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Improving the detection limits of antispasmodic drugs electrodes by using modified membrane sensors with inner solid contact

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Abstract

Three coated wire electrodes (CWEs) for the antispasmodic drugs; dicyclomine (Dc), mebeverine (Mv) and drotaverine (Dv) hydrochlorides were developed. Each electrode based on ion-associate of a heteropoly anion with the drug cation incorporated in membrane sensor modified with graphite and deposited on silver internal solid contact. The influence of addition of graphite to the membranes and the type of the internal solid contact on the potentiometric responses of the electrodes was investigated. The characteristics of the new electrodes were compared to the characteristics of previously reported traditional liquid inner contact electrodes of the same drugs. The lower detection limits of the proposed electrodes were somewhat better than those observed with the corresponding liquid contact ISEs and reached (1.2–2.0) $\times 10^{-7}$ M. The potentiometric selectivity of the CWEs revealed a significant improvement and much faster response times compared to the liquid contact ISEs. The practical utility of each electrode has been demonstrated by using it successfully in potentiometric determination of its respective drug in pharmaceutical preparations both in batch and flow injection conditions. Each electrode was also used as an indicator electrode in the potentiometric titration of the drug against standard silicotungstic acid and in potentiometric determination of the drug concentration in urine samples.

Keywords: Coated wire electrodes; Dicyclomine; Mebeverine; Drotaverine; Lowering of detection limits

1. Introduction

Previous work from our laboratory described the development of a group of conventional PVC membrane electrodes for the antispasmodic drugs dicyclomine [1], mebeverine [2] and drotaverine hydrochlorides [3]. The detection limits of these electrodes are in the range of 2×10^{-6}–8×10^{-6} M. These moderately high values of detection limits prohibit the use of these electrodes in determination of the drugs in real (unspiked) serum and urine samples. The detection limits of this type of electrodes are adversely affected by zero-current transmembrane fluxes of the measured (primary) ions [4]. Already very small changes of <1% in the total ionic concentration of the ISE membrane are sufficient to induce leaking of primary ions into the sample and thus cause the concentration in the contacting aqueous layer to be ca. 10^{-6} M, even if the bulk sample virtually did not contain primary ions. Consequently, the lower detection limit was found to be around 10^{-6} M [4]. It was, therefore, felt worthwhile to develop better sensors for these drugs using the facilities of coated wire electrodes (CWEs). Electrodes of this sort are simple in fabrication, inexpensive, durable, flexible, i.e. the electrode can be used at any angle, and easy to miniaturize. Also, more extensive usable concentration ranges and lower detection limits are obtained for a wide variety of both organic and inorganic cations and anions with the use of coated wire electrodes which contained no internal solutions to leak away into the test solution. However, irreproducible results and drift of the electrode potential often obtained with this type of electrodes. These effects are attributed to poorly defined charge transfer at the interface between the ionically conducting selective membrane and the electronically conducting substrate [5].

A significant improvement of CWEs was obtained when transducer layer with electronic conductivity was applied between the substrate and the ion-selective membrane, to yield so-called all-solid-state ion-selective electrode [6]. In this type of electrodes, electrochemically synthesized conducting polymer is electrodeposited directly onto an electronic conducting

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substrate or a conducting polymer is included in the cocktail of ion-selective membrane components and the mixture is cast directly on a solid conducting substrate [7–9].

In this work, we present a new type of solid potentiometric sensors, where no conducting polymers were used, but very fine pure graphite powder was added as electrically conducting additive to the membrane matrix. It was found that just a few mass percentage of graphite powder added to the plastic ion-selective membrane phase of Dc, Mv or Dv sensors resulted in a significant improvement of charge transfer between the substrate and the membrane phase. As a result, stability of the sensor potential and lowering of detection limits were observed with the present electrodes compared to the simple coated wire arrangements with no additive.

Dicyclomine (2-(diethylamino)ethylbicyclo-hexyl-1-carboxylate hydrochloride), mebeverine ((3,4-dimethoxybenzoic acid 4-[ethyl-(2-[4-methoxyphenyl]-1-methylethyl)-amino] butyl ester) and drotaverine (1-(3,4-diethoxybenzylidene)-6,7-diethoxy-1,2,3,4-tetrahydro-isoquinoline) are three of the most important antispasmodic drugs which are mainly used to reduce the effect of acetylcholine on smooth muscles. They have been used for treatment of irritable bowel syndrome, gastrointestinal diseases, biliary dyskinesia, nephrolithiasis, gynecologiicial diseases and vasomotor diseases associated with smooth muscle spasms [10]. The reported methods for determination of the antispasmodic drugs; dicyclomine [11,12], mebeverine [13,14] and drotaverine [15,16] are mainly chromatographic, which in spite of their high sensitivities, are very expensive, involve the use of complex procedures with several sample manipulations and requiring long analysis time.

The aim of this study was not only lowering the detection limits of antispasmodic drug electrodes but to propose a new type of miniature, solid, selective sensor for each drug and to investigate the optimized composition of the sensor and the experimental variables that contribute to the electrode response and led to the development of a simple, sensitive and reliable method for determination of each drug in different media in batch and in flow injection system.

