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Voltammetric Determination of *Azelastine*-HCl and *Emedastine* Difumarate in Micellar Solution at Glassy Carbon and Carbon Paste Electrodes

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Abstract: The electrochemical behavior of the two antihistaminic drugs *Azelastine*-HCl and *Emedastine* difumarate is studied in micellar solutions. Anodic oxidation is obtained at the glassy carbon electrode (GCE) and a carbon paste electrode (CPE) using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) in Britton-Robinson buffers (pH 8 and pH 6) containing 0.8×10^{-4} M sodium dodecylsulphate (SDS). The peak potential shifts to more positive value in anionic surfactant (sodium dodecylsulphate solution) than in presence of cationic surfactant (cetyltrimethylammonium bromide) or the non-ionic surfactant (*Triton X-100*). The oxidation was characterized by the single one-electron wave. The method has been validated according to the ICH Guidelines, when the limit of quantitation ranges between 0.4×10^{-7} and 0.8×10^{-7} mol L^{-1}.

Key words: Electrochemistry; Glassy carbon electrode; Carbon paste electrode; Surfactants; *Azelastine*-HCl; *Emedastine* difumarate; Determination; Pharmaceuticals.

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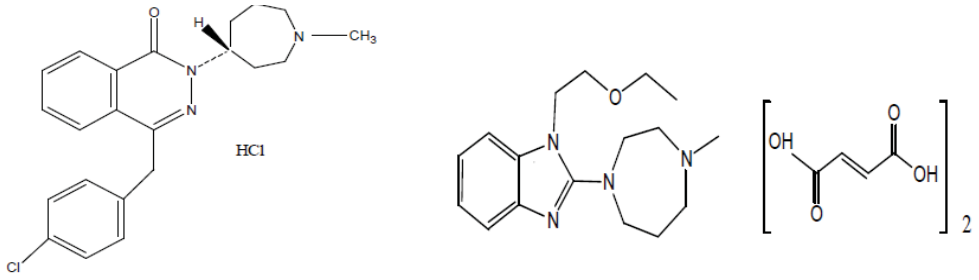
Introduction

Azelastine-HCl (AZT) is 4-(4-chlorobenzyl)-2-[(4RS)-1-methylhexahydro-1H-azepin-4-yl] phthalazin-1(2H)-one hydrochloride ([1]; see also Scheme 1). It is an intranasal antihistamine indicated as a appropriate medical treatment for patients suffering from the seasonal allergic rhinitis (SAR) and non-allergic vasomotor rhinitis (VMR).

Reportedly, this medicament is also used topically in the symptomatic relief of both acute and chronic allergic conditions, including rhinitis and conjunctivitis [2,3].

Emedastine difumarate (EDD) is 1H-benzimidazole, 1-(2-ethoxyethyl)-2-(hexahydro-4-methyl-1H-1, 4- diazepin-1-yl), (E)-2-butenedioate (1:2) (*Scheme 1* [4]). It is a second generation antihistamine used in eye drops to treat allergic conjunctivitis [5].



Scheme 1: *The chemical structure of the drugs studied.*
Left: *Azelastine hydrochloride,* right: *Emedastine difumarate*

The methods available for analysis of *Azelastine*-HCl in pharmaceutical dosage forms and biological fluids are those utilizing the principles of volumetric [6], UV- and VIS-spectrophotometry (colorimetry) [7], thin-layer chromatography, TLC [8], column chromatography, HPLC [9-11], or capillary electrophoresis, CE [12]. Few methods were then reported for analysis of *Emedastine* difumarate that combine HPLC with tandem mass spectrometry, MS [13,14] or a radioreceptor assay [15].

The micellar system affects peak potential, charge transfer coefficients, and stability of electrogenerated anion or cation radicals. Micellar effect may be of many kinds including electrostatic and surface interactions, hydrophobic forces and solute partition between the micelle and the water phases [16-20].

In this article studies, we present a comparative study gathering the observations and results obteined during of electrochemical oxidation of *Azelastine-HCl* and *Emedastine difumarate* in various micellar solutions. Our main intention was to purposely develop new electroanalytical methods for the detection and determination of the two title drugs in their formulations and related pharmaceutical products.

Experimental

Apparatus

Computer-driven Analytical electrochemical workstation with together with electrochemistry software (model "AEW2 + ECProg3"; Sycopel, England) were used in combination with a three-electrode configured stand (model "C-2"; the same manufacturer). The working electrode was a glassy carbon electrode (GCE; model "MF-2012"; BAS Instruments, USA) or a carbon paste electrode (CPE, model "MF-2010"; BAS), the reference electrode Ag/AgCl 3M KCl (model "MW-1032"; BAS). A digital pH-meter (model "Cyberscan 500"; EUTECH Instruments, USA) with combined glass electrode was also used. Finally, Origin 7.0 software was used for the transformation of the analytical signals.

