9.0 ml/min using Tol-PTA and Tol-STA electrodes. The obtained peak heights were then compared to those obtained from injecting standard solutions of the same concentrations prepared from pure drug at the same flow rate and the recovery % can then be calculated as the ratio of the peak heights of the preparations to that of the equivalent concentration of the standard.

Results given in Table 2, show that the mean recovery values for determining tolterodine using Tol-PTA electrode ranged from 99.8 to 101.2% for Detrusitol® capsule, 102.0–102.7% for spiked artificial urine samples and 98.7–100.5% for spiked artificial plasma samples with coefficient of variation ranging from 0.3 to 0.5 while the mean recovery values for determining tolterodine on using Tol-STA electrode ranged from 99.2 to 100.9% for Detrusitol® capsule, 100.8–102.0% for spiked artificial urine samples and 98.3–100.2% for spiked artificial plasma samples with coefficient of variation ranging from 0.3 to 0.5. The values of the recoveries obtained reflected high precision of the studied electrodes as sensors for their respective drug.

Table 2
Determination of Tol in pure solutions, pharmaceutical preparations, urine and plasma sample in batch and FIA conditions.

Taken	Tol-PTA				Tol-STA			
(mg)	Batch		FIA		Batch		FIA	
	Recovery (%)	RSD	Recovery (%)	RSD	Recovery (%)	RSD	Recovery (%)	RSD
Pure solutions								
1.189	99.7	0.5	–	–	97.4	0.30	–	–
2.378	100.4	1.0	–	–	99.5	0.54	–	–
11.890	99.9	0.6	–	–	98.6	0.95	–	–
23.780	100.5	0.7	–	–	97.6	0.54	–	–
Detrusitol capsule (4 mg/capsule)								
1.189	97.4	0.5	101.2	0.4	98.2	0.47	99.2	0.5
2.378	97.0	0.2	100.3	0.5	99.5	0.89	100.9	0.4
11.890	97.7	0.8	100.2	0.5	100.3	0.77	98.7	0.5
23.780	99.5	0.7	99.8	0.3	102.5	0.73	100.7	0.3
Urine								
1.189	98.2	0.7	100.7	0.5	97.8	0.29	101.4	0.4
2.378	100.5	0.6	102.8	0.5	97.0	0.54	100.8	0.4
11.890	99.9	0.3	102.8	0.5	97.5	0.63	101.9	0.5
23.780	100.4	0.8	101.9	0.4	97.1	0.43	102.0	0.3
Plasma								
1.189	97.4	0.5	98.7	0.3	100.5	0.69	98.3	0.5
2.378	99.5	0.5	99.0	0.4	98.3	0.38	99.1	0.5
11.890	98.5	0.9	99.5	0.6	100.0	0.48	98.6	0.4
23.780	97.7	0.4	100.5	0.4	98.1	0.50	100.2	0.3

RSD% is calculated for five determinations.

Table 3
Statistical treatment of data obtained for the determination of Tolterodine using Tol-PTA and Tol-STA electrodes, in comparison with the referenced method [5,7].

Item	Tol-PTA		Tol-STA		Referenced method [5,34]
	Batch	FIA	Batch	FIA	
Pure solutions					
Mean ± SD	100.1 ± 0.3	–	98.7 ± 1.3	–	94.2 ± 1.7
F-test	0.03 (5.41)	–	0.55 (5.41)	–	
Student *t*-test	8.4 (10.21)	–	5.02 (5.84)	–	
Probability	>0.001		>0.005		
Detrusitol (4 mg/cap)					
Mean ± SD	98.2 ± 1.2	100.2 ± 0.5	99.8 ± 1.8	99.9 ± 0.9	94.2 ± 1.7
F-test	0.50 (5.41)	0.1 (5.19)	0.90 (5.41)	0.31 (5.19)	
Student *t*-test	4.48 (4.54)	8.28 (8.6)	4.92 (5.84)	7.09 (7.17)	
Probability	>0.01	>0.0005	>0.005	>0.001	
Urine					
Mean ± SD	99.6 ± 0.9	101.7 ± 1.2	97.0 ± 0.8	101.4 ± 0.6	98.0 ± 3.0
F-test	0.1 (3.34)	0.17 (3.11)	0.06 (3.34)	0.03 (3.11)	
Student *t*-test	1.82 (2.35)	3.85 (4.60)	1.13 (1.25)	4.22 (4.60)	
Probability	>0.05	>0.005	>0.15	>0.005	
Plasma					
Mean ± SD	98.3 ± 0.8	99.3 ± 0.7	99.1 ± 1.1	99.1 ± 0.7	100.0 ± 6.9
F-test	0.01 (3.34)	0.01 (3.11)	0.02 (3.34)	0.01 (3.11)	
Student *t*-test	0.49 (0.76)	0.22 (0.74)	0.47 (0.76)	0.49 (0.74)	
Probability	>0.25	>0.25	>0.25	>0.25	

Figures between parentheses are the corresponding tabulated values.