2. Experimental

2.1. Materials and reagents

All chemicals used were of analytical reagent grade unless otherwise stated and doubly distilled water was used throughout. Dicyclomine hydrochloride (DcCl) and its dosage forms Spasmorest (tablets and ampoules) were obtained from Misr Co. for Pharm. Ind. S.A.E. (Cairo, Egypt). Mebeverine hydrochloride (MvCl) and its pharmaceutical preparation Colospasmin (tablets) from EIPICO (Cairo, Egypt). Drotaverine hydrochloride and its pharmaceutical preparation Do-Spa (tablets) were obtained from Alexandria Co. for Pharmaceuticals (Alexandria, Egypt). Silicotugstic acid (STA) and silicomolybdic acid (SMA) were purchased from Sigma. High molecular weight poly (vinyl chloride), tetrahydrofuran (THF), dibutyl phthalate (DBP) and graphite powder were obtained from Aldrich Chemical Company. Standard solutions of drug salts were prepared in double distilled water and standardized by recommended methods wherever necessary [17].

2.2. Preparation of the coated wire electrodes

Varying amounts of the ion-exchanger dicyclomine-silicotungstate (Dc-ST), mebeverine-silicotungstate (Mv-ST) or drotaverine-silicomolybdate (Dv-SM) and PVC were dissolved in about 5 ml of THF along with DBP as solvent mediator. About 0.1–0.6 wt.% graphite powder is then added with contentious mixing until a concentrated slurry was obtained. Metallic or graphite rod, about 1 mm diameter and 30 mm length was first polished on a cloth pad, then washed with water followed by acetone and sequentially air-dried. One end of the wire (about 20 mm length) was then coated by repeated dipping (about five times, a few minutes between dips) into the stirred membrane slurry. A membrane was formed on the wire rod surface, which was allowed to dry overnight.

2.3. Conditioning and storage

The prepared electrodes were conditioned for 1 h in 0.001 M drug solution. The slurries prepared for the membranes preparation were stored in an airtight chamber when not in use. A fresh electrode was prepared by adding THF into the slurry and repeating the above process.

2.4. Apparatus

The Potentiometric measurements in batch mode were carried out at $25 \pm 1\,^{\circ}C$ using a Jenway 3010 digital pH/mV meter with drug-coated wire electrode (CWE) in conjunction with Ag/AgCl reference electrode. A techne circulator thermostat Model C-100 (Cambridge, England) was used to control the temperature of the test solutions.

In flow measurements, a single-stream FIA system was used. It is composed of a four channels peristaltic pump (Ismatec, ISM 827) propelling a carrier solution of 0.033 M sodium sulphate through polypropylene tubing at a flow rates of 2.5–3.0 ml min^{-1}. An injection valve (Rheodyne, model 5020), fitted with an exchangeable sample loop was used for sample injection. The electrode (with homemade Teflon holder) was connected to a WTW micro-processor pH/ion-meter pMx 2000 (Weilheim, Germany) and interfaced to a strip chart recorder model BD111 from Kipp and Zonen (Deflt, Netherlands).

2.5. Selectivity of the sensors

Potentiometric selectivity factors of the antispasmodic drugs-CWEs were evaluated using the matched potential method (MPM) [18]. According to this method, the activity of each drug was increased from $a_A = 1 \times 10^{-6}$ M (reference solution) to $\grave{a}_A = 1 \times 10^{-3}$ M, and the changes in potential (ΔE) corresponding to this increase is measured. Next a solution of an interfering ion of concentration a_B in the range of (1×10^{-1}–1×10^{-2} M) is added to new 1×10^{-6} M reference solution until the same

potential change (ΔE) is recorded. The selectivity factor $k^{MPM}_{A,B}$ for each interferent was calculated using the following equation:

$$k^{MPM}_{A,B} = \frac{(\grave{a}_A - a_A)}{a_B} \quad (1)$$

2.6. Potentiometric determination

In batch measurements, the standard addition method was applied [19]. In this method the proposed electrode was immersed into a sample of 25 ml with unknown concentration ($\sim 10^{-7}$–10^{-4} M) drug solution and the equilibrium potential of E_u was recorded, then 0.1 ml of 1.0×10^{-3} M of standard drug solution was added into the testing solution and the equilibrium potential of E_s was obtained. From the potential change $\Delta E = (E_u - E_s)$ one can determine the concentration of the testing sample using the equation:

$$C_x = \frac{(C_s \times V_s)}{[(V_x + V_s)10^{\Delta E/S} - V_x]}$$

where C_x is the drug concentration of testing sample, C_s the concentration of the standard, V_x and V_s are the corresponding volumes, S the slope of the electrode response and ΔE is the change in potential.

For determination of the drugs in urine samples, 10 ml portion of urine was transferred to a 25 ml measuring flask, completed to the mark with water. The drug is then determined in this solution according to the described standard addition method.

