FLOW-INJECTION POTENTIOMETRIC AND CONDUCTOMETRIC DETERMINATION OF PAPAVERINE HYDROCHLORIDE IN THE PARENT SUBSTANCE AND A RELATED PHARMACEUTICAL PREPARATION

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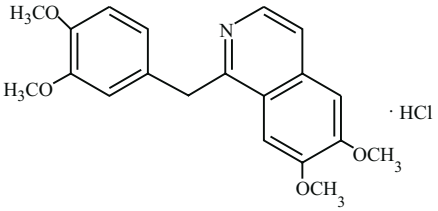
Papaverine (Pap) ion-selective plastic membrane electrodes based on ion associates of papaverine with phosphotungstic acid (Pap – PTA) and phosphomolybdic acid (Pap – PMA) were prepared and fully characterized in terms of membrane composition, life span, pH, and temperature. The proposed electrodes were applied to potentiometeric determination of papaverine in the parent substance and a related pharmaceutical preparation under batch and flow-injection analysis conditions. In addition, conductometric titrations were used for the assay of papaverine in the parent substance and the related pharmaceutical preparation. The selectivity of electrodes with respect to a large number of foreign inorganic cations, amino acids, and sugars was tested. The solubility product of the ion associate and the rate constant of the precipitation reaction leading to the ion associate formation were determined conductometrically.

1. INTRODUCTION

Papaverine hydrochloride (Pap-HCl), 1-(3,4-dimethoxybenzyl)-6,7-dimethoxyisoquinoline hydrochloride [1], is an alkaloid found in opium, which acts as a nonspecific smooth-muscle relaxant and vasodilator. Papaverine acts on the arterioles of all vascular beds, including the cerebral and coronary circulation systems. The drug decreases total peripheral resistance, produces coronary vasodilatation in myocardial ischemia, decreases cerebrovascular resistance, and increases blood flow in the region ischemized as a result of cerebral atherosclerosis. These effects are probably mediated through the potent inhibitory effect of papaverine on phosphodiesterase, which leads to increased concentration of cyclic adenosine monophosphate. Papaverine produces relaxation of smooth muscles in the digestive and biliary tracts and ureters.

Pap-HCl has been determined by various techniques, including chromatography [2, 3], spectrophotometry [4, 5], potentiometry [6 – 9], GC/MS [10], chemiluminescence [11, 12], capillary electrophoresis [13, 14], Raman spectroscopy [15], atomic absorption [16], extractive titration [17], coulometry [18], alkalimetric two-phase titration [19], and cerimetry [20].



Papaverine hydrochloride

In the present study, plastic membrane electrodes selective to Pap cations have been constructed based on the incorporation of papaverine – phosphotungstic acid (Pap – PTA) and papaverine – phosphomolybdic acid (Pap – PMA) ion associates (ion exchangers) in PVC membranes plasticized with dioctylphthalate (DOP). The electrodes were fully characterized under batch analysis conditions and then used to determine the drug under both batch analysis and flow-injection analysis (FIA) conditions. In addition, conductometric determination of Pap-HCl in the parent substance and a related pharmaceutical preparation was performed using PTA and PMA as titrants.

2. EXPERIMENTAL

2.1. Reagents and materials. All chemicals used for the preparation of solutions were of analytical and reagent grade. Doubly distilled water was used for preparing sample solutions and as a mobile medium in flow-injection measurements.

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The carrier and reagent solutions were degassed by means of vacuum suction. Sample solutions used for FIA were freshly prepared before measurements. The parent substance of Pap-HCl and a related pharmaceutical preparation (Vasorin in ampules, 60 mg Pap-HCl/2 ml) were provided the by Memphis Company (Cairo, Egypt).

2.2. Preparation of ion-exchangers. The Pap – PTA (yellowish brown precipitate) and Pap – PMA (faint brown precipitate) ion exchangers were prepared by mixing 100 ml of 1.0×10^{-2} mole/liter of PTA or PMA solutions with 100 ml $\times 10^{-2}$ mole/liter Pap-HCl solution. The target compounds, which were formed after precipitation on standing in a dark place overnight, were filtered, washed with doubly distilled water until chloride free, and dried at room temperature. The products were subjected to elemental analysis for C, H, and N, at the Microanalytical Center (Cairo University). The results of elemental analyses are presented in Table 1.

