Research Paper

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"Compact Cell" for the Selective Determination of Atropine

Chemistry

KEYWORDS : Atropine determination, PVC membrane, compact cell

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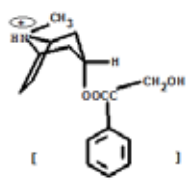
ABSTRACT

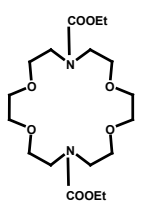
Compact cell for atropine ATR-CC was introduced for the first time. The cell was based on the combination of reference electrode and the membrane selective electrode in one set. Here, the atropine-selective electrode depends on using PVC-membrane containaing N,Ń-bis-ethoxycarbonyl-1,10-diaza-4,7,13,16-tetraoxacyclo-octadecane (DZCE) as an ionophoric matter. Potassium tetrakis-[3,5-bis-(triflоromethyl)-phenyl] borate (K-TFPB) was added to study its effect on the cell behavior. Very good sensitivity was found for determining atropine down to 1.01x10-7 M. The slope of the calibration graph was typically Nernstian 55.3-61.6 mV/ decade. The optimum pH was 8 when DZCE was used. When (K-TFPB) was added, the pH range was a wide range 3.15-8.5. Most common cations, drugs and amines were tested for their effect on the cell selectivity. Real atropine samples were analysed using the proposed ATR-CC.

Introduction

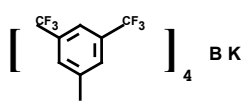
Atropine sulfate $[C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ (694.83)] is benzeneacetic, α-(hydroxymethyl)-, 8-methyl-8-azabicyclo-[3.2.1]oct-3-yl ester, endo-(±)-sulfate (2:1) (salt) monohydrate[1]. It is a tertiary amine, which can be obtained from plants of the solanaceous family, or prepared by synthesis [2]. It is an antimuscarinic alkaloid with both central and peripheral anticholinergic effects. Atropine is employed topically as a mydriatic and cyclopleggic in ophthalmology.



Atropinium ion (ATH^+)



N, N-bis-ethoxycarbonyl-1,10-diaza-4,7,13,16-tetraoxacyclo-octadecane (DZCE)



Potassium tetra-*kis*-[3,5-*bis*(trifloromethyl)-phenyl] borate (KTFPB)

Figure 1: Structural formulas of atropine (ATR), N, N-bis-ethoxycarbonyl-1,10-diaza-

4,7,13,16-tetraoxacyclo-octadecane(DZCE), and potassium tetra-kis-[3,5-bis-(trifloro-methyl)-phenyl] borate (K-TFPB).

Several ion-selective electrodes sensitive to atropine ion were described in the literature. An electrochemiluminescence electrospun carbon nanofiber based sensor for atropine was constructed by Yang et al [3]. It was based on tri(2,2-bipyridyl)Ru(II) carbon nanofibers/Nafion fibre. The linear range was 10^{-4}-10^{-7}M. The recovery range was 81-88%. In another work, an electrochemical sensor for isocarbophos based on a glassy carbon electrode modified with electropolymerized molecularly imprinted terpolymer was applied by Yan et al [4]. It showed a linear concentration range between $5x10^{-5}$ -$7.5x10^{-8}$M, and recovery range 95.5-110%. Some sensors were based on the tetraphenylborate (TPB) salts [5-9]. Some other electrodes were based on different atropine-ionophores, like calix[6]arene [10], valinomycin [11], or charged ionophores, like *o*-phenylenediamine copolymer [12], atropine-picrolinate and atropinium 5-nitrobarbiturate liquid membranes [13], or atropine-reineckate [14].

All of the aforementioned electrodes were applied for atropine determination. However, most of the previous work had the ability to analyse atropine in the microscale range. Here, selective electrodes for atropine were prepared by immobilizing diaza-18-crown-6 ether derivative (DZCE) as a new ionophore for atropine was applied. In addition, the most lipophilic borate salts, TFPB was added into the PVC-membrane. Superior to other 18-crown-6 ethers derivatives, DZCE showed a response to atropine at submicroscale concentration levels. Accordingly, it is of importance to explain the properties of this electrode. The drug-sensor allows assessments of pharmaceutical formulations down to 10^{-7} M concentration without the use of large volumes of solvents, e.g. trimethylsilyl derivatives [15], or toxic mercury-compounds in AAS [16], and is not highly expensive such as LC-MS [17].