In FIA, samples of different concentrations of drug samples were injected to the optimized FIA system. The 0.01 M Na_2SO_4 solution was propelled by the peristaltic pump at a flow rate of 2.5–3.0 ml min^{-1}. The drug-CWE was used as a working sensor against Ag/AgCl reference electrode. The average peak height of three signals for each sample was measured. A calibration graph (signal potential versus log [drug]) was obtained using 1.0×10^{-6}–1×10^{-3} M of standard drug solution.

3. Results and discussion

3.1. Preliminary studies

Ion-exchangers for use in ion-selective membrane sensors should have rapid exchange kinetics and adequate stability in the membrane. In addition, they should be well soluble in the membrane matrix and have a sufficient lipophilicity to prevent leaching from the membrane into the sample solution [20]. Leaching of ion-exchanger from the membrane not only influence the lower detection limits of ISEs but also bias the selectivity coefficients since it may be potential-determining, even if measurements are taken in pure solutions of a strongly discriminated ion [21].

In previous studies we described a group of ion-exchanger-based PVC membrane electrodes, with liquid inner contact, for the selective determination of dicyclomine [1], mebeverine [2] and drotaverine [3]. The ion-exchanger incorporated in each electrode was an ion-association of the drug cation with the heteropoly anion silicotungstate, silicomolybdate, phosphotungstate or phosphmolybdate. These heteropoly anions are promising candidates for the formation of highly lipophilic ion associates with many organic cations [22,23]. This is associated with the high molecular weights of these anions, which range from 1823 to 2880. In our previous works [1–3], the solubility product constants (K_{SP}) of these ion-associates were determined conductometrically [24] and the calculated solubility (S) values of these compounds revealed that the ion-associate dicyclomine-silicotungstate, mebeverine-silicotungstate and drotaverine-silicomolybdate have the lowest solubilities between the prepared ion-associates. Therefore, these compounds were selected in this study to be used as active recognition elements in the proposed CWEs.

The performance characteristics reported for a given ion-exchanger based PVC membrane electrode extremely depending on the membrane composition. Therefore, several membranes of varying nature and ratios of ion-exchanger/PVC/plasticizer/graphite were prepared for the systematic investigation of the optimum membrane composition for each of Dc-CWE, Mv-CWE and Dv-CWE electrodes. In the preliminarily experiments, silver wires were used as internal solid contacts of the studied electrodes. The results are summarized in Table 1. As expected, the amount of ion-exchanger is strongly affect the sensitivity of the electrode. The slope of the calibration graph increased with increasing ion-exchanger content until a values of 1.0, 1.0 and 0.5 wt.% were reached for Dc-CWE, Mv-CWE and Dv-CWE electrodes, respectively. However, further addition of ion-exchanger resulted in a diminished response slope of its corresponding electrode, most probably due to some inhomogenities and possible saturation of the membrane. DBP was used as plasticizer and solvent mediator in all membranes of the constructed CWEs, since it shows more appropriate conditions for extracting and incorporating the drug cations prior to their exchange with the soft ion-exchangers in the cases of similar liquid contact electrodes (LCE) incorporating the same ion-exchangers [1–3].

The important parameter of these new compositions was the modification of the membranes with graphite. As it is clear from results in Table 1, graphite can be an effective component for obtaining a much better sensitivity for these electrodes compared to the CWEs without graphite or to the previously reported traditional liquid contact electrodes for these drugs. Electrodes with no graphite (nos. 1–5, 9–13 and 17–20) exhibit near Nernstian slopes and limited ranges of concentration. The improvement of the response behavior and lowering the detection limits of the electrodes (nos. 7, 15 and 21) clearly demonstrate the important role of addition of graphite to the membranes of these electrodes. The best detection limits of 2.0×10^{-7}, 1.2×10^{-7} and 1.5×10^{-7} M were obtained for Dc, Mv and Dv-electrodes with membrane coats containing 0.5, 0.3 and 0.2 wt.% graphite, respectively.

A coated wire electrode with optimal detection limits must not contain an internal water film in which primary ions accumulate during conditioning because they would leach into the sample during measurements in dilute samples [25]. Additionally, a redox-active internal layer must be present in order to avoid interference from O_2. It seems that the presence of

