DRUG FORMULATIONS AND CLINICAL METHODS

A New Platform for Profiling Degradation-Related Impurities Via Exploiting the Opportunities Offered by Ion-Selective Electrodes: Determination of Both Diatrizoate Sodium and Its Cytotoxic Degradation Product

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Although the ultimate goal of administering active pharmaceutical ingredients (APIs) is to save countless lives, the presence of impurities and/or degradation products in APIs or formulations may cause harmful physiological effects. Today, impurity profiling (i.e., the identity as well as the quantity of impurity in a pharmaceutical) is receiving critical attention from regulatory authorities. Despite the predominant use of spectroscopic and chromatographic methods over electrochemical methods for impurity profiling of APIs, this work investigates the opportunities offered by electroanalytical methods, particularly, ion-selective electrodes (ISEs), for profiling degradation-related impurities (DRIs) compared with conventional spectroscopic and chromatographic methods. For a meaningful comparison, diatrizoate sodium (DTA) was chosen as the anionic X-ray contrast agent based on its susceptibility to deacetylation into its cytotoxic and mutagenic degradation product, 3,5-diamino-2,4,6 triiodobenzoic acid (DTB). This cationic diamino compound can be also detected as an impurity in the final product because it is used as a synthetic precursor for the synthesis of DTA. In this study, four novel sensitive and selective sensors for the determination of both DTA and its cytotoxic degradation products are presented. Sensors I and II were developed for the determination of the anionic drug, DTA, and sensors III and IV were developed for the determination of the cationic cytotoxic impurity. The use of these novel sensors not only provides a stability-indicating method for the selective determination of DTA in the presence of its degradation product, but also permits DRI profiling. Moreover, a great advantage of these proposed ISE systems is their higher sensitivity for the quantification of DTB relative to other spectroscopic and chromatographic methods, so it can measure trace amounts of DTB impurities in DTA bulk powder and pharmaceutical formulation without a need for preliminary separation.

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Today, regulations of the U.S. Food and Drug Administration and the European Medicines Agency require full proof that drugs admitted to the market are consistent with safety and efficacy (1, 2). Impurities and potential degradation products can cause changes in the chemical, pharmacological, and toxicological properties of drugs and thereby have a significant impact on product efficacy and safety (3). Accordingly, in modern analytical laboratories, impurity profiling is viewed as the most important tool for solving impurity-related problems, which is of vital importance to guarantee effectiveness and confirm the quality of medicines commercialized for the population (4).

As outlined by the International Conference on Harmonization (5), a stability-indicating analytical method is needed in order to accurately detect and to quantify (i.e., profile) degradation-related impurities (DRIs). In an ideal case, such a method should resolve all DRIs from the parent and among each other and should detect and accurately quantify all DRIs (6). Indeed, thanks to improvements in chromatographic and spectroscopic methods and their combinations, advances in DRI profiling have become possible (7). A careful survey of the literature indicates the predominant use of spectroscopic and chromatographic methods over electrochemical methods for the impurity profiling of active pharmaceutical ingredients (APIs). However, from a practical point of view, some of these presently used techniques suffer from several challenges, such as the use of lengthy procedures, a need for sample pretreatment, long analysis times, expensive instruments, and an inability to directly monitor finished product matrixes within the matrix because of particularly turbid and viscous solutions. Thus, it is considered essential to explore faster ways of DRI profiling that are relevant to real-world storage, distribution, and even patient in-use conditions, with low cost and no need for hazardous solvents, sample pretreatment, or extraction steps. In this regard, electrochemical methods, especially potentiometric ion-selective electrodes (ISEs), have several unique advantages for this purpose.

Over the last two decades, the field of potentiometry with ISEs has been more extensively explored and has acquired increasing prominence in pharmaceutical drug analysis. The outstanding

opportunities offered by this technique greatly expanded the field of application of ISEs for novel approaches beyond the direct potentiometric assay of pharmaceutical products (8–11). Their extremely low LODs and high selectivity, together with important operational features, such as portability and simplicity, place this technique at the forefront of electrochemical methodologies used in different disciplines for drug-related analytical investigations (12). Moreover, a very attractive feature of potentiometric ISEs is their ability to provide a rapid route for the simultaneous analysis of mixtures of cationic and anionic drugs without a need for sample preparation and extraction steps thanks to a compact, portable, low-cost instrument (13).

The development of the present contribution was motivated not only by further exploring how potentiometry can be used for the reliable quantitative determination of concentrations of API, but also by evaluating the opportunities offered by ISE for DRI profiling that have been shown to mediate harmful physiological effects. Diatrizoate sodium (DTA) was selected as the model based on its carboxylate group, which gives it the characteristic of a strong anionic electrolyte. In addition, DTA was chosen because of its propensity to deacetylate into a mutagenic and cytotoxic degradation product, 3,5-diamino-2,4,6 triiodobenzoate (DTB; 14; Figure 1). Besides being a major DRI, this cationic diamino compound can also be detected as an impurity in a final product because it is used as a starting material for the synthesis of DTA (14). In this study, four sensitive and selective sensors for the determination of both DTA and its cytotoxic degradation products are presented.