Materials

Azelastine-HCl (AZT) was kindly supplied from European Egyptian Pharm Co., Egypt, with 99.00 % purity. *Zalastine*® nasal spray (product "BN 7579001"; European Pharm Co., Egypt) was labeled to contain 1 mg AZT per 1 mL and Azelast® eye drops ("BN 86872", El-Kahira Pharm and Chem Ind Co., for EPCI, Cairo Egypt) were labeled to contain 0.5 mg AZT per 1 mL. *Emedastine* difumarate (EDD) was kindly supplied from Chem., Swiss, SIGMA, Co., Egypt, with 99.00 % purity. Finally, *Emedastine,* 0.05 % ophthalmic solution ("BN 190409-F_1"; Sigma, Cairo, Egypt) was labeled to contain 0.5 mg EDD per 1 mL.

Chemicals and Reagents

All pharmaceutical preparations were purchased from the local market. Britton-Robinson buffer (B-R buffer) [21] was prepared with pH 2.0 – 9.0 and kept in a refrigerator for about 7 days. Stock standard solutions of drugs 1×10^{-2} M were prepared by dissolving 209.2 mg of AZT or 267.29 of EDD in 50 mL deionized water in a volumetric flask. They were found to be stable for about one month at 4 ^{o}C. Each solution was diluted with water to obtain a working solution of 1×10^{-3} M of each drug.

For measurements, we also employ the anionic surfactant, sodium dodecyl sulphate (SDS; Sigma-Aldrich), the cationic surfactant cetyltrimethylammonium bromide (CTAB; Across Organics, USA) and non-ionic surfactant *Triton® X-100* (Mp Biomedical, France). Finally, methanol (p.a. grade; Adwic co., Egypt) was also used.

Preparation of the Working Carbon Electrodes

A *carbon paste electrode* (CPE) was freshly prepared by mixing graphite powder (0.5 g) with Nujol (0.3 mL) in a mortar. The carbon paste was packed into the hole of the electrode body and smoothed on a filter paper, until it had had a shiny appearance.

The *glassy carbon electrode* (GCE) was polished manually with 0.5 mm diameter alumina powder on a smooth polishing cloth prior to each measurement. Then, it was thoroughly rinsed with ethanol then with water, dried with a tissue paper and fitted to electrochemical cell.

Electrochemical (Genereal) Procedure

For voltammetric measurements, 5 mL of the B-R buffer tested (with pH 8 for AZT and pH 6 for EDD) were transferred into the cell; then, the electrode — either GCE or CPE — was immersed in the solution. All scans were run in positive direction with a potential scanning from +0.4 to +1.4 V vs. ref. for AZT and +0.7 to +1.5 V for EDD. After measurement(s) in blank solution, the cell was filled with 4.5 mL B-R buffer and the appropriate volume 0.5 mL 1×10^{-2} M solution of each drug was added; the respective voltammetric response at the working electrode being recorded. All the measurements were carried out at room temperature, 25±2 ^{o}C.

Determination of Azelastine HCl and Emedastine Difumarate in Pharmaceutical Samples

The content of three bottles of *Zalastine* or *Azelast* samples were mixed and the aliquots equivalent to 4.2 mg AZT were transferred to a 10 mL volumetric flask and then diluted to the mark with deionized water to obtain a working solution (with c = 1×10^{-3} M). For *Emedastine* (ophthalmic solution), the content of three bottles were mixed and the aliquots equivalent to 5 mg EDD evaporated, then diluted with 10 mL deionized water to obtain again the working solution (1×10^{-3} M). Then, the aliquots of the drug solution were introduced into the cell and the measurement carried out (see previous section).

Results and Discussion

Cyclic voltammogram of 1×10^{-3} M of each AZT or EDD in BR buffer of pH 8 and pH 6 in presence of 0.8×10^{-4} M SDS at both GCE and CPE was found to exhibit a single anodic peak. The peak potential was found to be 0.903 V and 0.857 V for AZT, and 1.074 V and 1.053 V for EDD at GCE or CPE, resp., at scan rate 100 mV s^{-1} (see Fig. 1 overleaf). No peaks were observed on the reverse scan, suggesting the irreversible nature of the electrode reaction.

Optimization of Experimental Parameters

Effect of pH. The electrochemical behavior of the two drugs at GCE or CPE was studied at different pH 2 - 9 using BR buffer solutions containing 0.8×10^{-4} M SDS. Fig. 2 indicates that pH 8 was optimum for AZT, above which the free base precipitate.