Table 1
Optimization of membrane compositions

Electrode	Composition (wt.%)				Detection limit (M)	Slope (mV/d)	LCR[b] (M)
	IE[a]	PVC	DBP	Graphite			
Dc-electrodes	Dc-ST						
CWE							
1	0.2	49.0	50.8	–	4.0×10^{-6}	54.5	6.0×10^{-6}–1.0×10^{-3}
2	0.5	49.0	50.5	–	1.5×10^{-6}	56.1	5.0×10^{-6}–5.0×10^{-3}
3	1.0	49.0	50.0	–	7.1×10^{-7}	58.5	1.0×10^{-6}–1.0×10^{-2}
4	1.5	49.0	49.5	–	5.2×10^{-6}	55.7	8.0×10^{-6}–1.0×10^{-2}
5	2.0	49.0	49.0	–	8.4×10^{-6}	53.7	1.0×10^{-5}–1.0×10^{-2}
6	1.0	49.0	49.8	0.2	4.5×10^{-7}	57.3	8.0×10^{-7}–1.0×10^{-2}
7	1.0	49.0	49.5	0.5	2.0×10^{-7}	58.5	6.0×10^{-7}–1.0×10^{-2}
8	1.0	49.0	49.0	1.0	2.0×10^{-7}	57.8	6.0×10^{-7}–1.0×10^{-2}
LCE[c] [1]	1.5	49.0	49.5	–	3.2×10^{-6}	58.5	4.0×10^{-6}–1.0×10^{-2}
Mv-electrodes	Mv-ST						
CWE							
9	0.2	49.0	50.8	–	2.2×10^{-6}	50.7	6.0×10^{-6}–5.0×10^{-3}
10	0.5	49.0	50.5	–	8.0×10^{-7}	54.4	2.0×10^{-6}–5.0×10^{-3}
11	1.0	49.0	50.0	–	5.0×10^{-7}	57.5	1.0×10^{-6}–1.0×10^{-2}
12	1.5	49.0	49.5	–	1.5×10^{-6}	57.0	4.0×10^{-6}–1.0×10^{-2}
13	2.0	49.0	49.0	–	8.0×10^{-6}	52.5	1.0×10^{-5}–1.0×10^{-2}
14	1.0	49.0	49.9	0.1	2.0×10^{-7}	55.4	4.0×10^{-7}–1.0×10^{-2}
15	1.0	49.0	49.7	0.3	1.2×10^{-7}	57.8	5.0×10^{-7}–1.0×10^{-2}
16	1.0	49.0	49.4	0.6	4.0×10^{-7}	56.8	6.0×10^{-7}–1.0×10^{-2}
LCE [2]	1.0	49.0	50.0	–	3.1×10^{-6}	56.0	5.0×10^{-6}–1.0×10^{-2}
Dv-electrodes	Dv-SM						
CWE							
17	0.2	49.0	50.8	–	1.6×10^{-6}	55.8	5.0×10^{-6}–5.0×10^{-3}
18	0.5	49.0	50.5	–	4.3×10^{-7}	60.1	8.0×10^{-7}–1.0×10^{-2}
19	1.0	49.0	50.0	–	8.5×10^{-7}	57.9	1.5×10^{-6}–1.0×10^{-2}
20	1.5	49.0	49.5	–	1.0×10^{-6}	57.0	4.0×10^{-6}–1.0×10^{-2}
21	0.5	49.0	50.3	0.2	1.5×10^{-7}	58.5	6.0×10^{-7}–1.0×10^{-2}
22	0.5	49.0	50.1	0.4	3.5×10^{-7}	59.1	8.0×10^{-7}–1.0×10^{-2}
23	0.5	49.0	49.9	0.6	4.7×10^{-7}	59.2	9.5×10^{-7}–1.0×10^{-2}
LCE [3]	0.5	49.0	50.5	–	2.0×10^{-6}	60.0	5.1×10^{-6}–1.0×10^{-2}

[a] IE, ion-exchanger.
[b] LCR, linear concentration range.
[c] LCE, liquid contact electrode.

graphite in PVC membrane can help ion-to-electron transduction between the membrane and the internal solid-contact and decreases the diffusion property of this membrane, consequently avoiding the formation of aqueous film between the membrane and this solid-contact. Also, the reducing characteristics of carbon minimize the interference from oxygen.

To investigate the effect of the conductive bed (inner solid contact) nature on the efficiency of the CWEs, each optimized coating mixture of Dc, Mv and Dv-membranes was used in preparation of electrodes with different conductive beds, namely, silver, platinum, graphite and glassy carbon. After conditioning, the electrodes of each drug were examined in the concentration range of 1×10^{-7}–1×10^{-2} M of drug by determining their linear concentration ranges, slopes of the calibration graphs and detection limits. The results indicated that the detection limits and dynamic ranges of these CWEs are influenced by the nature of the conductive bed, and the detection limit decreases with decreasing the resistivity of the beds (Table 2). This is true as the electrode potential performance is attributed to the electron exchange mechanism at the coating–bed interface and to the ion-exchange process at the coating–solution interface [26,27]. Silver has the lowest resistivity between the studied elements ($1.62\ \mu\Omega\ cm^{-1}$), and as shown in Table 2, the CWEs based on this element have the lowest detection limits. Therefore, silver wires were used as the inner solid contacts of the electrodes in this study.

The reproducibility of each drug-CWE was evaluated by preparing a series of five electrodes with similar composition and the response of these electrodes to its respective drug was tested by constructing five calibration graphs. The results of the averages of slopes, detection limits, linear dynamic ranges, standard deviation of measurements and the other response characteristics of the three drugs electrodes were specified according to IUPAC recommendations [19] and given in Table 2.

The results clearly indicated that the limits of detection of the CWEs proposed for Dc, Mv and Dv are somewhat better than those observed with the corresponding liquid contact ISEs. Representative calibration graphs for Dc-CWE and Dc-liquid contact electrode shown in Fig. 1, indicate the improvement of detection limit of Dc-sensor with the use of the modified CWE.