Experimental

Instruments

(a) *Potentiometric measurements.*—Potentiometric measurements were carried out using a silver (Ag)–silver chloride (AgCl) double-junction-type external reference electrode (Thermo Scientific Orion 900200, Massachusetts; 3.0 M KCl saturated with AgCl as an inner filling solution and 10% KNO_3 as a bridge electrolyte) and Jenway digital ion analyzer (Model No. 3330; Essex, United Kingdom). A Jenway pH glass electrode was used for pH adjustments.

(b) *Spectrophotometric measurements.*—UV-spectrophotometric measurements were carried out with a Shimadzu dual-beam UV–visible spectrophotometer (Model No. UV-1601 PC; Kyoto, Japan).

(c) *Chromatographic (HPLC) measurements.*—The LC consisted of an isocratic pump (Model No. G1310A; Agilent, United States), a UV variable-wavelength detector (Model No. G1314 A; Agilent 1100 Series), and a Rheodyne injector (Model No. 7725I; Rohnert Park, CA) equipped with a 20 μL injector loop (Agilent). The stationary phase was a C18 Zorbax TM 5 μM analytical column (250 × 4.6 mm i.d.; Agilent). The mobile phase was filtered through a 0.45 μm Millipore membrane filter and degassed for ~15 min in an ultrasonic bath prior to use. UV detection was carried out at 238 nm. The samples were also filtered through a 0.45 μm membrane filter and injected with the aid of a 25 μL Hamilton analytical syringe.

Materials and Reagents

Polyvinyl chloride (PVC), dodecyltrimethyl ammonium bromide (DTMAB), tetraheptyl ammonium bromide (THAB), tridodecylmethyl ammonium chloride (TDMAC), sodium tetraphenylborate (NaTPB), calix[6]arene (CX6), and 2-nitrophenyl octyl ether (2-NPOE) were obtained from Fluka Chemie GmbH (St. Louis, MO), and tetrahydrofuran (THF) was obtained from BDH (Poole, England). Potassium chloride, sodium hydroxide, and hydrochloric acid (HCl) were kindly supplied by El-Nasr Pharmaceutical Chemical Co. (Cairo, Egypt).

Samples

(a) *Reference samples.*—DTA reference standard was purchased from the Sigma-Aldrich Co., and its purity was certified to be ≥99.99%.

(b) *Preparation of the degradation product.*—The degradation product, DTB, was prepared according to our recent work (15). Briefly, complete degradation of DTA was induced by refluxing with 2 M HCl for 6 h. The induced acidic degradation was evaluated by TLC plates and silica gel 60 F_{254} and with chloroform–methanol–ammonium hydroxide (20 + 10 + 2, v/v/v) as the mobile phase. The degraded solution was neutralized, transferred quantitatively into a 100 mL volumetric flask, and diluted to volume with distilled water. The structure of the degradation product was elucidated by IR and MS.

(c) *Pharmaceutical formulation.*—Gastrografin solution, manufactured by the Schering Co. (Batch No. 51424A; Belimed, Spain), was labeled to contain a 0.6 g/mL diatrizoate anhydrous base.

Solutions

DTA stock standard solution with a concentration of 1×10^{-2} M in distilled water was prepared in a 25 mL volumetric flask by dissolving 0.168 g pure DTA in distilled water.

Degradation product stock solution with a concentration of 1×10^{-2} M was prepared in 0.1 M HCl.

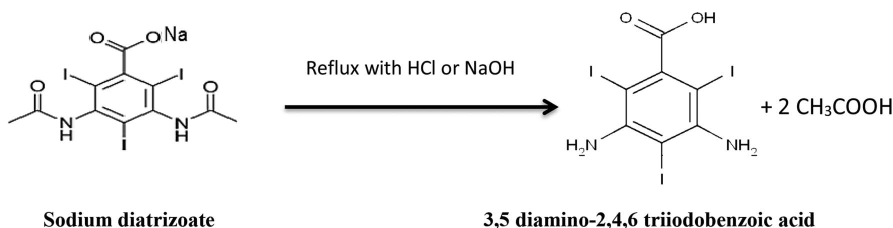

Figure 1. Suggested mechanism of degradation.

Table 1. Response characteristics of the investigated ion-selective electrodes and the validation parameters of the response and the regression equations

	Diatrizoate sodium		Degradation product	
Validation parameter	Sensor I	Sensor II	Sensor III	Sensor IV
Slope	–57.5	–60.1	29.5	30.2
SE slope	0.818	0.288	0.33	0.4
Correlation coefficient (r)	1	0.9998	0.9998	0.9996
Concentration range, M	10^{-4} to 10^{-2}	10^{-5} to 10^{-2}	10^{-5} to 10^{-2}	10^{-6} to 10^{-2}
Working pH range	6–10	6–10	1–4	1–4
Response time, s	10	5	10	10
Accuracy, %	99.99 ± 0.178	99.94 ± 0.762	100.05 ± 1.04	100.11 ± 1.08
Repeatability, %[a]	101.34 ± 0.848	100.89 ± 0.657	101.33 ± 0.943	99.85 ± 0.977
Intermediate precision, %[a]	99.37 ± 0.336	100.44 ± 0.662	100.85 ± 1.23	101.23 ± 1.04
Specificity, %	100.47 ± 1.271	100.66 ± 0.834	99.99 ± 1.25	101.05 ± 0.412
LOD[b]	8.33×10^{-5}	7.50×10^{-6}	7.86×10^{-6}	8.77×10^{-7}

[a] Three concentrations of DTA and DTB (10^{-2}, 10^{-3}, and 10^{-4} M) were analyzed three times intra- and interdaily for repeatability and intermediate precision, respectively.