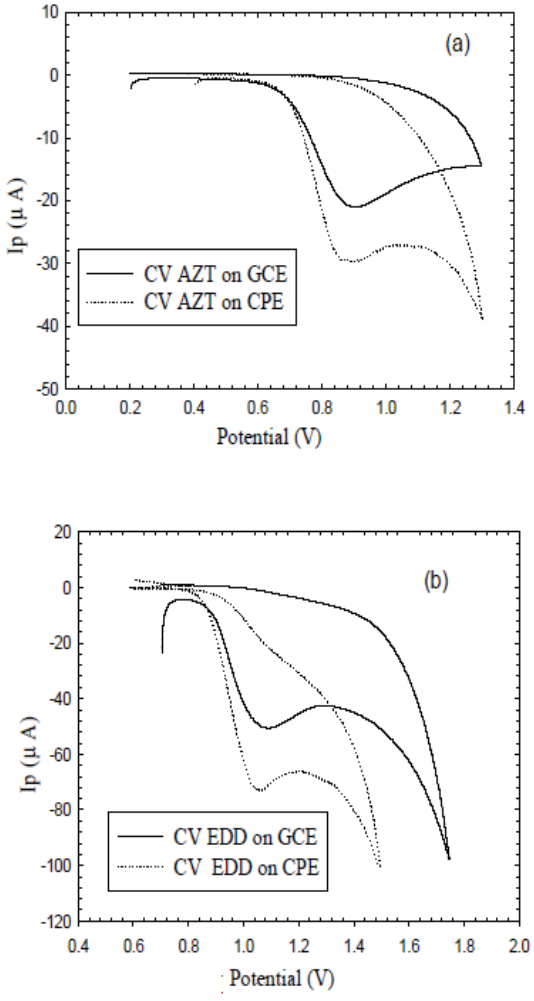
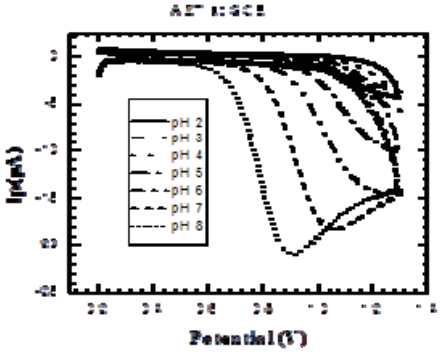


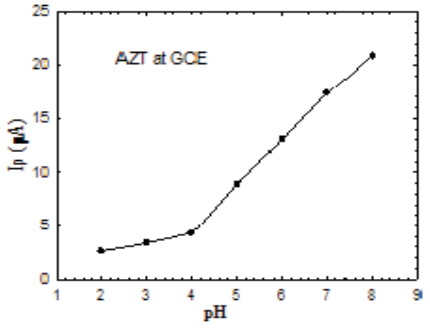
Fig. 1:

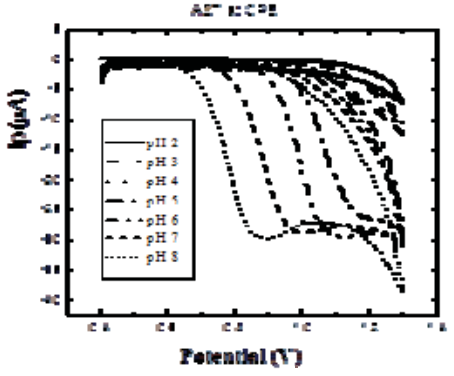
Cyclic voltammograms of 1×10^{-3} M Azelastine-HCl (pH 8) and of Emedastine difumarate (pH 6) on bare glassy carbon and carbon paste electrode in BR-buffer, when using a scan rate of 100 mV s^{-1}.

As ascertained in measurements with *Emedastine* difumarate (EDD), the current increased with the increasing pH until pH 6; after that, the peak current decreases, showing a distinct broadening. Thus, the value of pH 6 was chosen throughout the further work.

Effect of Scan Rate. The interfacial reaction of each drug at each electrode was identified by recording its cyclic voltammograms of 1×10^{-3} M at different scan rates (v) 10-250 mV s^{-1} and potential at 0.903 V and 0.857 V for AZT or 1.074 V and 1.053 V for EDD at GCE and CPE respectively. Direct proportionality was observed between log current, and log scan rate in range from 10 – 250 mV s^{-1}, with equations:







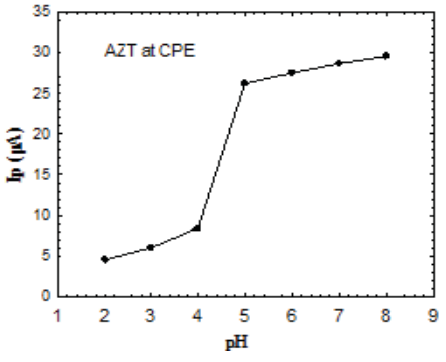
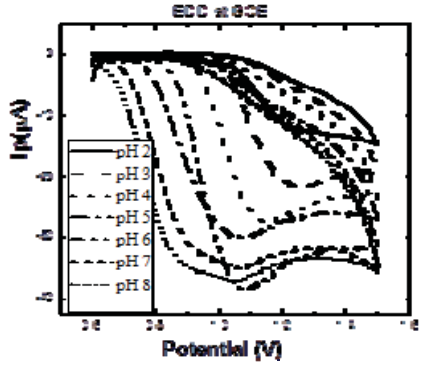
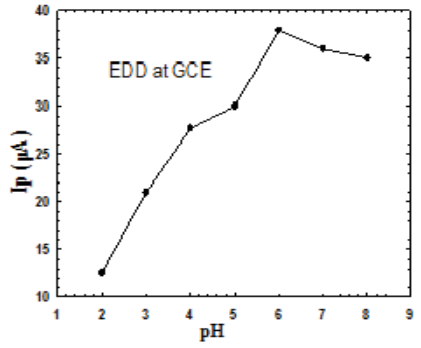
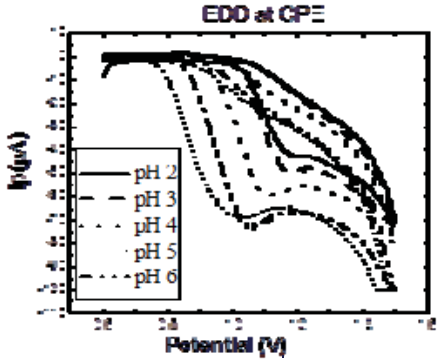