2.3. Preparation of electrodes. The conventional electrodes were constructed as described previously [21, 22]. The membrane composition was selected by varying the percentage content (w/w) of the ion exchanger, PVC and DOP until optimum conditions were reached. The electrode body was filled by a solution that was 1.0×10^{-2} mole/liter with respect to both KCl and parent drug and preconditioned by soaking in 1.0×10^{-3} mole/liter Pap – HCl solution. An Ag/AgCl wire was immersed in the electrolyte solution to act as the internal reference.

2.4. Apparatus for batch and flow-injection analysis. Potentiometric measurements in the batch analysis mode were carried out with a Jenway 3010 digital pH meter (Jenway LTD, Essex, UK). A Model C-100 circulator thermostat (Techne Co, UK) was used to control the temperature of a test solution. The conductivites were measured using a Jenway 4330 conductivity meter. The electrochemical system can be represented as follows:

Ag/AgCl/filling solution/membrane/test solution/
KCl salt bridge//SCE.

The potential of the ion-selective electrode (ISE) was measured using saturated calomel electrode (SCE) as the external reference electrode under open circuit conditions at a given experimental temperature. The FIA setup comprised an ISM 827 four-channel peristaltic pump (Ismatec, Zurich, Switzerland) and a Model 5020 injection valve with exchangeable sample loop (Rheodyne, Cotati, CA, USA). The electrode was connected to a WTW pMX 2000 microprocessor pH/ion meter and interfaced to a Model BD111 strip chart recorder (Kipp & Zonn, Delft, Netherlands). A wall-jet cell, providing fast response, ease of construction, and compatibility with electrodes of different shapes and size, was used in flow measurements.

2.5. General procedure for conductometric measurements. Various volumes containing 11.27 – 45.10 mg of pure Pap-HCl or the related pharmaceutical preparation (Vasorin) were transferred to 50 ml volumetric flask and diluted to the mark with distilled water. The contents of the volumetric flask were quantitatively transferred to a beaker and the conductivity cell was filled. Then, 1.0×10^{-2} mole/liter PTA or PMA solution was added with a microburette, and the conductance was measured after addition of each portion of the titrant solution with thorough stirring. The conductance readings, taken after 1 – 2 min after each addition, were corrected for solvent dilution [23] by means of the following equation, assuming that conductivity is a linear function of dilution:

$$\Omega_{corr} = \Omega_{ods}[(v_1 + v_2)/v_1],$$

where Ω is the electrolyte conductivity (corr, corrected; obs, observed), v_1 is the initial volume, and v_2 is the volume of the added reagent. A graph of the corrected conductivity versus volume of titrant added was constructed, and the titration end point was determined. One milliliter of 1.0×10^{-2} mole/liter PTA or PMA solution is theoretically equivalent to 11.27 mg Pap-HCl.

2.6. Conductometric determination of the solubility product and the rate constant of ion associate formation. The solubility products and the rate constants of Pap – PTA and Pap – PMA ion pair formation were conductometrically determined as described previously [24]. For this purpose, a

TABLE 1. Elemental Analysis of Papaverine Ion Associates with PTA and PMA

Associate	C%		H%		N%	
	Found	Calcd.	Found	Calcd.	Found	Calcd.
Pap – PTA[a]	20.05	18.74	2.39	2.84	1.19	1.08
Pap – PMA[b]	22.11	21.98	2.32	3.14	1.27	1.28

[a] $[C_{20}H_{22}NO_4]_3[P(W_3O_{10})_4]$, [b] $[C_{20}H_{22}NO_4]_3[PO_4 12MoO_2]$.

TABLE 2. Compositions of Various Papaverine-Containing Membranes and the Slopes of the Corresponding Calibration Graphs

Ion exchanger	Composition, wt.%				Slope, mv/decade	RSD*, %
	Pap – PTA	Pap – PMA	DOP	PVC		
Pap-PTA **	3.0	–	48.5	48.5	58.0	0.46
	5.0	–	47.5	47.5	53.0	0.59
	7.0	–	46.5	46.5	54.5	0.48
	10.0	–	45.0	45.0	50.8	1.00
Pap-PMA**	–	2.0	49.0	49.0	56.0	0.57
	–	3.0	48.5	48.5	54.0	0.71
	–	5.0	47.5	47.5	52.8	0.62
	–	7.0	46.5	46.5	51.5	0.93