Table 2
Response characteristics of optimized Dc, Mv and Dv-CWEs prepared by using different inner contacts

Electrodes (Inner contact)	R ($\mu\Omega\,cm^{-1}$)	Slope (mV/d)	Limit of detection (M)	Linear range (M)	t_{resp} (s)	r^2	Accuracy (%)
Dc-Electrodes							
Silver	1.62	58.5	2.0×10^{-7}	6.0×10^{-7}–1.0×10^{-2}	≤8	0.98	98.5
Platinum	10.70	54.3	6.9×10^{-7}	1.6×10^{-6}–1.0×10^{-2}	≤10	0.97	97.1
Graphit	1375	60.9	8.8×10^{-7}	2.0×10^{-6}–1.0×10^{-2}	≤15	0.96	97.4
Glassy carbon	4000	55.7	3.9×10^{-6}	4.2×10^{-6}–1.0×10^{-2}	≤15	0.98	98.6
Mv-Electrodes							
Silver	1.62	57.8	1.2×10^{-7}	5.0×10^{-7}–1.0×10^{-2}	≤10	0.99	98.4
Platinum	10.70	55.5	4.5×10^{-7}	7.9×10^{-7}–1.0×10^{-2}	≤12	0.96	99.6
Graphite	1375	61.7	1.2×10^{-6}	3.4×10^{-6}–1.0×10^{-2}	≤12	0.98	97.6
Glassy carbon	4000	59.3	1.3×10^{-6}	3.2×10^{-6}–1.0×10^{-2}	≤15	0.95	98.7
Dv-Electrodes							
Silver	1.62	58.5	1.5×10^{-7}	6.0×10^{-7}–1.0×10^{-2}	≤8	0.96	96.8
Platinum	10.70	56.2	6.4×10^{-7}	1.6×10^{-6}–1.0×10^{-2}	≤10	0.97	97.7
Graphite	1375	61.4	9.0×10^{-7}	2.4×10^{-6}–1.0×10^{-2}	≤14	0.97	97.1
Glassy carbon	4000	57.3	1.3×10^{-6}	2.0×10^{-6}–1.0×10^{-2}	≤15	0.98	98.6

R, resistivity of inner contact (Reproduced from C.R.C. Handbook of Chemistry and Physics, 58th ed., CRC Press, West Palm Beach, Florida, F–170,171); t_{resp}, response time; r^2, correlation coefficient.

The internal filling solution in liquid contact ISEs, however is a major cause of transmembrane fluxes of the primary ions which drastically affect the lower detection limits of these electrodes.

An important advantage of the new CWEs, based on membranes modified with graphite, is their lower response time relative to the previously developed liquid contact electrodes. The higher response time of the electrodes of the latter type (≤20 s) may be attributed to the higher noise originated from the higher resistance of the liquid-contact membranes, which was a consequence of their large thickness [25].

In the cases of all CWEs, the average response time required for an electrode to reach potential response within ±1 mV of final equilibrium values after successive immersion in a series of solutions each having a 10-fold difference in concentration was measured. The resulting potential-time plots for the CWEs obtained upon changing the Mv, and Dv concentration from 1.0×10^{-5} to 1.0×10^{-4} M and from 1.0×10^{-4} to 1.0×10^{-5} M are shown in Fig. 2. The graphs clearly indicated that the potentiometric response of the electrodes is reversible,

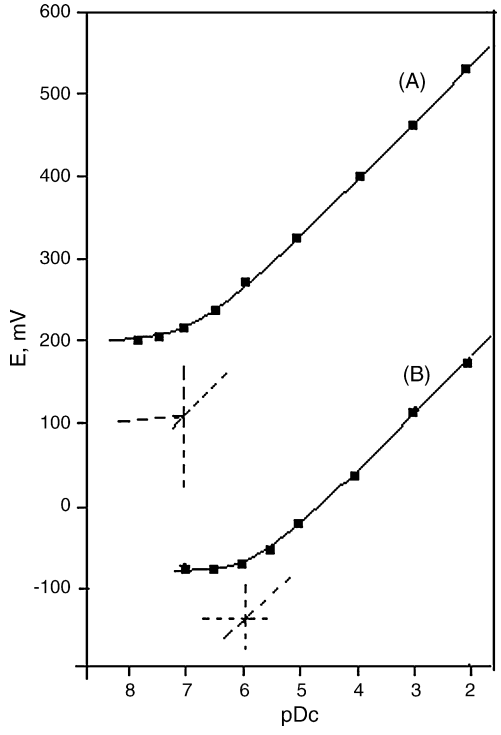

Fig. 1. Improving of the lower detection limit of Dc-selective electrode. Calibration graphs of Dc-CWE (A) and Dc-liquid contact electrode (B).