Fig. 2, Part **A**:

Effect of pH on the oxidation of 1×10^{-3} *M Azelastine-HCl in BR-buffer at the GCE and CPE* (scan rate: 100 mV s^{-1})







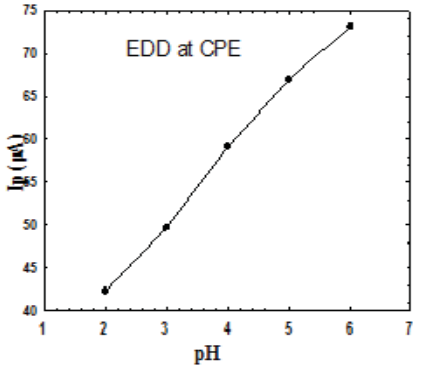


Fig. 2, Part **B**:

Effect of pH on the oxidation of 1×10^{-3} *mol* L^{-1} *Emedastine* difumarate *in BR-buffer at GCE and CPE* (recorded at a scan rate of 100 mV s^{-1})

$\log I_p = 0.321 + 0.439 \log \nu$ r = 0.9995 for AZT at GCE

$\log I_p = 0.794 + 0.481 \log \nu$ r = 0.9994 for AZT at CPE

$\log I_p = 0.494 + 0.483 \log \nu$ r = 0.9998 for EDD at GCE

$\log I_p = 1.123 + 0.316 \log \nu$ r = 0.9998 for EDD at CPE

The slope being less than 0.50 indicates diffusion controlled process [22]. The relation between anodic peak current, i_{pa} (μA), diffusion coefficient of the electro active species D_o ($cm^2 s^{-1}$), and scan rate ν (Vs^{-1}), is given by Randles-Sevcik equation [23,24],

$$i_{pa} = (2.69\times10^5)\, n^{3/2}\, A\, C_o^{*}\, D_o^{1/2}\, \nu^{1/2} \qquad (1)$$

where

n ... number of electrons exchanged in oxidation (one electron for AZT and EDD [25]),
A ... apparent surface area of the electrode (cm^2),
C_o ... concentration of the electro active species (mol cm^{-3}),
D_o ... diffusion coefficient.

A plot of i_{pa} versus $\nu^{1/2}$ (10 to 250 mV s^{-1}) for AZT and EDD gave a straight line according to equation (1) (Fig. 3), which is realized up to a scan rate of 100 mVs^{-1} followed by a deviation with increasing scan rate. D_o was calculated and the results are listed in Table I. The size of the diffusion layer at the electrode surface proximity changes with the voltage scan used. At relatively slow voltage scans (as for CPE), the diffusion layer grows towards the solution side and further from the electrode surface and peak current increase in case of CPE than of GCE.

Table I: *Electrochemical parameters of Azelastine-HCl and Emedastine difumarate for the glassy carbon and carbon paste electrodes.* The oxidation peak potential, E_{pa}, and current, I_{pa}, were determined at scan rate, ν = 100 mV s^{-1}.

Parameters	AZT		EDD	
	GCE	CPE	GCE	CPE
E_{pa} / mV (vs. Ag/AgCl)	898	887	1075	1051
I_{pa} / mA	0.023	0.064	0.038	0.073
D_o / cm^2 s^{-1}	1.5×10^{-5}	9.98×10^{-5}	4.14×10^{-5}	1.27×10^{-4}

Notes: AZT ... *Azelastine* hydrochloride, EDD ... Emedastione difumarate; GCE ... Glassy carbon electrode, CPE ... carbon paste electrode;