[b] The LOD was measured by interception of the extrapolated arms of calibration curves.

Procedure

(a) *Sensor fabrication.—(1) Fabrication of sensors I and II.—* In two separate glass Petri dishes (5 cm in diameter), 0.4 mL 2-NPOE was thoroughly mixed with 190 mg PVC and 10 mg THAB or 10 mg TDMAC to produce sensors I and II, respectively. Each mixture was dissolved in 6 mL THF by stirring with a glass rod. The Petri dish was then covered with Whatman No. 3 filter paper and left to stand overnight to allow solvent evaporation at room temperature. A master membrane with a thickness of 0.1 mm was obtained. From the prepared membrane, a disk (about 8 mm in diameter) was cut using a cork borer and fixed using THF to a transposable PVC tip that was clipped to the end of the electrode glass.

Equal volumes of 1×10^{-4} M DTA and 1×10^{-4} M potassium chloride (prepared in distilled water) were mixed and used as an internal reference solution. An Ag/AgCl wire (1 mm in diameter) was immersed in the internal reference solution as an internal reference electrode. The sensor was conditioned by soaking in 1×10^{-4} M DTA stock standard solution for 24 h and storing in the same solutions when not in use.

*(2) Fabrication of sensors III and IV.—*In a glass Petri dish (5 cm in diameter), 0.4 mL 2-NPOE was thoroughly mixed with 190 mg PVC and 1 mg NaTPB. The mixture was dissolved in 6 mL THF by stirring with a glass rod. The Petri dish was then covered with Whatman No. 3 filter paper and left to stand overnight to allow solvent evaporation at room temperature. A master membrane with a thickness of 0.1 mm was obtained. From the prepared membrane, a disk (about 8 mm in diameter) was cut using a cork borer and fixed using THF to a transposable PVC tip that was clipped to the end of the electrode glass.

Equal volumes of 1×10^{-4} M DTB and 1×10^{-4} M potassium chloride (prepared in distilled water) were mixed and used as an internal reference solution. An Ag/AgCl wire (1 mm in diameter) was immersed in the internal reference solution as an internal reference electrode. The sensor was conditioned by soaking in 1×10^{-4} M DTB stock standard solution for 24 h and storing in the same solutions when not in use. The same previously mentioned procedure was carried out for the fabrication of sensor IV with the addition of 3.8 mg CX6.

(b) *Sensor calibration.—*Each sensor was separately conjugated with a double-junction Ag/AgCl reference electrode, calibrated by immersion in the respective drug solutions (1×10^{-7} to 1×10^{-2} M) and allowed to equilibrate while stirring under constant reading of the potentiometer. The electromotive forces were recorded within ±1 mV. Calibration graphs were plotted, relating the recorded electrode potentials obtained from the four proposed sensors versus log molar concentrations of the corresponding compounds. Sensors I and II were washed with distilled water before and after each run until they reached a constant potential, whereas sensors III and IV were washed with 0.1 N HCl.

(c) *Direct potentiometric determination of laboratory-prepared mixtures containing different ratios of DTA and DTB.—(1) Determination of DTA using sensors I and II.—* Into a series of 25 mL volumetric flasks, different volumes of

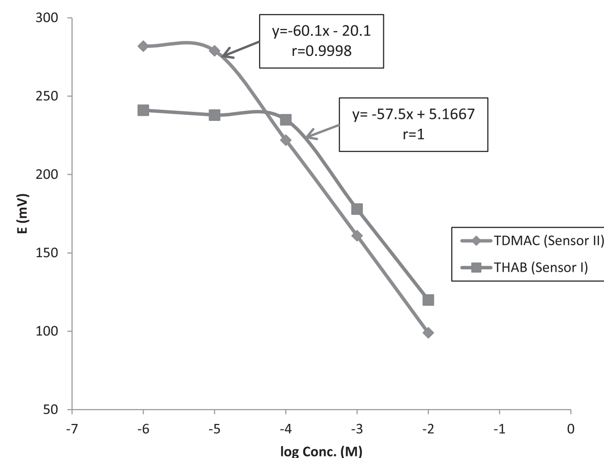

Figure 2. Profile of the potential in millivolts versus log molar concentration of DTA using sensor I (1×10^{-4} to 1×10^{-2} M) and sensor II (1×10^{-5} to 1×10^{-2} M).

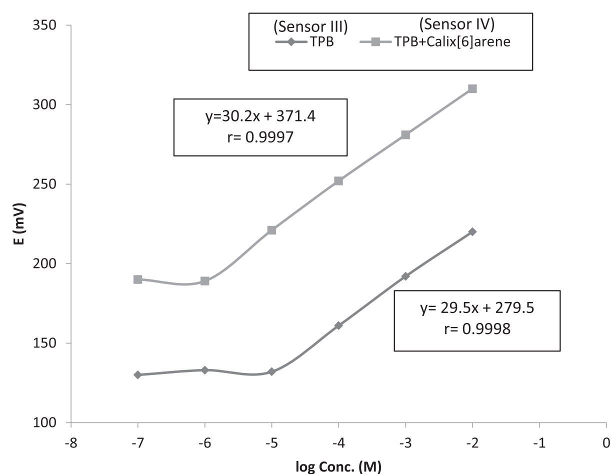

Figure 3. Profile of the potential in millivolts versus log molar concentration using sensor III (1×10^{-5} to 1×10^{-2} M) and sensor IV (1×10^{-6} to 1×10^{-2} M).