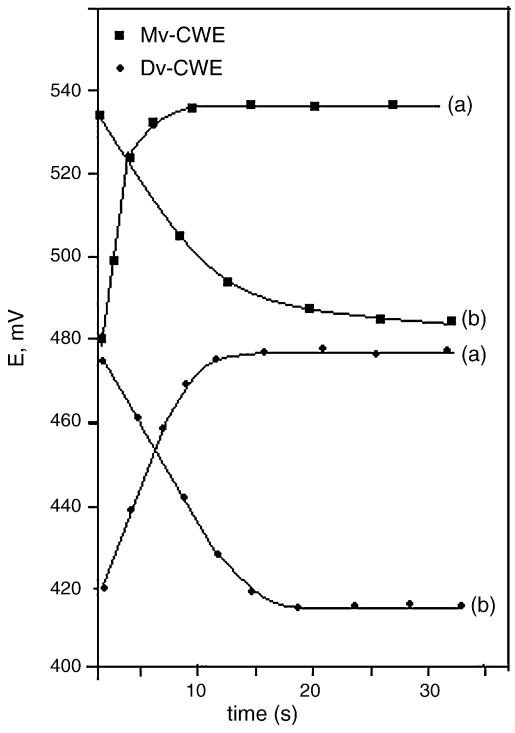

Fig. 2. Response time characteristics of Mv-CWE and Dv-CWE: (a) 1.0×10^{-5}–1.0×10^{4} M and (b) 1.0×10^{-4}–1.0×10^{-5} M.

although the time needed to reach the equilibrium value for the case of high-to-low sample concentration is longer than that of the low-to-high sample concentration. The average response time for all electrodes are included in Table 2.

3.2. Effect of pH

The effect of pH on the response of each coated wire electrode was examined at 25 °C with a series of sample solutions (1×10^{-5}–1×10^{-3} M of its respective drug). The pH was adjusted by adding small volumes of (0.1–1 M) hydrochloric acid or sodium hydroxide to the test solutions and the variation of potential was followed. It was found that the behaviors of the CWEs are closely similar to the previously reported liquid contact electrodes. The CWEs showed virtually no appreciable variation in potential due to pH change in the ranges 1.5–6.5, 1.5–7.2 and 2.5–7.5 for the electrodes of Dc, Mv and Dv, respectively.

3.3. Lifetime

Freshly prepared CWE can be used after soaking in 1×10^{-3} M of its respective drug solution for at least 30 min. The effect of soaking on the performance of the CWEs was studied by soaking each electrode in 10^{-3} M solution of its respective drug for variable intervals of time starting from 30 min reaching to 30 days. The slopes of the electrodes were observed to show gradual decrease after 20 ± 2 days. The life spans of the CWEs, in general, are less than those of the corresponding liquid contact electrodes. This may be attributed to the poor mechanical adhesion of the PVC-based sensitive layer to the conductive bed [28]

3.4. Selectivity of the antispasmodic drugs electrodes

The selectivity factors obtained for the antispasmodic drugs CWEs toward some inorganic cations, organic cations, sugars, urea and amino acids were investigated and presented in Table 3. These values are compared to those reported for the corresponding liquid-contact electrodes. The selectivity factors were calculated for both electrode types using the matched potential method at the activities between 10^{-6} and 10^{-2} M of each drug [18]. This method is recommended by IUPAC for determination of potentiometric selectivity factors to overcome the limitations of Nicolsky–Eisenman equation. As described in the experimental part the selectivity factor is obtained as the ratio of the changes in the activity of the analytes ($\grave{a}_A - a_A$) and interfering ion a_B.

The selectivity factors obtained with the proposed CWEs reflect a very high selectivity of each electrode for its respective drug. The selectivity factors of the two types of electrodes (coated wire and liquid contact electrodes) are comparable but they are somewhat better throughout with the CWEs than for the liquid-contact type. It is well documented in the literature that the transmembrane ion-fluxes taking place within the membrane of the electrode of the latter type, strongly influence the selectivity pattern of the sensor. If substantial amounts of analyte ions are displaced from the membrane during a reconditioning process to determine selectivity coefficients, the membrane effectively responds at the interface to a mixed ion sample. In the worst case, the measured potential values are completely independent of the interfering ion concentration, while apparent sub-Nernstian response slopes are normally observed [29].

Table 3
Selectivity factor values ($-\log k^{MPM}_{A,B}$) of CWEs in comparison with liquid contact electrodes (LCE)