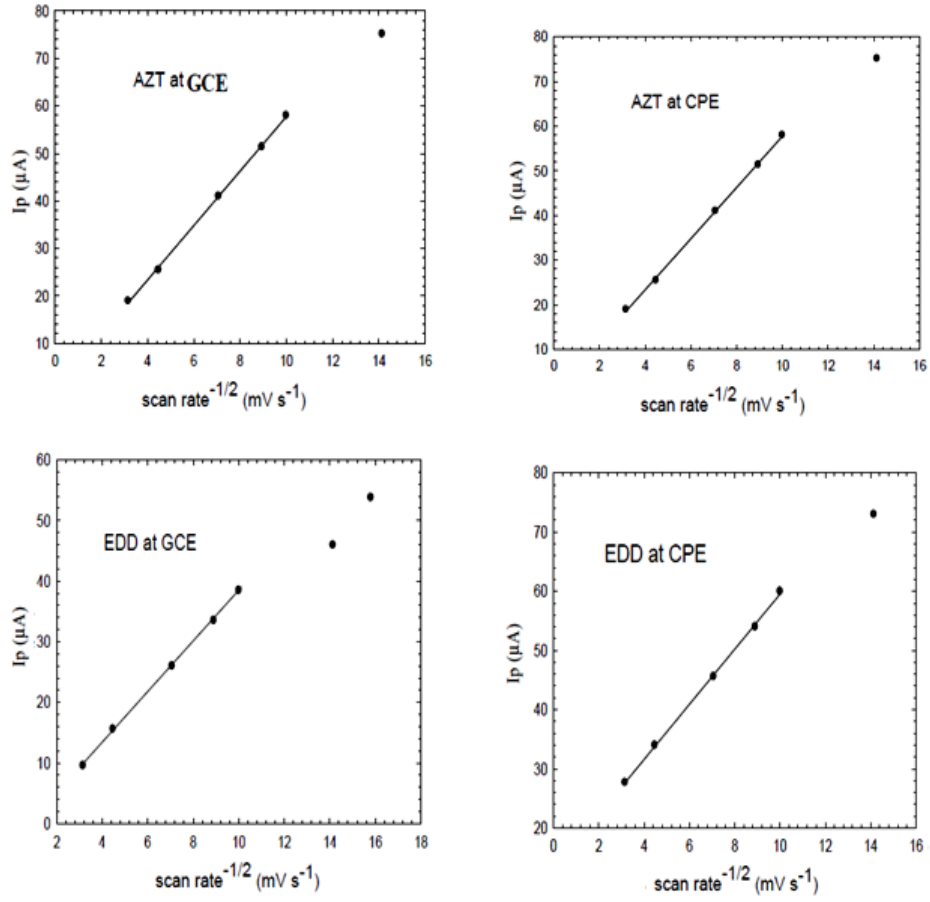


Fig. 3: *Plots of the oxidation peak currents of Azelastine-HCl and Emedastine difumarate versus square root of scan rate ($v^{1/2}$) at GCE and CPE.*

Effect of Accumulation Time. The effect of accumulation time on the anodic peak current of both drugs at pH 8 and pH 6 for AZT and EDD respectively, was studied at GCE and CPE at open circuit condition. The peak currents disappeared after 15 sec, this means that the electrode surface was immediately covered completely with the electroactive species (diffusion controlled process), and thus the current was instantaneously measured.

Effect of Surfactants. The effect of 0.8×10^{-4} M of different surfactants, namely SDS, CTAB and *Triton® X-100* on the voltammetric response of 1×10^{-3} M of each drug was shown in Fig 4. The anionic surfactant, SDS, increases the anodic peak currents of both drugs, while the cationic, CTAB, decrease it. The non-ionic surfactants, Triton® X-100, stabilized the anodic peak currents for AZT, while for EDD the anodic peak currents were decreased.

The presence of anionic surfactant (SDS) results in electrostatic interaction between the positively charged drug and the negatively adsorbed surfactant film. As a result, the surface concentration of the drug increased, facilitating the oxidation process. The cationic and the non-anionic surfactants have no attraction to the drug.

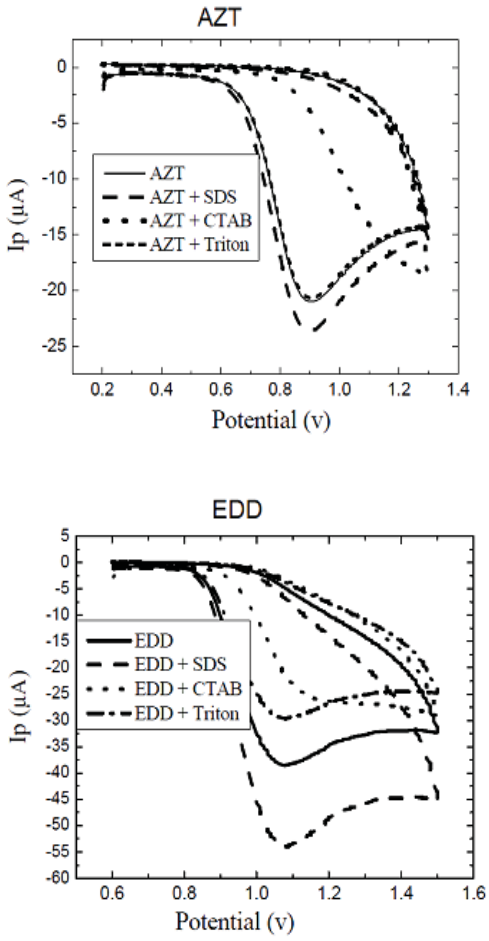


Fig. 4:

Effect of choice of different surfactant on the voltammetric current response of Azelastine-HCl (pH 8) and Emedastine difumarate (pH 6) in BR buffer on GCE at scan rate of 100 mV s^{-1}.

Differential Pulse Voltammetry

In this study, the scan rate 10 mV s^{-1} was chosen because at this value the sensitivity was relatively high and the voltammetric curves were of well-shaped with a relatively narrow peak width (Fig. 6,7). Under the above-optimized conditions, linear relation between the peak current and concentration for AZT was found in ranges of 4.0×10^{-6} – 1.6×10^{-4} mol L^{-1} and 4.0×10^{-6} – 2.0×10^{-4} mol L^{-1} at GCE and CPE, respectively.