3.6. Dissolution test

Drug absorption from a solid dosage form after oral administration is a very important factor to study in drug administration and quality control and it depends on the release of the drug substance from the drug product, the dissolution or the drug under physiological conditions, and the permeability across the gastro-intestinal tract. In vitro dissolution testing is usually relevant to predict the in vivo performance.

In vitro dissolution testing can be used to: assess the batch-to-batch consistency and to signal potential problems with in vivo bioavailability of a drug product; and ensure continuing product quality and performance after certain changes, such as changes in the formulation during the manufacturing process, the site of manufacture, and the scale-up of the manufacturing process and finally, help in suggesting new formulations that might have faster or slower release according to the use of the pharmaceutical compound.

The proposed electrodes were applied to following the dissolution profile of Tol in the sustained release Detrusitol® capsule. It was found that the released amount of Tol increase with time till a plateau is reached after 12 h, Tol-PTA detects the release of 96.4% of the drug while Tol-STA detects 97.3% release; these results meet the specifications of the Pharmaceutical Company (Pfizer) which states that not less than 90% of the labeled amount of Tol is dissolved after 12 h.

In this work, the ISE and the Calomel reference electrode were dipped inside the vessel during 18 h to measure the potential continuously without any need to take a sample from the dissolution medium at each time interval and measuring it Spectrophotometrically (UV) or using HPLC and without the need for any complicated logarithmic volume correction procedure followed commonly in these two techniques. ISE is not affected by turbidity due to excipients, there was no need to centrifuge the sample before measuring it and this is another advantage of the ion-selective electrode procedure. Thus, the suggested method in this study offers a dissolution profile in agreement with the spectrophotometric method but with minimizing error, and consuming less time and effort. Dissolution profiles and the used calibration curve for Tol in Detrusitol® capsules on using Tol-STA electrode are presented in Fig. 3.

3.7. Statistical analysis and validity of the proposed method

The reproducibility (interday precision) of the presented electrodes was tested by measuring a series of different concentrations (1.0×10^{-5}–1.0×10^{-2}) of Tol in using the presented electrodes

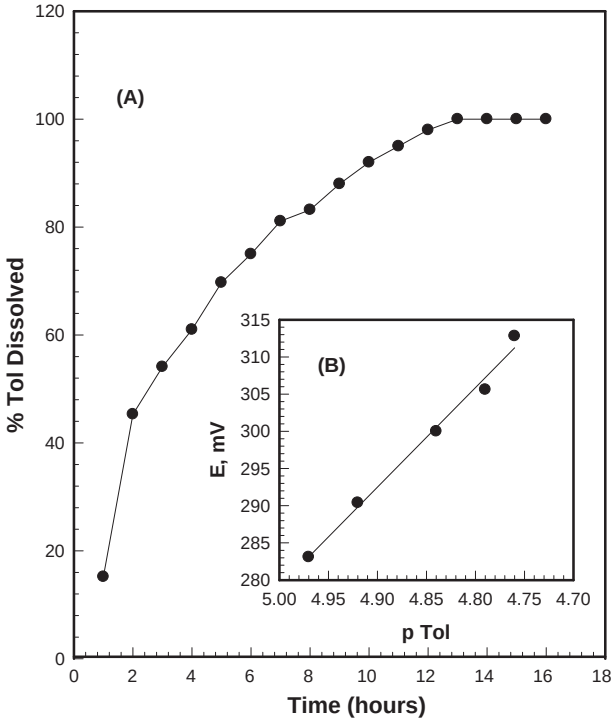

Fig. 3. Dissolution profile of Tol in Detrusitol® capsule on using Tol-STA electrode (A) and the calibration curve used in dissolution testing to convert the resulting potentials into concentrations (M) of drug and consequently into % dissolved.

for 3 consecutive days, and these results were subjected to linear regression analysis, using sigma plot 10.0, in order to establish whether the investigated electrodes exhibit any fixed bias. The slopes and intercepts of the regression lines did not differ significantly from the ideal values, revealing the absence of a systematic error during the measurements within the investigated concentration range.