* Relative standard deviation (four preparations).
** Optimum composition.

series of Pap-HCl, PTA, and PMA solutions with various concentrations (C) was prepared. The conductivities of these solutions at 25°C were measured and used to calculate the specific conductivities (K) corrected for the effect of dilution, after which the equivalent conductivities (λ) of solutions were determined as $\lambda = 1000K/C$. Straight line plots of λ versus $C^{1/2}$ were constructed, and the equivalent conductivities at infinite dilution were determined for Pap-HCl ($\lambda_{0(\text{Pap-HCl})}$), PTA ($\lambda_{0(\text{Pap}-\text{PTA})}$), and PMA ($\lambda_{0(\text{Pap}-\text{PMA})}$) solutions from the intercept of the corresponding line with the λ axis. The activity coefficients of the ions employed were taken equal to unity because all solutions were sufficiently dilute (from 1.0×10^{-4} to 1.0×10^{-2} mole/liter) and, hence, less affected by changes in the ionic strength of the solution [25]. The values of $\lambda_{0(\text{Pap}-\text{PTA})}$ and $\lambda_{0(\text{Pap}-\text{PMA})}$ were calculated using the Kohlrausch law of independent ion migration [26]. The solubility (S) and solubility product (K_{sp}) of each ion pair were obtained from the following relations:

$$S = K_s \times 1000/\lambda_0;\ K_{sp} = 27S^4,$$

where K_s is the specific conductivity of a saturated solution of Pap – PTA or Pap – PMA determined at 25°C and corrected for the solvent effect. Stirring a suspension of the ion-pair of a saturated solution precipitate in distilled water for 3 h yielded a saturated solution.

3. RESULTS AND DISCUSSION

3.1. Composition of a membrane. In the plastic membrane of ion selective electrodes, the amount of lipophilic salt should be sufficient to ensure reasonable ion exchange at the gel layer/test solution interface, which is responsible for the membrane potential. In addition, the amount of plasticizer should be selected so as to provide a membrane with good physical properties, which simultaneously acts as an effective solvent mediator for the lipophilic ion exchanger salts. An increase in the amount of the plasticizer, on the one hand, improves the adhesive properties of the membrane and, on the other hand, favors deterioration of the membrane depending

TABLE 3. Performance Characteristics of Papaverine-Selective Electrodes at Various Temperatures

Electrode	Temperature, °C	Slope, mV/decade	Working concentration range, mole/liter	E^0, mV
Pap-PTA	25	57.0	1.0×10^{-5}–6.3×10^{-4}	206
	30	58.0	$4.0 \times 10^{-6} – 1.0 \times 10^{-3}$	210
	35	62.0	$1.0 \times 10^{-5} – 2.5 \times 10^{-3}$	214
	40	67.0	$4.0 \times 10^{-6} – 1.2 \times 10^{-3}$	216
	50	72.0	$1.0 \times 10^{-5} – 2.5 \times 10^{-3}$	222
	60	74.0	$1.0 \times 10^{-5} – 2.5 \times 10^{-3}$	222
	70	76.0	$1.0 \times 10^{-5} – 2.5 \times 10^{-3}$	226
Pap-PMA	25	52.0	$1.0 \times 10^{-5} – 2.5 \times 10^{-3}$	202
	30	58.0	$1.0 \times 10^{-5} – 2.5 \times 10^{-3}$	212
	35	58.0	$4.0 \times 10^{-6} – 2.5 \times 10^{-3}$	240
	40	65.0	$4.0 \times 10^{-6} – 2.5 \times 10^{-3}$	252
	50	66.0	$1.0 \times 10^{-5} – 2.5 \times 10^{-3}$	270
	60	70.0	$1.0 \times 10^{-5} – 2.5 \times 10^{-3}$	298
	70	74.0	$1.0 \times 10^{-5} – 2.5 \times 10^{-3}$	324

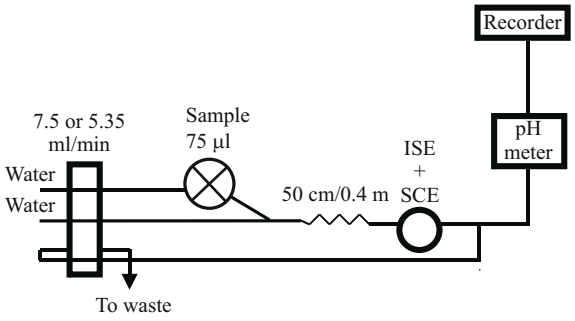

Fig. 1. Schematic diagram of the flow injection system.