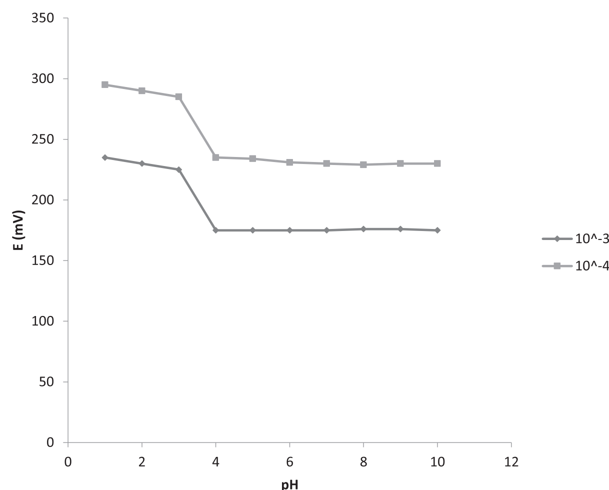

Figure 4. Effect of pH on the response of sensor I (working pH range of 6–10).

DTA and DTB (1×10^{-2} M prepared in distilled water) were accurately measured, transferred, and diluted to volume with distilled water.

(*2*) *Determination of DTB using sensors III and IV.*—Into another series of 25 mL volumetric flasks, different volumes of DTB and DTA (1×10^{-2} M prepared in 0.1 N HCl) were accurately measured, transferred, and diluted to volume with 0.1 N HCl.

(d) *Direct potentiometric determination of DTA in the Gastrografin solution.*—The Gastrografin solution is labeled to contain 0.6 g/mL anhydrous DTA. A solution with a concentration of 10^{-2} M was prepared, and fabricated sensors I and II in conjunction with the double-junction Ag/AgCl reference electrode were immersed in the prepared solution, the resulting potential recorded, and the respective concentration calculated from the corresponding regression equation.

(e) *Estimation of the slope, response time, and operative life of the proposed sensors.*—The electrochemical performance of the three proposed sensors was evaluated according to International Union of Pure and Applied Chemistry recommendations (16).

(f) *Effect of pH.*—The effect of pH on the potential values of the three sensors was studied over ranges in pH of 1–10 for all the proposed sensors. The pH was manipulated by adding diluted aliquots of 0.1 M HCl and 0.1 M sodium hydroxide solutions to 1×10^{-4} and 1×10^{-3} M solutions of both DTA and DTB. The potential obtained at each pH value was recorded.

(g) *Effect of interfering substances on the electrode selectivity.*—The potential response of the proposed sensors in the presence of a number of related substances was studied, and the potentiometric selectivity coefficient, $-\log(K^{pot}_{primary\ ion\ interferent})$, was calculated to estimate the degree to which a foreign substance would interfere with the response of the electrodes to their primary ion (DTA for sensors I and II and DTB for sensors III and IV). The selectivity coefficients were calculated by a separate solutions method (SSM) (16), using the following equation:

$$-\log\left(K^{pot}_{primary\ ion\ interferent}\right) = E_1 - E_2/S$$

where E_1 = potential measured in 10^{-3} M primary ion solution; E_2 = potential measured in 10^{-3} M of interferent solution; and S = slope of the investigated sensor.

Results and Discussion

This paper is positioned at a promising junction between two areas of recent and increasing interest: impurity profiling and chemical sensing. To the best of our knowledge, no ISE for DRI profiling has been reported to date. From this perspective, we exploit the chance of having two compounds with different ionic characteristics under study; thus, our scientific motivation was to develop a simple, accurate, reproducible, and rapid electrochemical method for the determination of the anionic drug, DTA, and its cationic cytotoxic DRI, DTB, with no need for pretreatment or prior separation. In order to achieve this, we structured our experimental work so that we could initially compare the responses of the three membranes with different ion exchangers to select the optimum one for the determination of the anionic drug, DTA. Subsequently, we fabricated ISEs for the measurement of the cationic cytotoxic degradation product by exploiting the binding properties of calixarene ionophores. Finally, we analyzed different laboratory-prepared mixtures containing each component to investigate the opportunities offered by each electrode.