The repeatability (intraday) precision was tested by measuring a solution of concentration 1.0×10^{-2} M three times in the same day using the same electrode, relative standard deviation of less than 1.00% for both Tol-PTA and Tol-STA electrodes were obtained [32,33].

For potentiometric detection using ion-selective electrodes, the limit of detection (LOD), defined as the Tol concentration corresponding to the intersection of the extrapolation of the linear part of the calibration curve; was found to be 20.5 μg and 25.7 μg for Tol-PTA and Tol-STA electrodes, respectively, while the limit of quantification (LOQ), defined as the last point corresponding to the intersection of the linear part of the calibration curve, was found to be 32.5 μg for both electrode. While under FIA conditions, the LOD values were found to be 25.7 μg and 23.0 μg for Tol-PTA and Tol-STA electrodes, respectively and the LOQ values were found to be 62.7 μg for Tol-PTA and Tol-STA electrodes, respectively. The difference in the limits of detection and quantification between batch and FIA measurements, can be attributed to the decrease in the working concentration range of the studied electrodes in FIA conditions in comparison to batch.

F- and *t*-test [35] were used in comparing standard deviations values obtained on using the proposed electrodes versus a referenced method previously presented in literature. The referenced method used in case of pure capsules is based on liquid chromatography–electrospray tandem mass spectrometry [2]. The assay was based on liquid–liquid extraction of the compounds from plasma with tert-butylmethylether and hydrophilic interaction chromatography performed on a silica column.

Another reference method was used in case of biological samples based on extraction of the analytes was performed with liquid/liquid or solid-phase extraction prior to derivatization with a silyl reagent [7]. The accuracy (inter- and intra-day) for Tolterodine and its 5-hydroxymethyl metabolite was within 87–110% and precision was better than 90%. Comparing the obtained *F*-and *t*-values with the tabulated ones as given in Table 3, it is clear that the obtained values were fairly lower than the theoretical tabulated values, i.e., the methods applied do not exhibit significant differences in comparison to those of the referenced method which reflects the accuracy and precision of the present method.

Table 4
Comparison between the performance characteristic of Tol-PTA and Tol-STA electrodes.

Factor	Tol-PTA	Tol-STA
Ion-exchanger	7.00%	5.00%
DOP	46.50%	47.50%
PVC	46.50%	47.50%
Usable conc. range (M)		
Batch	1.0×10^{-5}–1.0×10^{-2}	1.0×10^{-5}–1.0×10^{-2}
FIA	5.0×10^{-5}–1.0×10^{-2}	5.0×10^{-5}–1.0×10^{-2}
Life span		
Wet	30 days	30 days
Dry	More than 60 days	More than 60 days
pH range		
Batch	2.00–6.00	2.00–6.50
FIA	2.00–5.50	2.00–6.00
LOD		
Batch	6.30×10^{-6} M	7.90×10^{-6} M
FIA	7.90×10^{-5} M	7.07×10^{-5} M
LOQ		
Batch	1.00×10^{-5} M	1.00×10^{-5} M
FIA	5.00×10^{-5} M	5.00×10^{-5} M
Standard additions methods (batch)		
Detrusitol® Cap	97.4–99.5%	98.23–102.55%
Urine	98.2–100.5%	97.01–98.81%
Plasma	97.4–99.5%	98.07–100.50%
Applications (FIA)		
Detrusitol® Cap	99.8–101.2	99.2–100.9%
Urine	100.0–102.8	100.8–102.0%
Plasma	98.7–100.5	98.3–100.2%
Dissolution		
% Released	97.40%	98.4%

4. Conclusion

Comparison of the results of the two presented electrodes, given in Table 4, indicates that the electrodes proposed in this study are of comparable performance characteristics and both have a wide range of selectivity with respect to a large number of inorganic cations, and neutral molecules. The electrodes exhibit long life span (more than 60 days) in dry conditions, and have a stable usable pH range and were successfully applied in the assay of Tol in its dosage form and in complex biological matrixes under both batch and FIA conditions. Thus, they can be used for routine analysis and verification in quality assurance during manufacture of tolterodine and related pharmaceuticals, as well as for constructing its dissolution profile.

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