TABLE 4. Selectivity Coefficients $[-\log K^{pot}_{\text{Pap},J^{(z+)}}]$ for Papaverine-Sensitive Electrodes under Batch Analysis and FIA Conditions

Interfering agent	Batch analysis				FIA	
	Pap–PTA		Pap–PMA		Pap–PTA	Pap–PMA
	SSM[a]	MSM[b]	SSM[a]	MSM[b]		
Na^+	2.29	3.99	4.47	–	8.50	5.10
K^+	2.56	4.04	4.66	–	6.20	5.70
Ni^{2+}	3.79	–	5.22	–	4.60	4.86
Ca^{2+}	3.87	–	4.58	–	5.70	4.76
Ba^{2+}	3.60	–	5.11	–	6.50	4.46
Sr^{2+}	3.75	–	4.60	–	8.90	5.83
Mg^{2+}	4.81	–	4.69	–	6.20	4.57
Cu^{2+}	3.96	–	4.90	–	5.20	5.57
Co^{2+}	4.01	–	4.94	–	6.50	4.62
Cr^{3+}	4.40	–	6.12	–	8.60	5.95
Maltose	–	4.58	–	5.39	22.50	22.30
Leucine	–	5.10	–	6.30	16.00	16.70
Glucose	–	4.24	–	5.28	25.40	29.20
Fructose	–	5.08	–	6.75	35.00	33.50
Serine	–	5.56	–	6.56	34.90	33.50
Glycine	–	5.48	–	5.58	15.90	19.10
Alanine	–	5.48	–	7.01	23.30	26.80
Vitamin C	–	5.03	–	5.67	18.50	14.80
Vitamin B_1	–	4.80	–	6.09	16.25	12.10
Vitamin B_6	–	5.05	–	5.45	11.80	10.33

[a] Separate solution method, [b] mixed solution method.

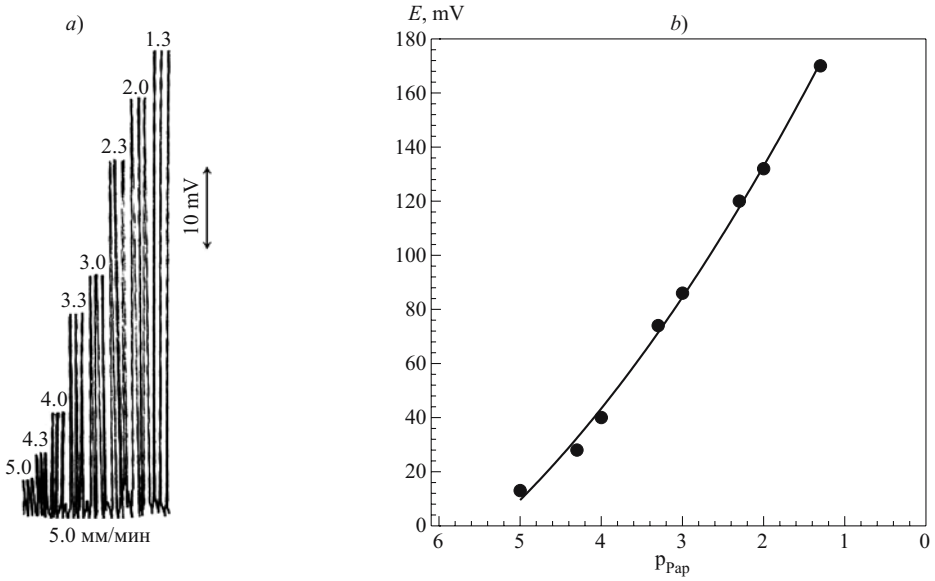

Fig. 2. (*a*) The typical recordings obtained on measuring Pap-HCl solutions of various concentrations (left to right: 1.0×10^{-5}, 5.0×10^{-5}, 1.0×10^{-4}, 5.0×10^{-4}, 1.0×10^{-3}, 5.0×10^{-3}, 1.0×10^{-3}, 5.0×10^{-2}, mole/liter using Pap-PTA electrode under optimum FIA conditions and (*b*) the corresponding calibration graph.