Compact Cell "CC" is a cell that comprises both the reference and the ISE in one set. This type of cells facilitates the determination procedures. The first known compact cell was introduced by Zareh [18] for ascorbic acid. To my knowledge, none tried to introduce such type of cells for determination of other substances. Nowadays, the ISE is widely constructed and applied for the common cations, anions, and even complex organic ions. Actually, it is needed to expand the benefits of using such cells. In this work, a compact cell (CC) for atropine was introduced for the first time. The cell based on the combination of atropine selective electrode with a reference electrode. The present atropine electrodes were constructed depending on the use of a synthesized *N,Ń-bis*-ethoxycarbonyl-1,10-diaza-4,7,13,16-tetraoxacyclo-octadecane (diaza-18-crown-6) (DZCE), figure1. The application of this cell facilitates the measurement and analysis of atropine.

Materials and Methods

Reagents and materials:

Materials used for the preparation of electrodes were tetrahydrofuran (THF) (Merck) (after its distillation), dodecyl phthalate (DDP) (Fluka), potassium tetra-*kis*-[3,5-*bis*-(triflоromethyl) phenyl]borate (K-TFPB) (Fluka), high molecular weight poly(vinylchloride) (PVC) (Fluka) were used for the preparation of all membranes. The host molecule (DZCE) *N,Ń-bis*-ethoxycarbonyl-1,10-diaza-4,7,13,16-tetraoxacyclo-octadecane (diaza-18-crown-6) was kindly provided by E. Malinowska (Warsaw Technical University, Poland), which was synthesized according to the procedure described previously [19]. Atropine

sulfate (Sigma), ephedrine HCl (Sigma), caffeine (Sigma), pilocarpine hydrochloride (Sigma); glycine, arginin, and sodium glutamate (Aldrich) were used. Nitrate salts of inorganic cations (Na^+, K^+, Li^+, NH_4^+, Ca^{++}, Mg^{++}, and Ba^{++}) were purchased from (Fluka). Injection ampoules of atropine sulfate (1 mg/ml) products of (Hospira, USA) and 0.6 mg/ml (Hameln, UK) were used. Atropine ophthalmic solution 1% (Bausch &Lomb Incop., USA) was used. De-ionized water was operated through the whole work for the preparation of different solutions and for rinsing the electrodes.

Instruments:

The cell-EMF values were measured using a bench top model pH-meter (Jenway, UK), The instrument was loaded to a computer system through RS-232 connection. The same instrument was applied for pH-measurements. Spectrophotometric measurements were carried by flow-injection spectrophotometer (UV1800-Shimadzu, Japan).

Membrane preparation and potentiometric measurements:

2 mg DZCE or (1mg KTFPB + 2mg DZCE) were applied as sensor materials for membranes Ia-Ic and IIc (table 1), respectively. The mentioned ionophores were mixed with 62-67mg either NPOE, DOS, or DDP plasticizers and 30-31.5mg PVC. Table1, shows the prepared membrane compositions. The membrane was prepared according to procedures described before [10]. The membrane discs were mounted onto CC-ATR for electromotive force measurements.

Table 1: Composition of membranes Ia, Ib Ic and IIc used for preparing compact-cells for atropine.

Name	PVC, mg	DZCE, mg	TFPB, mg	NPOE, mg	DOS, mg	DDP, mg	Slope, mV/ Decade	R^2
Ia-membrane	31	2	0	66	0	0	55.9	0.9986
Ib-membrane	60	2	0	0	65	0	55.9	0.9986
Ic-membrane	30	2	0	0	0	62	61.6	0.9988
IIc-membrane	31.5	2	1	0	0	67	55.3	0.9991

Cell preparation and potential measurements:

2mg of DZCE (for type-I), DZCE+ TFPB (for type-II) were the sensor materials for all the used membrane types Ia-Ic, and IIc. The mentioned ionophores were mixed with 60-67mg NPOE (Ia,), DOS (Ib), or DDP (Ic and IIc) plasticizer and 30-31.5 mg PVC to prepare their corresponding membranes. The mixture was dissolved in THF, poured in a glass ring 24 mm i.d. placed on a glass plate. The mixture was left overnight for evaporation, and then the resulting membrane was cut into discs of 7 mm i.d. The obtained discs were fixed to the end of ISE-compartment of a CC made from Teflon. The electrode-compartment was filled with an aqueous inner filling solution (0.01 M ATR and KCl).

The measurement was carried using a compact-cell, which was assembled using Teflon rod (length = 10 cm, diameter = 12 mm). It is composed of two separate compartments. The first compartment contains ascorbic ISE, while the second compartment contains the reference Ag/AgCl electrode. Figure 2 shows a schematic representation of atropine compact-cell (CC-ATR).