Performance Characteristics of the Proposed Sensors

Lipophilic mobile ion-exchanger sites play a key role when used as added components to ISE membranes (17, 18). Their main function is to render an ion-selective membrane perm-selective (in order to observe Nernstian response slopes) and to reduce bulk membrane impedance. In practice, alkali salts of tetraphenylborate derivatives are used for cation-selective membranes and tetra-alkylammonium salts for anion-selective membranes (17, 18). It is well demonstrated that lipophilicity of the ion exchanger dramatically affects the performance characteristics of ISEs (19). In particular, the LOD of ionophore-free ion-exchanger membranes is directly dependent on the lipophilicity behavior of the ion exchanger used, with more lipophilic analogs yielding lower LODs. Indeed, at the LOD, the concentration of primary ions at the membrane phase boundary is ultimately given by the leaching of ion-exchanger additives because the leaching process is described by a coextraction process of the ion exchanger and its counterion. Furthermore, it has also been

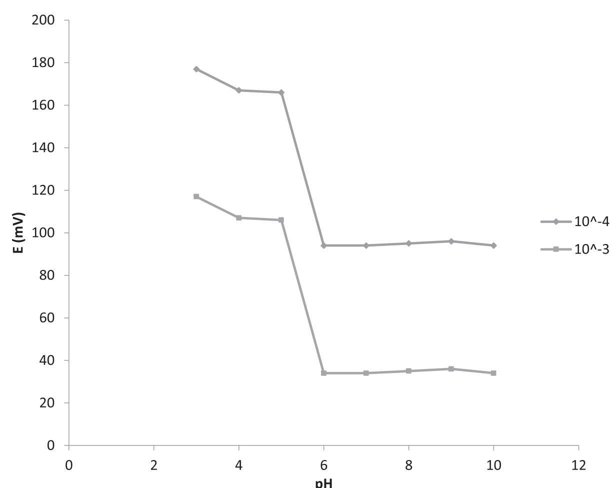

Figure 5. Effect of pH on the response of sensor II (working pH range of 6–10).

shown that the lifetime of such ion sensors is directly related to the lipophilicity behavior of the ion exchanger in the sensing membrane and the rate of its leaching out of the membrane (19).

Anionic DTA Sensors

DTAs possess a carboxylate moiety with a pKa value of 1.13 (14); thus, it behaves like anion in neutral and basic media, and, therefore, the DTA ISE membrane must exhibit an anion exchange capacity. This was achieved by investigating the response of different lipophilic anionic exchangers—DTMAB, THAB, and TDMAC—in which each membrane was initially conditioned in 1×10^{-4} M DTA for 1 day in order to replace the original exchangeable counterion (Br^- or Cl^-) of the ion exchanger with DTA.

The results showed that the preliminary fabricated sensor using DTMAB with a log$P$ value of 0.91 exhibited weak lipophilicity, so it displayed poor solubility in the membrane matrix. This membrane gave non-Nernstian responses and, therefore, was excluded from further investigations. On the other hand, sensor I, which was fabricated using THAB with a log$P$ value of 6.66, displayed good lipophilicity and solubility

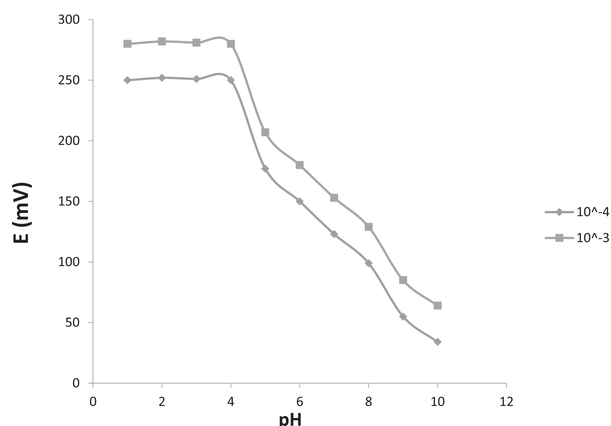

Figure 6. Effect of pH on the response of sensor IV (working pH range of 1–4).

Table 2. Potentiometric selectivity coefficients ($\log K^{pot}_{DTA,I}$) of the proposed sensors

	Selectivity coefficient (logK)[a]			
Interferents, 10^{-2} M	Sensor I	Sensor II	Sensor III	Sensor IV
Sodium chloride	–1.46	–3.43	–6.22	–6.89
Potassium bromide	–2.09	–3.52	–5.72	–6.66
Sodium oxalate	–1.83	–3.37	–6.45	–6.79
Lactose	–2.26	–3.62	–8.41	–8.44
Glucose	–2.68	–3.80	–8.44	–8.91
Degradate	–1.77	–3.53		
Diatrizoate sodium			–6.82	–6.99

[a] Average of three determinations.

in the membrane matrix. This membrane gave Nernstian responses over a limited concentration range, from 1×10^{-4} to 1×10^{-2} M, with anionic slopes of –57.50 ± 1.46 and an LOD of 8.3×10^{-5} (Table 1). The best performance characteristics were obtained with sensor II, which was fabricated using TDMAC with a log$P$ value of 10.67, which had high lipophilicity and solubility in the membrane matrix. This membrane gave very good Nernstian responses over a wider concentration range, from 1×10^{-5} to 1×10^{-2} M, with anionic slopes of –60.1 ± 0.57 and an LOD of 7.5×10^{-6} M (Figure 2). This was directly related to the partition coefficients of these ion exchangers between an organic phase and an aqueous phase that increases in the order of 0.91 (DTMAB) < 6.66 (THAB) < 10.67 (TDMAC), which parallels the order of their hydrophobicity. This result may explain the sequence of LODs for the DTA electrodes with membranes using these ion exchangers. One may readily notice that the partition coefficient for TDMAC is about 10 times greater than that for DTMAB and about 2 times greater than THAB. This result leads to an increase in sensitivity of and a long lifetime for the DTA selective electrode based on TDMAC.