on the properties of both the ion exchanger and the matrix [27, 28]. In this study, the ratio of the plasticizer (DOP) to the matrix (PVC) was kept constant at 1:1, while the amount of ion exchanger was varied. The electrodes under investigation could operate in a broad range of working concentrations, from 1.0×10^{-5} to 2.5×10^{-3} mole/liter. Four different membrane compositions were investigated, with an ion pair content of 3.0 (I), 5.0 (II), 7.0 (III) and 10.0% (IV) for Pap – PTA and 2.0 (I), 3.0 (II), 5.0 (III), and 7.0% (IV) for Pap – PMA. The best performance was achieved using compositions of 3.0% Pap – PTA, 48.5% PVC, 48.5% DOP and 2.0% Pap-PMA, 49.0% PVC and 49.0% DOP. These optimum compositions were used to prepare membrane electrodes for all subsequent investigations. The preparation process was highly reproducible, as revealed by the low relative standard deviation (RSD) values of the slopes (Table 2).

3.2. Effect of soaking. Freshly prepared electrodes must be soaked in order to activate the surface of the membrane by forming an infinitesimally thin gel layer, at which ion exchange takes place. This preconditioning process requires different times depending on the diffusion characteristics and the equilibrium at the electrode/test solution interface; fast establishment of the equilibrium is certainly a condition for a

TABLE 5. Determination of Pap-HCl by Potentiometric Titration Using PTA and PMA as Titrants

Added, mg	Parent substance			Vasorin (60 mg/2 ml ampule)		
	Found, mg	Recovery, %	RSD*, %	Found, mg	Recovery, %	RSD*, %
PTA as titant						
11.27	10.42	92.50	0.35	11.02	97.80	0.69
22.55	21.70	96.25	0.85	21.83	96.85	1.01
33.83	32.98	97.50	0.24	32.95	97.40	0.74
45.10	44.25	98.12	0.12	44.24	98.10	0.55
PMA as titrant						
11.27	10.98	97.50	0.91	10.99	97.60	1.34
22.55	21.70	96.25	0.48	21.83	96.85	0.82
33.83	32.13	95.00	0.56	32.88	97.20	0.69
45.10	45.37	100.60	0.25	44.29	98.21	0.46

* Four determinations.

TABLE 6. Determination of Pap-HCl in Parent Substance Solution and Pharmaceutical Preparation by the Standard Additions Method under Batch Conditions

Added, mg	Pap – PTA			Pap – PMA		
	Found, mg	Recovery, %	RSD*, %	Found, mg	Recovery, %	RSD*, %
Parent substance						
0.94	0.93	98.58	0.59	0.94	99.99	0.35
1.88	1.86	99.28	0.66	1.90	101.10	1.39
9.39	9.31	99.19	0.69	9.18	97.80	0.79
18.79	17.91	95.32	0.23	18.75	99.80	0.13
93.97	95.12	101.23	0.64	91.63	97.52	0.65
Vasorin (60 mg/2 ml ampule)						
0.94	0.94	100.20	0.60	0.92	98.40	0.68
1.88	1.86	99.30	0.21	1.90	101.51	0.56
9.39	9.13	97.21	0.59	9.38	99.90	0.48
18.79	18.88	100.50	0.79	18.12	96.46	0.63
93.97	94.13	100.17	0.30	93.15	99.13	0.69

* Four determinations.

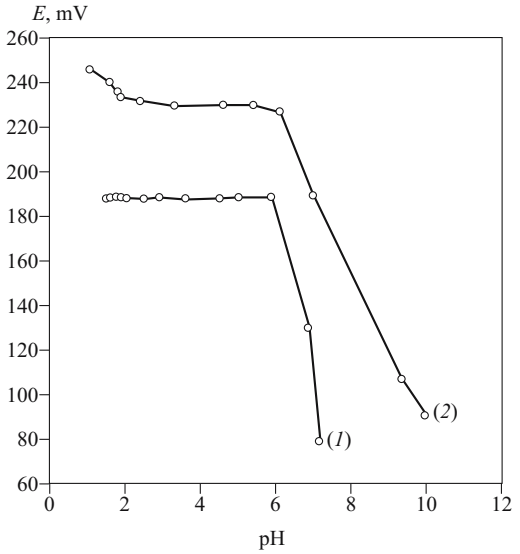

Fig. 3. Effect of pH of a test Pap-HCl solution with a concentration of 10^{-2} mole/liter on the potential response of (*1*) Pap – PTA and (*2*) Pap – PMA electrodes.