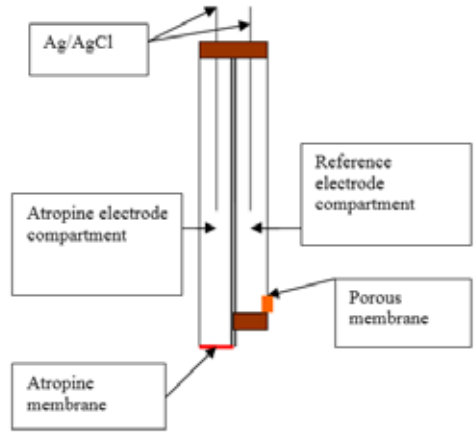


Figure 2: Schematic representation of compact cell for atropine CC-ATR.

The potential was measured at room temperature by immersing the proposed ATR-CC into 50 ml water. Different aliquots of 10^{-2} and 10^{-1} M of atropine were added to cover a concentration range of 10^{-7}-10^{-2} M atropine. The obtained data were recorded and graphically represented. The electrochemical cell for the potential measurement can be represented as:

Ag-AgCl/Li-acetate/Sample// Ion-selective membrane// Inner filling solution/ Ag-AgCl

where 0.1M lithium acetate solution was the filling of the reference electrode compartment.

The potential values were recorded and plotted versus p[ATR]. For studying the pH-effect on the emf of CC-ATR, NaOH or HCl (0.1M) were used for the pH-adjustments. The emf values for CC-ATR were recorded at different pH for 10^{-4}, 10^{-3}, and 10^{-2} M ATR solutions.

The selectivity coefficient values for several cations (Na^+, K^+, Li^+, NH_4^+, Ca^{++}, Mg^{++}, and Ba^{++}), aminoacids (glycine, arginine, and sodium glutamate) and pharmaceutical amines (ephedrine HCl, caffeine, and pilocarpine hydrochloride) were calculated (table 2) by the use of the separate solution method (SSM) [20]. The emf of the interference solution (0.01 M) and that for the same concentration of ATR solution were measured. Then, the selectivity coefficient values of CC-ATR ($K_{i,j}^{pot}$) were estimated for the different interferents according to the equation:

$$\log K^{pot}_{ATR^+, J^{z+}} = (E_j - E_{ATR^+}) / S + [1-(Z_{ATR^+}/Z_j)] \log a_{ATR^+}$$

Where: E represents the emf readings for the primary ion ATR^+ and the interfering ion (J^{z+}); and (S) is the observed slope for the primary ion.

Injection ampoules of atropine sulfate (1 and 0.6 mg/ml) (products of Hospira, USA and Hameln. UK) were diluted to 50 ml solutions. The obtained solutions were transferred to the potentiometric cell. ATR-CC was immersed into the solutions and the cell EMF was measured. The potential readings of the sample solutions were compared to previously prepared calibration graph under the same condition.

Results and discussion

Two ATR-CC with different membrane-compositions were prepared. One has a membrane containing DZCE (type-I), and the other constitutes (KTFPB+DZCE) (type-II). Both electrodes exhibit typical Nernstain slope (55.8- 61.6 and 55.3 mV/decade). They work linearly down to 1.9×10^{-6}, and 10^{-7} M ATR. The calibration graphs representing both ATR-CC types are displayed in fig-

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ure 2. The lower linear limit of the calibration graph for ATR-CC type- Ic is the best among all the studied ATR-CC compositions. This is because of the highest lipophobicity of DDP, which helps the exchange of ATR^+.

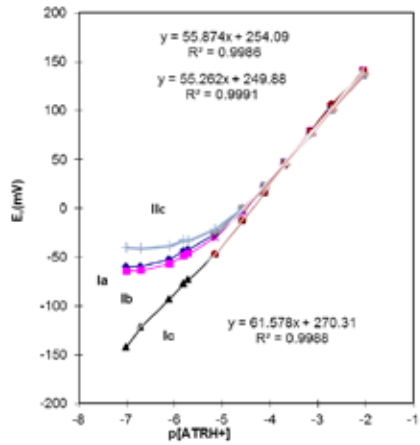

Figure 3: Calibration graphs ATRH-CC based on different membrane compositions: Ia) DZCE/NPOE, Ib) DZCE/DOS and Ic) DZCE/DDP, and IIc)DZCE+TFPB/DDP.