As for *Emedastine* difumarate, the electrochemical oxidation at GCE and CPE gave linear relations between the peak current and concentration in the ranges of 8.0×10^{-6} – 2.0×10^{-4} mol L^{-1} and 1.0×10^{-6} – 8.0×10^{-5} mol L^{-1} at GCE and CPE, respectively (see Table II).

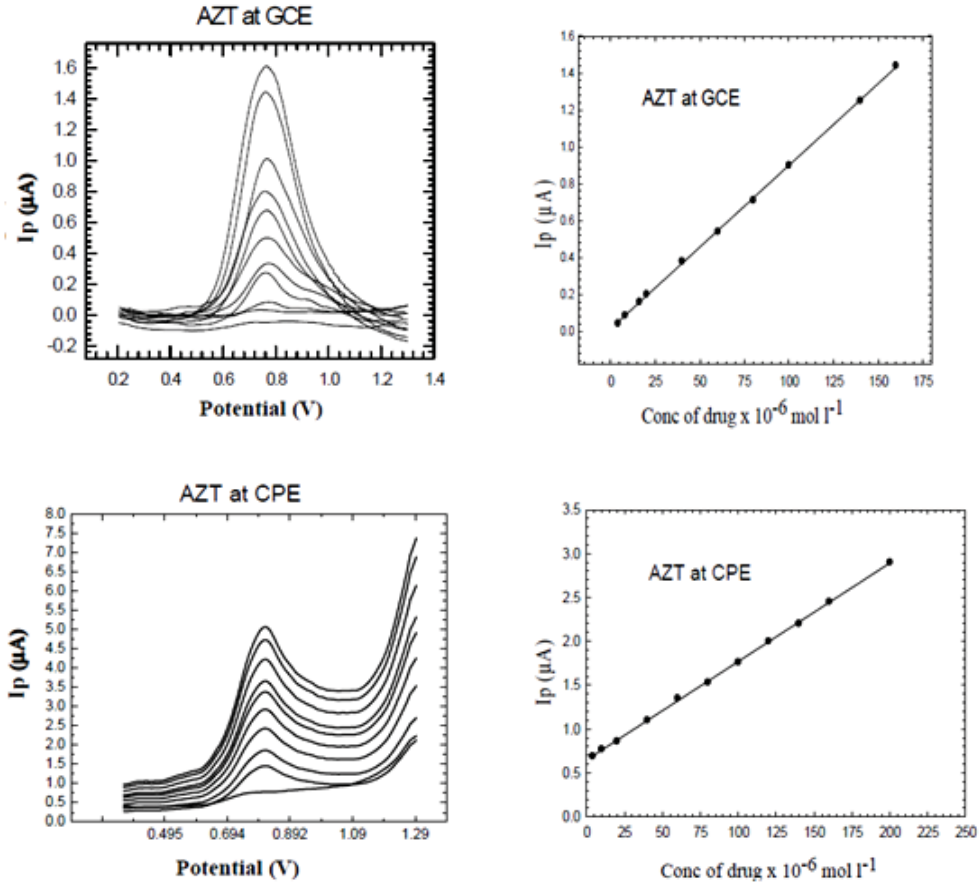


Fig. 6: *DP-voltammograms and the respective calibration curves of Azelastine-HCl at GCE and CPE at a scan rate of 10 mV* s^{-1}.

Method Validation

Limits of Detection (LOD) and Limits of Quantification (LOQ). LOD and LOQ [26] for AZT were calculated and found to be 1.42×10^{-7} and 4.72×10^{-7} mol L^{-1} for GCE, and 1.11×10^{-7} and 3.71×10^{-7} mol L^{-1} for CPE. For EDD, the respective estimates were 1.01×10^{-7} and 3.36×10^{-7} mol L^{-1} for GCE, and 1.23×10^{-7} and 4.11×10^{-7} mol L^{-1} for CPE. All these results are surveyed in Table II (see overleaf).