fast potential response [29]. For the electrodes used in this study, the soaking time was 1/2 h with a slope of 56.0 and 54.0 mV per concentration decade (mV/decade) for Pap – PTA and PAP – PMA, respectively. Continuous soaking of the electrodes whith 1.0×10^{-3} mole/liter Pap-HCl solution negatively affected their response to papaverine cations. This may be attributed to leaching of the active components (ion exchanger and plasticizer) to the solution. It should be noted that the slopes of the calibration graphs obtained for preconditioned electrodes exhibited almost perfect Nernstian behavior in the first 12 days; then the response decreased gradually to about 50.0 mV/decade after 16 and 14 days and eventually reached 40.0 mV/decade after 25 and 24 days for Pap – PTA and Pap – PMA electrodes, respectively. However, it was noticed that, in all cases, an electrode that had been kept dry in a closed vessel and stored in a refrigerator, showed a good preservation of the slope value and the response properties. Thus, it is recommended that unused electrodes should be kept dry in closed vessels in a refrigerator.

3.3. Effect of the temperature of a test solution. Calibration graphs (electrode potential E_{el} versus p_{Pap}) were constructed for various test solution temperatures (25, 30, 35, 40, 50, 60, and 70°C) for both electrodes. The slopes, working concentration ranges, and the standard electrode potentials (E^0) of the electrodes at each temperature are listed in Table 3. The isothermal coefficient (dE^0/dt) of the electrode and the standard electrode potentials (E^0) against the normal hydrogen electrode (NHE) at various temperatures were obtained from calibration graphs as the intercepts at $p_{Pap} = 0$ after subtraction of the corresponding values of the standard electrode potential (vs. SCE) at these temperatures, and were plotted versus $T - 25$°C, where T is the temperature of a test solution on the centigrade scale. A straight-line plot is obtained according to the Antropov equation [30]:

$$E^0 = E^0 + (dE^0_{(25)}/dt)(t - 25),$$

where $dE^0_{(25)}$ is the standard electrode potential at 25°C.

The slopes of the calibration graphs represent the isothermal coefficients of the electrodes (6.0×10^{-4} and 26.0×10^{-4} V/°C for Pap – PTA and Pap – PMA, respectively). These slopes were compared with the corresponding theoretical values at the given temperature and were found to be in good agreement and, in addition, revealed a fairly high thermal stability of the electrodes within the temperature range investigated. The proposed electrodes were found to be usable up to temperatures reaching 70°C without noticeable

TABLE 7. Statistical Treatment of Data Obtained for the Determination of Papaverine Using Pap-Selective Electrodes in Comparison to Reference Method under Batch Analysis Conditions

Parameter	Official Method	Pap – PTA	Pap – PMA
Parent substance			
Mean recovery	98.60	99.53	99.20
RSD	0.90	0.54	0.63
Probability		0.01	0.05
$F^{5,5}$ value (5.05)		0.36	0.49
t-Value (2.30)		2.72	1.52
Vasorin (60 mg/2 ml ampule)			
Mean recovery	99.19	99.31	99.81
RSD	1.30	0.50	0.57
Probability		0.05	0.05
$F^{5,5}$ value (5.05)		0.15	0.19
t-Value (2.30)		0.39	1.72

TABLE 8. Determination of Pap – HCl by Conductometric Titration Using PTA and PMA as Titrant

Added, mg	Parent substance			Vasorin (60 mg/2 ml ampule)		
	Found, mg	Recovery, %	RSD[a], %	Found, mg	Recovery, %	RSD[a], %
PTA as titrant						
11.27	11.49	102.00	0.94	10.81	96.00	0.82
22.55	22.77	101.00	0.98	22.32	99.00	0.86
33.83	34.39	101.66	0.55	34.39	101.66	1.33
45.10	45.66	101.25	0.32	44.82	99.60	0.75
PMA as titrant						
11.27	10.81	96.00	1.22	11.04	98.00	0.99
22.55	22.32	99.00	1.54	22.36	99.20	0.71
33.83	33.26	98.33	0.45	33.83	100.00	0.73
45.10	44.19	98.00	0.61	45.55	101.00	0.85

[a] Four determinations.

deviation from the Nernstian behavior in entire temperature range studied.