Interferent	Selectivity factor values, $-\log k^{MPM}_{A,B}$					
	Dc-electrodes		Mv-electrodes		Dv-electrodes	
	CWE	LCE	CWE	LCE	CWE	LCE
Na^+	5.16	5.10	4.93	4.64	5.16	4.76
NH_4^+	4.26	4.24	4.12	3.8	3.53	3.3
K^+	5.22	4.97	3.41	3.58	4.65	4.84
Zn^{2+}	4.38	4.25	4.66	4.12	4.22	3.94
Co^{2+}	3.92	3.65	3.74	–	3.34	–
Ni^{2+}	4.57	4.19	3.85	4.07	4.69	4.19
Ba^{2+}	4.34	4.00	4.65	4.16	4.75	4.23
Mg^{2+}	4.27	4.21	4.58	4.22	4.32	4.11
Mn^{2+}	4.48	4.35	4.68	4.11	4.55	4.20
Hg^{2+}	3.13	3.80	4.51	4.24	4.43	–
Cd^{2+}	4.21	–	4.49	4.10	4.24	4.18
Cu^{2+}	4.46	4.21	4.11	4.05	4.35	4.15
Sr^{2+}	4.94	4.22	3.93	4.07	4.73	4.30
Cr^{3+}	4.97	4.11	3.75	4.17	4.57	4.18
Glucose	4.83	4.34	4.67	4.23	4.95	5.40
Lactose	5.38	–	4.53	4.12	3.73	3.19
Maltose	5.53	4.11	4.72	4.31	4.95	5.14
Fructose	5.43	4.32	4.78	4.21	5.24	5.20
Urea	4.89	4.11	3.33	3.67	4.71	4.15
Vit. C	4.64	4.19	3.25	3.02	4.73	4.81
Thiamine	2.56	1.39	3.17	2.25	5.21	5.32
Pyridoxamine	4.04	4.03	4.62	3.17	4.78	4.38
Papaverine	1.35	1.53	3.83	3.87	1.68	0.44
Theronin	4.14	3.97	3.95	–	4.85	5.15
Valine	5.19	4.12	4.54	4.18	4.76	4.38
Leucine	4.94	4.00	3.75	3.00	4.42	4.16
Asparagine	5.22	4.17	3.51	3.43	5.11	5.17
L-cystine	4.55	4.24	4.11	4.34	4.90	4.24
DL-serine	5.31	4.35	4.63	4.23	4.93	5.10
Alanine	4.90	4.00	4.03	3.52	4.81	4.53

3.5. Optimization of FIA system

Potentiometric flow injection analysis of the antispasmodic drugs Dc, Mv and Dv in different samples using CWEs was performed to assess the feasibility of use of these electrodes in flow measurements. In order to obtain the best response of the electrodes, the following factors were optimized: (a) composition of the adjusting carrier stream; (b) the injection volume and (c) the flow rate.

The composition of the adjusting solution is a variable of great significance. A certain saline level is required to buffer the ionic strength of the samples and to define an appropriate conductivity of the carrier solution flowing through the system. The saline composition also influences the response rate, the wash-out time of the ISEs and their performance characteristics. Several salts were assayed for the adjusting solution including sodium sulphate, sodium chloride, potassium nitrate and barium chloride

Table 4
Practical applications of the proposed sensors

Sample	Concentration (M)	Drug recovery (%)[a]				Max. difference (%)
		Standard method	Present Methods			
			SAM[b]	Pot. Titration	FIA	
Dicyclomine						
Aqueous solutions	1×10^{-6}–1×10^{-3}	98.7 ± 0.6	99.2 ± 0.5	98.4 ± 0.5	97.3 ± 0.4	2.4
Spasmorest, tablets	5×10^{-5}–1×10^{-3}	97.4 ± 0.4	98.4 ± 0.6	97.8 ± 0.4	96.6 ± 0.5	2.0
Spasmorest, ampoule	1×10^{-4}–1×10^{-3}	98.1 ± 0.5	97.8 ± 0.7	96.5 ± 0.6	96.4 ± 0.7	2.9
Mebeverine						
Aqueous solutions	1×10^{-6}–1×10^{-3}	99.4 ± 0.7	99.1 ± 0.4	97.6 ± 0.5	97.5 ± 0.5	3.1
Colospasmin, tablets	5×10^{-5}–1×10^{-3}	98.4 ± 0.6	98.2 ± 0.5	96.4 ± 0.5	95.4 ± 0.6	4.2
Drotaverine						
Aqueous solutions	1×10^{-6}–1×10^{-3}	99.1 ± 0.5	99.4 ± 0.4	96.4 ± 0.7	97.4 ± 0.6	2.8
Do-Spa tablets	5×10^{-5}–1×10^{-3}	98.7 ± 0.5	98.7 ± 0.6	95.4 ± 0.6	95.7 ± 0.4	3.9

[a] Average of five measurements.
[b] SAM, standard addition method.

to study the cation or the anion influence on the response of the electrode. Good wash characteristics and higher sensitivities were obtained by the use of 0.033 M sodium sulphate as adjusting solution since sulphate was the anion that provided a better response among these tested anions.

The injection volume was studied using 0.033 M sodium sulphate as an ionic strength adjusting solution. It was verified that the increase of the injected volume of the sample using loops with different volumes (9.4–500 μl) of 1.0×10^{-4} M drug solution produced an increment of the measured signal until it reached a steady value for injected volumes greater than 340 μl. Nevertheless, 75 μl was taken as the optimum volume giving approximately 90% of the maximum peak obtained by 340 μl loop, in order to reduce the return-to-baseline time.

The flow rate influence on the response of the electrode was investigated by varying the total flow rate from 0.9 to 5.35 ml min^{-1}. It was found that, as the flow rate increased, the peaks become higher and narrower until a flow rate of 2.5, 3.0 and 3.0 ml min^{-1} are reached for Dc, Mv and Dv electrodes, respectively. At higher flow rates, the peaks obtained are nearly the same. These flow rates were used through this work providing approximately 90–95% of the maximum peak height obtained by higher flow rates, shorter time to reach the baseline and less consumption of the carrier.