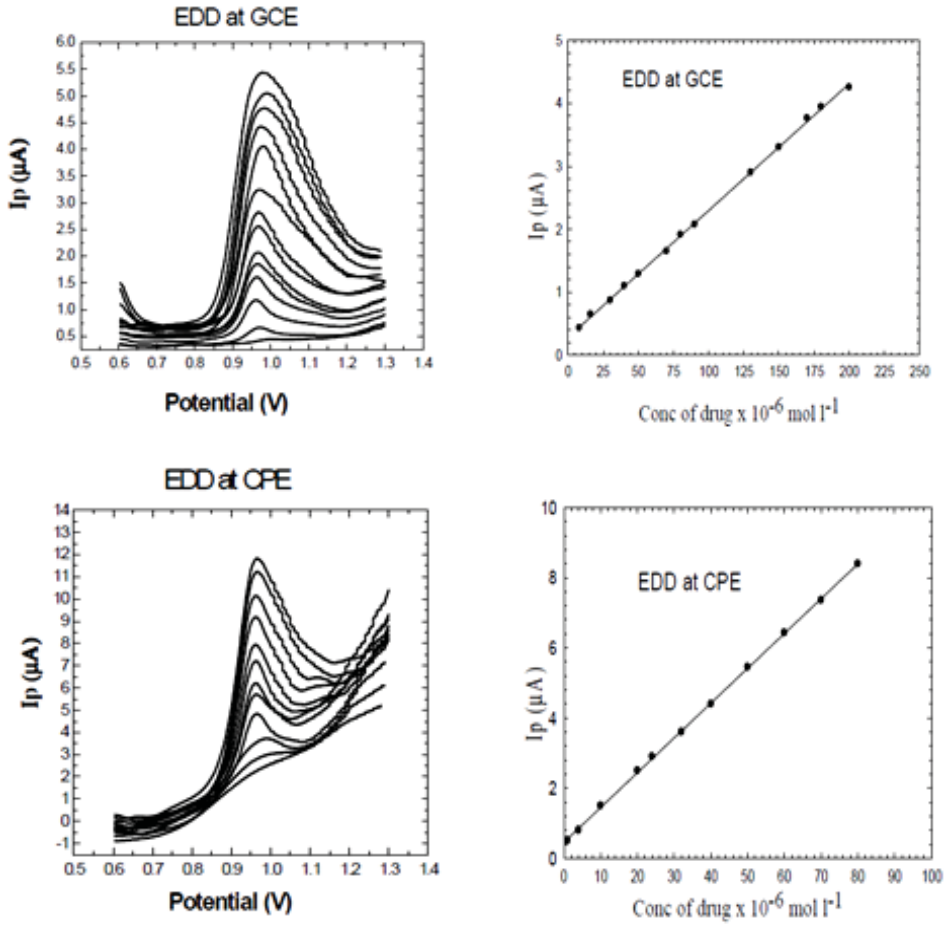


Fig. 7: *DP-voltammograms and the respective calibration curves of Emedastine difumarate at GCE and CPE at a scan rate of 10 mV* s^{-1}.

Accuracy. It was determined by triplicate analyses of the previously mentioned procedure for five different concentrations of both drug substances. The concentrations were calculated from the corresponding regression equations. The mean percentage recoveries and the relative standard deviation (RSD) were evaluated and the results are also given in Table II.

Precision. The intra-day precision was assessed by analyzing five concentration levels in triplicate in a single assay. The inter-day precision was assessed by analyzing the same sample concentration in triplicate, in 3 days; the RSDs were less than ±2 % and being adequate for quality control of *Azelastine*-HCl and *Emedastine* difumarate (see Tables III and IV).

Table II: *Regression data of the calibration curve for the quantitative determination of Azelastine-HCl and Emedastine difumarate at GCE and CPE surfaces by DPV technique.*

Parameters	*Azelastine* HCl		*Emedastine* difumarate	
	GCE	CPE	GCE	CPE
Linearity (mol $L^{-1}*10^{-6}$)	4 – 160	4 – 200	8 – 200	1 – 80
LOD (mol $L^{-1}*10^{-7}$)	1.42	1.11	1.01	1.23
LOQ (mol $L^{-1}*10^{-7}$)	4.72	3.71	3.36	4.11
Slope	0.0089	0.0112	0.0201	0.0993
Intercept	0.0147	0.6483	0.2843	0.4564
Correlation coefficient (r)	0.9999	0.9998	0.9997	0.9999
SE	0.0083	0.0147	0.0336	0.0480
Accuracy[a)] (mean±RSD, %)	100.51±0.814	100.06±0.814	99.65±0.692	100.31±0.344

Notes: a) average of five different determinations.

Specificity. It was confirmed by investigation of the voltammograms of both the standards and the drug test solution. Identical voltammograms were obtained. The addition of the standard drug solution to the test solution did not change the characteristics of the differential pulse voltammogram.

Robustness. The proposed method was also evaluated by the constancy of the peak area values with the deliberated small changes in the experimental parameters, which was realized by the method. The time between preparation of the solutions and the measurement gives an indication about this factor.

Determination of *Azelastine*-HCl and *Emedastine* Difumarate in Drug Products

The proposed DPV method was successfully applied for determination of each *Azelastine* and *Emedastine* in their drug substances and different drug products (*Azelast* eye drops, *Zalastin* nasal spray and, *Emedastine* eye drops) without interference from some common excipients used in pharmaceutical preparations. The results were compared statistically with those obtained with the official and manufacturer methods [1,4,27].

Table III: *Intraday and interday precisions for Azelastine in drug substance.*

Conc. taken (mol L^{-1} *10^{-6})	Intraday precision				Interday precision			
	GCE		CPE		GCE		CPE	
	Found (mol L^{-1} *10^{-6})	Recovery[a] (%)	Found (mol L^{-1} *10^{-6})	Recovery[a] (%)	Found (mol L^{-1} *10^{-6})	Recovery[a] (%)	Found (mol L^{-1} *10^{-6})	Recovery[a] (%)
4	3.98	99.5	3.99	99.75	4.02	100.5	4.04	101.00
40	40.2	100.5	40.05	100.13	39.88	99.7	39.97	99.93
80	80.5	100.63	79.2	99.00	80.4	100.5	80.3	100.38
120	119	99.16	121	100.83	120.5	100.42	120.9	100.75
160	160.5	100.31	160.9	100.56	160.7	100.44	161	100.63
200	-	-	201.4	100.7	-	-	201.4	100.7
Mean recovery ±RSD[b] (%)	100.02±0.651		100.16±0.695		100.31±0.344		100.57±0.369	

Notes: a) average of three determinations; b) average of fifteen determinations.