3.4. Optimization of the FIA response. The flow injection technique is a very effective way to improve the performance characteristics of ISEs for various reasons, including the following main factors:

(1) A permanent liquid flow has a conditioning effect on the sensor membrane, leading to a better sensitivity and increasing reproducibility of the emf readings.

(2) The liquid junctions and the flow potentials are stable.

In the present study, the flow injection measurements were carried out in a two-line system, whereby the sample was injected into a distilled water flow, which then merged with another flow of distilled water. In both lines, the same tube size was used, which ensured the same flow rate. The stabilization of the base line potential has been achieved by using both water and buffer electrolyte as sample carrying solutions, but the latter provided lower peak heights, so water was used throughout the whole work as the mobile medium. The junction of the two flows was connected to a detector by 50-cm-long tube with an internal diameter of 0.4 mm. Figure 1 shows the configuration of the system used in these measurements. The dispersion coefficient was found to be 1.20, which was indicative of limited dispersion that favors optimum sensitivity and fast response of the electrode [31].

3.4.1. Effect of injection volume. Samples of various volumes in the range from 20 to 500 μl were injected into the flow. In general, the greater the sample volume, the higher the peak height and the retention time of the sample at the electrode surface, requiring a longer period to reach the steady state and greater consumption of the sample and carrier [32]. A sample loop of 150 μl was used throughout this study, which gave about 98% of the maximum peak height obtained using 500-μl sample loop, but at a shorter time necessary to reach the base line and at a lower consumption of reagents.

3.4.2. Effect of flow rate. The dependence of the peak height and the time to reach the base line on the flow rate were studied. The electrode response to the drug solution with a fixed concentration (1.0×10^{-2} mole/liter) was studied for various flow rates (4.15, 5.35, 7.50, 9.70, 12.50, 17.85, 23.25, 25.00 and 27.00 ml/min). A flow rate of 7.50 ml/min was chosen for both Pap – PTA and Pap – PMA electrodes, providing 99% of the maximum peak height obtained at higher flow rates within a shorter time to reach the baseline and lower consumption of the carrier. Figure 2 shows typical recordings obtained for a Pap – PTA electrode under optimum FIA conditions.

3.5. Electrode response in the FIA regime. In the potentiometeric measurements performed in the flow injection regime, the electrode potential depends on the activity of the main detected ion, which is the main advantage of this technique. On the other hand, the main disadvantage of this detection method is a slow response of the electrode potential to concentration changes, especially when low concentrations are measured. The response depends mainly on the state of the membrane surface in contact with the analyzed solution. An increase in the slope of the calibration graphs was observed in the flow injection regime as compared to the batch analysis (where the potential is measured under conditions very close to the equilibrium at the membrane – solution interface [33]). The slopes of the calibration graphs were 57.2, 58.5, 59.4, 60.0, 62.5, 65.5, 67.7 and 69.9 mV/decade for Pap – PTA and 60.0, 62.5, 65.0, 64.2, 66.0, 66.0, 67.8 and 68.9 mV/decade for Pap – PMA at a flow rate of 4.15, 5.35, 7.50, 9.70, 12.50, 17.85, 23.25, 25.00 and 27.00 ml/min, respectively (compared to 51.5 and 52.4 mV/decade for Pap – PTA and Pap – PMA, respectively, under batch analysis conditions). The working concentration range for both Pap – PTA and Pap – PMA electrodes in the flow injection regime is 1.0×10^{-5} to 5.0×10^{-2} mole/liter. Therefore, under FIA conditions, both electrodes show a wider range of concentrations as compared to that under batch analysis conditions.

3.6. Effect of pH. The effect of pH of a test solution on the electrode potentials was studied under both batch analysis and FIA conditions. In batch measurements, the potential variations in response to pH change were followed by the addition of small volumes of HCl and NaOH (0.1 – 1.0 mole/liter) to the test solutions (1.0×10^{-2}, 1.0×10^{-3}, and 1.0×10^{-4} mole/liter Pap-HCl). In the flow injection regime, a series of 1.0×10^{-2} mole/liter Pap-HCl solutions with various pH values ranging from 1.0 to 10.0 was injected in the flow, and the peak heights representing variations of the potential response with pH were monitored.