The mechanistic equation that represents the exchange reaction at the membrane-solution interface for ATR-CC type-I (contains only DZCE) is written as below:

$$(ART)_2^{++}SO_4^{--} + [DZCE] \text{ ===== } [(DZCE\text{- } ATR)_2^{++}][SO_4^{--}]$$

$$K_{eq} = [(DZCE\text{- } ATR)_2^{++}][SO_4^{--}] / [(ART)_2^{++}SO_4^{--}][DZCE]$$

In case of ATR-CC type-II (contains DZCE/K-TFPB), the following equilibrium is expected:

$$[TFPB]^- K^+ + DZCE \text{ ===== } [(DZCE\text{-}K)^+ TFPB^-]$$

$$2[(DZCE\text{-}K)^+ TFPB^-] + (ART)_2^{++}SO_4^{--} \text{ ===== } 2[(DZCE\text{- } ATR)^+ TFPB^-] + K_2SO_4$$

$$K_{eq} = [(DZCE\text{-}ATR)^+ TFPB^-]^2 [K_2SO_4] / [(DZCE\text{-}K)^+ TFPB^-]^2 [(ART)_2^{++}SO_4^{--}]$$

The dynamic response of both electrodes showed instantaneous and stable potential readings. Figure 4, displays the obtained results. From the cited graphs it can be detected that the response time of the studied ATR-CC was < 5 seconds. This value reflects the fast response of each of the studied cell.

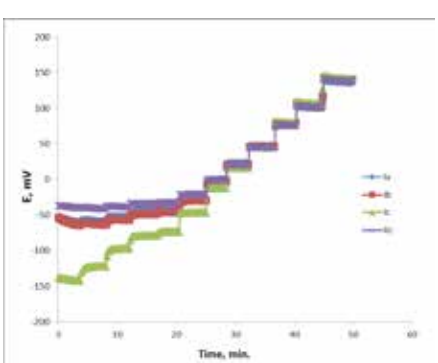

Figure 4: Dynamic response of ATR-CC when different membrane types Ia, Ib, Ic and IIc.

The mV-pH curves for both ATR-CC (types I and II) are shown in figure 4. The working pH-range is 3.5-8.3 for both cells when 10^{-3} M ATR^+ solution was measured. Whenever 10^{-3} M ATR^+ solution was measured, the plateau potential values were between 2.9 and 9.1. This is a wide pH-range compared to the previous electrodes, which are not working in alkaline medium (2.5-6 and 3.5-6). Figure 5, shows the mV-pH curves for the ATR-CC I and II-types at 10^{-3} M concentrations. The formation of the free base is the reason of the break in the basic part of the plateau. In acidic medium, the hydrogen ion interference is the reason of the curve break. This depends on the concentration of ATR^+. It showed lower pH break-values at higher ATR^+-concentrations.

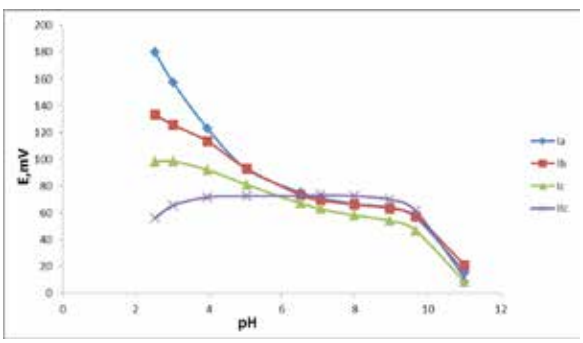

Figure 5: pH-effect on emf of ATR-CC with different membrane types (Ia, Ib, Ic and IIc) when measurements were applied for 10-3 M ATR+ solution.

Selectivity:

The selectivity coefficient values ($K^{pot}_{ATR^+,j^{z+}}$) for ATR-CC towards selected common inorganic cations, amino acids, and pharmaceutical amines were calculated (table 2). It is shown that the values of the selectivity coefficients for the inorganic cations range between 10^{-2} and 10^{-3} for Ia-Ic type ATR-CC cell, while it was 10^{-3}-10^{-4} for IIc. This is due to the presence of DDP enhances the selectivity interaction.

In case of the tested aminoacids, (glycine, glutamate, arginine) the $K^{pot}_{ATR^+,j^{z+}}$–values were of the order of 10^{-3} for Ia-Ic cells, which was similar to the recorded values for IIc-cell.