Table IV: *Intraday and interday precisions for Emedastine difumarate in drug substance.*

Conc. taken (mol L^{-1} *10^{-6})	Intraday precision				Interday precision			
	GCE		CPE		GCE		CPE	
	Found (mol L^{-1} *10^{-6})	Recovery[a] (%)	Found (mol L^{-1} *10^{-6})	Recovery[a] (%)	Found (mol L^{-1} *10^{-6})	Recovery[a] (%)	Found (mol L^{-1} *10^{-6})	Recovery[a] (%)
4	4.01	100.25	-	-	4.02	100.5	-	-
8	7.99	99.88	7.98	99.75	8.01	100.12	8.02	100.25
20	20.1	100.5	20.2	101	19.97	99.85	20.07	100.35
50	50.2	100.4	50.4	100.8	50.3	100.6	50.1	100.2
80	79.7	99.63	79.65	99.56	80.5	100.63	80.7	100.88
140	140.35	100.25	140.8	100.57	140.7	100.5	140.6	100.43
200	201	100.5	-	-	201.6	100.8	-	-
Mean recovery± RSD[b] (%)	100.02±0.651		100.16±0.695		100.31±0.344		100.57±0.369	

Notes: a) average of three determinations; b) average of fifteen determinations.

Table V: *Statistical analysis of the results obtained by the proposed DPV and manufacturer procedures to determine Azelastine-HCl in drug substance and drug products.*

Values	Drug substance			Zalastine Nasal Spray			Azelast Eye Drop		
	GCE	CPE	Official Method[a]	GCE	CPE	Manufac. Method[b]	GCE	CPE	Manufac. Method[b]
Mean	100.51	100.06	100.3	99.97	99.61	99.52	99.73	99.80	99.26
SD	0.818	0.813	0.543	0.532	0.741	1.02	0.821	0.654	1.11
SE	0.364	0.363	0.243	0.238	0.331	0.456	0.367	0.292	0.496
Variance	0.669	0.660	0.295	0.283	0.549	1.04	0.674	0.428	1.232
N	5	5	5	5	5	5	5	5	5
t-test (2.306)[c]	0.479	0.549		0.875	0.159		0.761	0.938	
F-test (6.400)[c]	2.268	2.238		3.675	1.894		1.828	2.878	
Standard addition mean[d] ± RSD (%)				99.90 ± 0.421	100.08 ± 0.354		99.95 ± 0.490	100.03 ± 0.378	

Notes: a) official HPLC method, BP 2010; b) manufacturer's UV-spectrophotometric method; c) the values between parentheses are the theoretical values t and F for p = 0.05; d) mean of five replicates**.**

Table VI: *Statistical analysis of the results obtained by DPV method and official method for the determintation of Emedastine difumarate in its drug substance and product.*

Values	Drug substance			*Emedastine* ophthalmic solution		
	GCE	CPE	Official method[a]	GCE	CPE	Official method[a]
Mean	99.65	100.10	100.3	100.5	100.4	100.6
SD	0.689	0.773	0.819	0.723	0.432	0.938
SE	0.308	0.346	0.367	0.323	0.193	0.419
Variance	0.476	0.598	0.671	0.523	0.187	0.879
N	5	5	5	5	5	5
t-test (2.306)[b]	1.357	0.396		0.189	0.434	
F-test (6.400)[b]	1.409	1.122		1.682	4.701	
Standard addition mean[c] ±RSD (%)				100.04 ± 0.553	99.77 ± 0.627	

Notes: a) HPLC method USP (2011); b) the values between paranthesis are the theoretical values of t and F at p = 0.05; c) mean of five replicates.

The results of the calculated student t-test and variance ratio F-test exclude any significant differences between both methods with respect to accuracy and precision. The validity of the methods was also assured with standard addition technique (Table V and VI).

Conclusions

The use of clean techniques, speed and simplicity of the analytical methods applied to obtain the results are the reasons behind the even growing importance of electroanalytical methods in the quality control of active ingredients for medications. It can be stated that the proposed DPV method can be used successfully to determine *Azelastine*-HCl and *Emedastine* difumarate in drug substances and drug products.

The methods developed were compared with the reported and official methods and it has been found out that they are a satisfactory alternative for the determination of these drugs because of simplicity, low cost, good sensitivity, sufficient accuracy and precision as indicated by the recovery rate analyses, the RSDs obtained, as well as LODs, and LOQs.

The procedure proposed has also shown some distinct advantages, such as short period of real time of drug analysis, and no pretreatment, or time consuming extraction steps are required prior to the analysis. Although carbon paste and glassy carbon electrodes give acceptable results in the analysis of these drugs, we prefer the carbon paste electrode for biological analysis due to its high sensitivity, as well as simpler and quicker preparation for measurements. By the way, this preference corresponds to the still quite high popularity of CPEs in pharmaceutical analysis [29,30].

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