Additionally, it is worth noting that in the initial experiments we used other lipophilic anionic exchangers for DTA determination, such as malachite green and bathophenanthroline–iron(II)-based membranes, but drifting signals and non-Nernstian responses were obtained.

Cationic DTB Sensors

For the analytical methodology to powerfully profile DRIs, it should be able to quantify low-level DRIs in the presence of the parent molecule (6). Practically speaking, the fact that DTB behaves as a cation suggests the use of ion exchanger of cationic type; NaTPB was chosen as the cationic exchanger in the fabrication of sensors III and IV. With the purpose of achieving a more selective and sensitive sensor for the detection of DTB, the ionophore, CX6, was selected because it is membrane-compatible and possesses outstanding complexation properties toward protonated ammonium ions (20). It should be noted that calixarenes (nanobaskets) have the ability to form supramolecular (inclusion) complexes with many appropriately sized organic ions and molecules by direct hydrogen bonding and cation–π interactions (21).

In order to examine the selective recognition of DTB by the CX6 ionophore in the membrane phase, the performance of an ionophore-based (CX6) ISE for DTB (sensor IV) was compared with an ionophore-free ion-exchanger NaTPB (sensor III) as a

Table 3. Potentiometric determination of diatrizoate sodium in laboratory-prepared mixtures with the proposed sensors

		Recovery, %[a]			
		Diatrizoate sodium		Degradate	
Degradation, %	DTA concn, M	Sensor I	Sensor II	Sensor III	Sensor IV
90	1×10^{-3}	101.87	101.23	98.87	100.88
70	3×10^{-3}	101.15	101.31	100.10	101.59
50	5×10^{-3}	99.84	100.31	100.91	101.31
10	9×10^{-3}	99.03	99.33	98.59	101.00
Mean		100.47	100.66	99.99	101.05
RSD, %		1.277	0.839	1.249	0.416

[a] Average of three determinations.

control experiment. It was found that the CX6-based ISE showed better sensitivity and could detect DTB at lower concentrations; a linear range of 1×10^{-6} to 1×10^{-2} M, with a cationic slope of 30.2 ± 0.80 and an LOD of 5.6×10^{-7} M compared with an LOD of 6.8×10^{-6} M for the blank NaTPB membrane. These data are summarized in Table 1. This behavior is attributed to the strong binding of the CX6 ionophore to DTB resulting in both buffering the activity of DTB in the sensing membrane to a low level and reducing its release from the membrane into the sample phase (Figure 3).

Effect of pH

For quantitative measurements with ISEs, the effect of pH on the response of the proposed sensors was studied to identify the optimum experimental conditions. Figures 4 and 5 show the potential–pH profile for 1×10^{-3} and 1×10^{-4} M DTA for sensors I and II. Below pH 4, there was a slight gradual decrease in potential with increasing pH, without a well-defined constant region. This could be explained by the medium not being basic enough below pH 4 to cause complete dissociation and ionization of the carboxylate group of DTA (pKa value of ~1.13). Alternatively, it was apparent that the sensor responses were fairly constant in solutions of pH 6–10; i.e., in these pH ranges, the ionizable COO^- group of DTA is completely ionized, dissociated, and sensed.

On the other hand, Figure 6 shows the potential–pH profile of 1×10^{-3} and 1×10^{-4} M DTB for sensors III and IV. It was apparent that below pH 4 the potentials displayed by the electrodes were fairly constant, with a well-defined constant region within pH values of 1–3. This acidic pH strongly favors the complete ionization of the two NH_2 groups present in the DTB structure (based on a pKa of ~7.9 for DTB); whereas above pH 5, the potential showed a sharp decrease, which could be attributed to the formation of nonprotonated primary NH_2 groups of DTB. Of particular importance, it should be pointed out that whole calibration curves performed at pH values close to the neutral pH range (6.5–7) showed significant differences in the corresponding calibration Nernstian slopes rather than in the low pH values. Under acidic conditions, the DTB molecule is doubly charged (divalent ion), whereas the two primary NH_2 groups were completely ionized and sensed, and hence a Nernstian slope of almost 30 mV was obtained. Under neutral conditions (pH 6.5), the DTB molecule is partially ionized, in which only about 50% of each NH_2 group is ionized, and, therefore, it was almost sensed as a monovalent ion and the corresponding Nernstian slope of about 55 mV obtained. For this reason, acidic pH values were chosen as the working pH range, and the calibration curves were carried out in 0.1 M HCl to ensure that the two NH_2 groups were in a completely ionized form.

Sensor Selectivity

DTA sensors.—The effect of interfering substances on the performance of the sensors was studied by SSM (16). The response of the two sensors in the presence of susceptible excipients, organic and inorganic-related substances, was assessed, and the results of the calculated selectivity coefficients showed that the proposed sensors displayed high selectivity and that no significant interference was observed from the susceptible interfering species (Table 2).

It is important to point out that DTB shows a marked sub-Nernstian response due to the presence of an electroactive group (COO^-). A justification for the ISE's observed greater selectivity toward DTA can be the higher lipophilicity of DTA compared with DTB, in which $\log K^{pot}_{DTA.DTB} = -3.53$ for the ion-exchanger membrane (TDMAC), as shown in Table 2. This is consistent with the fact that simple membranes containing only ion exchangers always favor ions that more lipophilic rather than less. This high selectivity facilitates the development of a DTA ISE that is a stability-indicating method for the determination of DTA.