For pharmaceutical amines (ephedrine, and pilocarpine), both of the ATR-CC types I and II showed similar values of $K^{pot}_{ATR^+,j^{z+}}$. They were of the order 10^{-1}. It can be recommended that ephedrine showed the highest value when measurements were carried by IIc-type. The $K^{pot}_{ATR^+,j^{z+}}$ in case of caffeine was 10^{-3} for Ia-Ic and 10^{-5} for IIc cell type. Here, the improvement of the selectivity values for ATR-CC in case of caffeine can be explained due to the absence of terminal amine group compared to the other tested drug amines.

Table 2: selectivity coefficient values ($K^{pot}_{ATR^+,j^{z+}}$) for ATR-CC comprising different membranes Ia-Ic, and IIc.

Interferent	Selectivity coefficient, ($K^{pot}_{ATR^+,j^{z+}}$)			
	Ia	Ib	Ic	IIc
K^+	$5.9x\ 10^{-2}$	$5.2x\ 10^{-2}$	$4.3x\ 10^{-2}$	$2.3x\ 10^{-3}$
Li^+	$3.2x\ 10^{-2}$	$4.0x\ 10^{-2}$	$4.0x\ 10^{-3}$	$1.3x\ 10^{-4}$
Na^+	$2.2x\ 10^{-2}$	$2.7x\ 10^{-2}$	$8.2x\ 10^{-3}$	$4.3x\ 10^{-4}$
Mg^{++}	$4.6x\ 10^{-3}$	$6.4x\ 10^{-3}$	$9.6x\ 10^{-4}$	$3.6x\ 10^{-5}$
Ca^{++}	$1.6x\ 10^{-3}$	$2.3x\ 10^{-2}$	$5.0x\ 10^{-3}$	$8.01x\ 10^{-5}$
Ba^{++}	$2.3x\ 10^{-3}$	$2.6x\ 10^{-2}$	$1.1x\ 10^{-2}$	$1.6x\ 10^{-4}$
Glycine	$2.9x\ 10^{-3}$	$2.9x\ 10^{-3}$	$2.5x\ 10^{-3}$	$4.0x\ 10^{-3}$
Sodium glutamate	$1.9x\ 10^{-3}$	$4.1x\ 10^{-3}$	$2.5x\ 10^{-3}$	$9.0x\ 10^{-4}$

Argnin	1.6x 10^{-4}	4.3x 10^{-4}	3.8x 10^{-4}	4.25x10^{-5}
Amonium	9.3x 10^{-3}	1.4x 10^{-2}	1.1x 10^{-2}	1.7x 10^{-3}
Ephedrine	8.0x 10^{-2}	6.1x 10^{-1}	4.3x 10^{-1}	1.13
Caffiene	4.7x 10^{-3}	6.0x 10^{-3}	5.0x 10^{-3}	3.19x10^{-5}
Pilocarpine	3.2x 10^{-1}	2.7x 10^{-1}	2.6x 10^{-1}	3.6x 10^{-1}

3.4.Analysis of atropine samples by using ATR-CC:
By using ATR-CC the atropine assessment becomes easier without sample pretreatment. Since ATR-CC based on IIc-membrane exhibited the best electrode performance, so it was applied for an actual analysis of atropine samples. Atropine sulfate injection (0.6-1 mg/ml), atropine eye drops (10 mg/ml) were assayed using the mentioned ATR-CC. The procedure was applied using both the direct potentiometric and the known addition techniques. The obtained results (table 3), showed that the found percentage values were 90.5-99.8% and the relative standard deviation values were 1.7-2.1 (4-determinations).

Table (3): Determination of atropine in its samples using ATR-CC based on DZCE:

Atropine sample	Taken amount	US-Pharm. method		Direct potentiometry		**Known addtion method**	
		Percentage found, %	RSD	Percentage found, %	RSD*	Percentage found, %	RSD*
Atropine sulfate (injection), Hameln	0.6 mg/ml	97.9	2.2	90.5	1.8	97.2	2.1
Atropine ophthalmic soln (eye drops), Bausch&Lomb	10 mg/ml	98.3	2.1	97.8	1.9	99.8	1.8
Atropine sulfate (injection), Hospira	3 mg	98.0	1.9	95.2	1.4	96.3	1.7

* Results for n=4-determinations.

5.Conclusions:
It can be concluded that atropine can be determined by using new generation of sensors based on using Compact Cell (CC).

The cell is applied successfully for analyzing atropine in range of (10^{-2} - 10^{-7} M).

This type of cells can be elaborated for use to determine other drugs.

The benefits of using such cell can be expanded especially for built-in devices.

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