Under these optimal conditions the FI system with CWE detector provides a low dead volume, fast response, good wash characteristic and possible measurements of 60–100 samples per hour. Once the FI system was optimized, standard Dc, Mv and Dv solutions in the range of 1×10^{-2}–1×10^{-7} M were injected in triplicates using the proposed FIA method, and calibration curves were constructed for the optimized flow injection system based on the peak heights which follow the expected Nernstian behavior.

3.6. Analytical applications

Each of the prepared CWEs has been successfully used for the determination of its respective drug in aqueous solutions and in pharmaceutical preparations (dicyclomine hydrochloride tablets and ampoules (Spasmorest), mebeverine hydrochloride tablets (Colospasmin) and drotaverine hydrochloride) tablets (Do-Spa)) by using the standard addition method, potentiometric titration and flow injection analysis and the results are summarized in Table 4.

In the potentiometric titration method, different volumes of 1.0×10^{-4}–1.0×10^{-3} M drug solution or its pharmaceutical preparation were taken (1, 2, 3 and 5 ml) and completed to 25 ml with doubly distilled water. The CWE in conjunction with calomel reference electrode was immersed in the solution and titrations were carried out with standard 1×10^{-3} M silicotungstic acid. Fairly high e.m.f jumps at the vicinity of the end points ranging from 80 to 240 mV were recorded which reflect very high degrees of completeness of the titration reactions.

Different Dc, Mv and Dv samples (aqueous and pharmaceutical preparations solutions) were analyzed by the optimized FIA system using the proposed CWEs as detectors. The peak height were measured, and then compared to the standard calibration graphs based on injection of standard solutions of each drug.

Table 4 shows recoveries of drugs samples applying the reported methods with the proposed electrodes and a comparison of the results with those obtained by official methods [17]. The values obtained show that the present methods are of comparable precision to that of the standard methods and the variation in recovery within ±4.2%.

For determination of the antispasmodic drugs in urine, two healthy male volunteers took 40 mg DcCl (two tablets of Spasmorest) in the morning. After that, urine samples were collected in pre-cleaned dry polypropylene containers after different intervals of time extending to 8 h. The total volume of each collected urine sample was determined and recorded. The 10.0 ml portions were then taken from each sample for determination of DcCl in urine using the drug-CWE and applying the standard addition method only to overcome the matrix effects in this real sample. The same was repeated after each volunteer swallowed two tablets of Do-Spa (80 mg DvCl) or Colospasmin (200 mg

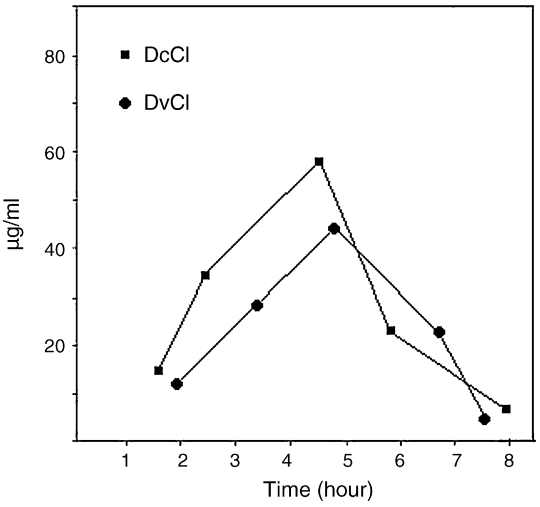

Fig. 3. The excretive profiles in urine after single oral doses of dicyclornme and drotaverme hydro chlorides.

MvCl). The results of trial determinations are summarized in Fig. 3.

It was found that excretive DcCl or DvCl reached a maximum in 4–5 h after taking the drug tablets. It was documented that the principle route of elimination of DcCl and DvCl is via the urine (79.5 and 40.0% of the doses, respectively) [30,31]. MvCl was not detected in urine samples using Mv-CWE. This is because about 95% of orally administered mebeverine in humans is metabolized and only about 2–5% of the dose reaches to urine [32], i.e. below the limit of detection of the Mv-CWE.

4. Conclusions

The present study shows that coated wire electrodes based on membranes modified with graphite and inner solid contact with low resistivity are very promising platforms to reach low detection limits. It was found that just a few mass percentage of graphite powder added to the plastic ion-selective membrane phase of dicyclomine, mebeverene or drotaverine sensors resulted in a significant improvement of charge transfer between the solid contact and the membrane phase. As a result, stability of the sensor potential and lowering of detection limits were observed with the present electrodes compared to the simple coated wire arrangements with no additive or to the corresponding liquid contact electrodes. It was demonstrated here that the selectivity behavior of the present CWEs have had a very good improvement in comparison to the conventional liquid contact electrodes. The results indicated also the superiority of the proposed CWEs in terms of response time and applicability.

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