DTB Sensors

A justification for ISE's observed greater selectivity toward DTB compared with DTA can be the absence of an electroactive cationic group (free NH_2 group) in the structure of the intact drug, where $\log K^{pot}_{DTB.DTA} = -6.20$, which means that sensor IV was at least 10^6 times more selective for DTB rather than DTA (Table 2). This remarkably high discrimination facilitates the

Table 4. Determination of diatrizoate sodium in the pharmaceutical formulation with the proposed sensors

Sensors	Pharmaceutical formulation	Found ± RSD, %[a]
Sensor I	Gastrografin solution	100.44 ± 1.323
Sensor II		99.81 ± 0.964

[a] Average of three determinations.

Table 5. Statistical analysis of the results obtained by the proposed sensors and the official method for the determination of DTA in pure powder form

Parameter	Sensor I	Sensor II	Official method[a]
Mean, %	99.99	99.94	100.85
SD	0.18	0.76	0.97
Variance	0.03	0.58	0.94
n	3	4	5
Student's *t*-test[b]	1.48 (1.94)	1.53 (1.89)	
F-value[b]	19.04 (19.25)	1.62 (6.39)	

[a] Precipitimetric titration using standard 0.05 N $AgNO_3$ and the tetrabromophenolphthalein indicator.

[b] Values in parentheses represent the corresponding tabulated values of *t* and *F* at *P* = 0.05.

development of ISE for profiling trace amounts of this impurity in the parent molecule.

Potentiometric Determination of Laboratory-Prepared Mixtures Containing Different Ratios of DTA and DTB

The results obtained upon analysis of the laboratory-prepared mixtures containing different ratios of DTA and DTB showed that proposed sensors I and II can be successfully used for the selective determination of anionic DTA in the presence of cationic DTB with no need for prior separation. It was also obvious that proposed sensors III and IV can be successfully used for the selective determination of DTB in the presence of DTA with no need for prior separation (Table 3).

Potentiometric Determination of DTA in the Gastrografin Solution

The proposed sensors were used to assay DTA in a pharmaceutical formulation (Gastrografin solution). The results proved the applicability of the sensors as demonstrated by the accurate and precise recovery percentages. Susceptible excipients did not show any interference. Thus, determination of DTA was carried out without prior treatment or extraction (Table 4). To examine the validity of the proposed sensor, the obtained results are compared with those of the official *United States Pharmacopeia* (USP) method (22), and no significant differences were found. It should be noted that the time required for sample analysis was short in the case of ISE (less than 1 min) compared with about 120 min for the official precipitimetric titration using standard 0.05 N $AgNO_3$ and a tetrabromophenolphthalein indicator (Table 5). Furthermore, the proposed sensor does not require any preliminary drug extraction, as described in the USP method.

Profiling Degradation-Related Impurities of DTA by UV-Spectrophotometry and HPLC

With the purpose of comparing the LODs obtained by the proposed ISE with those obtained by UV-spectrophotometry and HPLC for the profiling of DRIs of DTA, Figure 7 shows the absorption spectra of 24 μg/mL DTA and 2 μg/mL DTB solutions at neutral pH. Based on Figure 7, it is quite obvious that DTB cannot be expressed in a direct manner due to severe overlap in the spectrum for the DTA peak at 238 nm, and the lowest concentration of DTB that could be accurately detected by UV-spectrophotometry was 2 μg/mL.

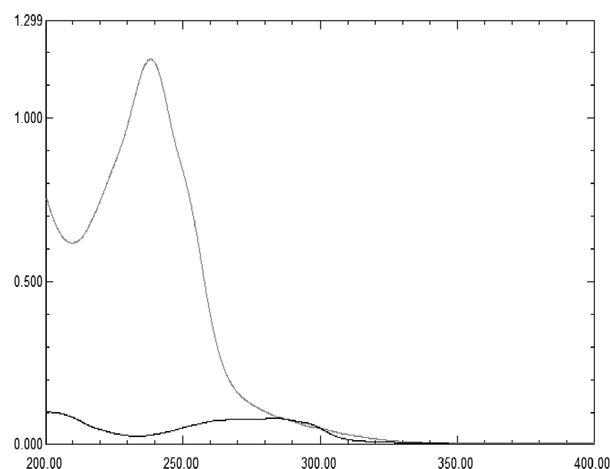

Figure 7. Zero-order absorption spectra of 24 μg/mL DTA and 2 μg/mL DTB.

Concerning the HPLC method, Figure 8 shows that best resolution, with symmetric and sharp peaks obtained using a mobile phase of water–methanol (75 + 25, v/v) at pH 3 (adjusted using orthophosphoric acid), a flow rate of 1 mL/min, and UV detection carried out at 238 nm. The retention times were 5.52 ± 0.1 min for DTA and 4.04 ± 0.1 min for the DTB (23). The linear range and LOD for DTB were 2–100 and 1.2 μg/mL, respectively.

In reference to the proposed ISE for the analysis of DTB, with an LOD of 0.063 μg/mL, the sensitivity of this ISE was almost 20 times greater than that of the UV-spectrophotometry and HPLC methods. The proposed method is thus a fast and sensitive means of detecting trace amounts of DTB impurities in a DTA pharmaceutical formulation.

Although the ISE, UV-spectrophotometry, and HPLC methods are in good agreement in pharmaceutical DRI profiling, the three analytical methodologies differ in a number of ways. ISEs offer unique advantages: (*1*) the capability of direct monitoring within the finished product matrixes (even in turbid and viscous solutions) without a need for sample preparation and extraction; (*2*) the possibility of miniaturization and portability, permitting their use as a bench-top, real-time analyzer or for in situ measurements; (*3*) a fast response time and virtually no power demand; (*4*) considerably cheaper, easy to use, nondestructive, and highly reproducible; (*5*) compatible with microfabrication processes, allowing mass production of cost-effective sensors; (*6*) the possibility of monitoring the degradation kinetics of UV-inactive drugs (chromophore-free drugs); (*7*) excellently suited for use in "electronic tongues," in which an array of carefully selected ISEs with appropriate cross-sensitivities may be used for the simultaneous detection of multiple targets; and (*8*) a wider concentration range, in which impressive LODs at about the nanomolar level are currently achievable owing to the in-depth understanding of transmembrane ion fluxes (24).

The only two limitations facing ISEs is their inability to elucidate the chemical structure of the degradation product and direct detection of the electrically neutral organic species because the transfer of these species across the interface of an

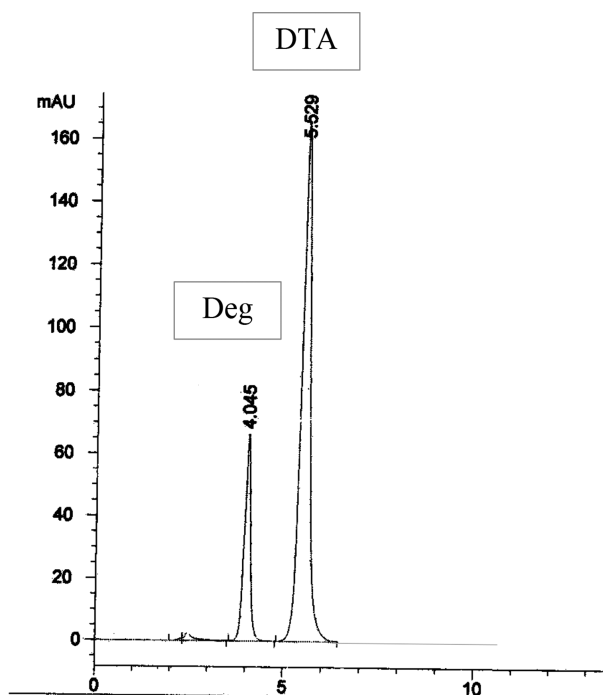

Figure 8. HPLC chromatogram of a resolved mixture (20 μg/mL DTA and 20 μg/mL DTB).

aqueous sample into an ISE membrane does not involve the transfer of an electric charge across the interface.

From a comparative point of view, although the UV-spectrophotometry technique is very robust, limitations to spectrophotometric analysis include a lack of selectivity and complications from interfering species that may be present at far higher concentration levels than the analyte of interest, and consequently requiring lengthy sample preparation and the manipulation of data to resolve overlapping peaks.

HPLC is undoubtedly the most important method in drug-impurity profiling. It is widely used for separating and quantifying impurities, and this technique is most frequently coupled with spectroscopic methods in the identification and elucidation of the structure of impurities. As must be realized from the above discussion, the process of DRI profiling by HPLC is partially time-consuming and difficult. In general, also, the HPLC method should require as many experimental runs as are necessary to achieve the desired result. Moreover, there are a large number of interdependent parameters that exist in the practice of HPLC, and, consequentially, the requirement to study these parameters during method development through multiple chromatographic runs makes the method somewhat difficult overall (25). However, HPLC is very much used, especially during initial impurity-profiling investigations to study the number of degradation products formed because it is a separation-based technique.

Compared with the traditional HPLC method, the newly established electrochemical method was more rapid because the DRI profiling of APIs proceeded simultaneously, and the sample was analyzed without a need for chromatographic separation. Consequently, costs and time spent in the optimization process are dramatically reduced. In addition, the method did not suffer from negative matrix effect problems and is environmentally friendly because no organic solvent was involved in the entire experimental procedure (i.e., it is an organic, solvent-free method).

Conclusions

This work addresses the promising junction of two areas of recent and increasing concern: pharmaceutical DRI profiling and electroanalysis. To this end, great opportunities exist for ISEs in the field of DRI profiling of organic APIs. ISEs, UV-spectrophotometry, and HPLC are in good agreement in DRI profiling. The successful application of the proposed ISEs to detect trace amounts of DTB in DTA samples represents the first use of potentiometric sensors in real-life samples and demonstrates the usefulness of this method in impurity profiling. This opens a venue to extend this simple and inexpensive procedure to other APIs without significant modification, permitting ISE application in routine QC laboratories. Alternatively, the quick and uncomplicated prescreening of large numbers of samples on site may be performed with these electrodes prior to the relatively time-consuming, off-site analysis of much smaller numbers of selected samples with conventional methods of analysis, such as HPLC. The HPLC would be indicated in particular in the case of initial impurity-profiling investigations to study the number of degradation products formed and any other related compounds anticipated to be present in the finished product.

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