It is evident that the Nernstian electrode response is not affected by pH changes in the range from 1.0 to 6.0 under batch analysis conditions, while in the FIA regime, the electrode does not respond to pH changes in the 3.0 – 5.0 range. Nevertheless, at pH values below these intervals, the potential exhibits a gradual decrease that can be related to the interference of hydroxonium ion; the decrease observed at higher pH values is most probably attributed to the formation of the free papaverine base in the solution, leading to a decrease in the concentration of the detected papaverine cation [34]. Figure 3 shows the typical curve illustrating the effect of pH on the potential response of the electrodes studied under batch analysis conditions.

3.7. Selectivity of electrodes. It was shown earlier for solid state membrane electrodes that the apparent selectivity coefficient $K^{pot}_{Pap,J^{(z+)}}$ measured under transient flow injection conditions may differ significantly from that measured under batch analysis conditions [35 – 38]. This was explained by the difference in the time of interaction of interfering species with the membrane surface. This difference increases with increase in the intensity of interaction of these species with the membrane in comparison to the main detected ion. In addition, the interference process is strongly dependent on the rates of diffusion and the exchange reaction of the inter-

fering ion [39]. Therefore, in FIA measurements, where the sample remains in contact with the electrode for a shorter period of time, the apparent selectivity is expected to be different from that found under batch analysis conditions. We have studied the influence of some inorganic cations, sugars, and amino acids on the Pap-HCl electrode.

Under FIA conditions, the values of selectivity coefficients were calculated using the potential values corresponding to the peak heights for the same concentrations of the drug and the interfering species (J^{z+}). Under batch analysis conditions, the separate solution method was applied by measuring the potentials for 1.0×10^{-2} mole/liter of both Pap-HCl (E_1) and the interfering ion (E_2) separately, and the selectivity coefficients $K^{\text{pot}}_{\text{Pap},J(z+)}$ were calculated using the slope of the calibration graph of the test electrode:

$$\log K^{\text{pot}}_{\text{Pap},J^{(z+)}} = \frac{E_2 + E_1}{S} + \log(\text{Pap}) - \log(J^{z+})^{\frac{1}{z}}.$$

A high concentration of interfering ions (1.0×10^{-2} mole/liter) was used to ensure that there would be no interference if lower concentrations than this were present under otherwise the same experimental conditions in both batch and FIA modes. This method is considered the simplest way to evaluate the degree of interference that might take place, and is used to perform measurements in important biological samples such as blood [40]. In this study, the tolerance in using the proposed electrodes for determining papaverine without interference was established for sugars and amino acids, which is related to the fact that the nature of charging of these interfering species is partially due to the induced polarity inside the molecule. The mixed solution method, which is time consuming owing to the need to prepare many solutions and performing many steps, was used only as a confirmation in cases when $-\log K^{\text{pot}}_{\text{Pap},J^{(z+)}} < 3.0$.

The selectivity coefficients of the proposed electrodes (Table 4) show evidence for a very high selectivity of these electrodes with respect to papaverine cations under both batch analysis and FIA conditions. The results obtained for amino acids and sugars under FIA are in good agreement with those obtained under batch analysis conditions.

3.8. Analytical applications. Pap-HCl was determined potentiometrically using the proposed electrodes under batch analysis conditions by both potentiometric titration and the standard addition method. The results of the determination of papaverine in the parent substance and the pharmaceutical preparation (Vasorin) by potentiometric titration method are presented in Table 5.

The standard addition method was applied by adding a small portion of 1.0×10^{-2} mole/liter standard Pap-HCl solution to 50 ml distilled water containing various concentrations of the parent substance or the pharmaceutical preparation (from 0.09 to 1.88 mg). A change in the millivolt response was recorded after each addition and used to calculate the concentration of Pap-HCl sample solutions. The required volumes of the preparation at various concentrations were taken and diluted to 50 ml with distilled water. The results of the determination of papaverine in the parent substance and the pharmaceutical preparation (Vasorin) by standard addition method are presented in Table 6.

The results of analyses using the standard addition method were compared with the data provided by the official method in terms of the *F*- and *t*-tests [41]. The results are shown in Table 7. A comparison of the obtained *F* and *t* values with the theoretical values shows that the proposed method does not exhibit significant differences in comparison to the official method, which confirms the accuracy and precision of the proposed method.

Table 8 shows the results of papaverine determination in the parent substance and the pharmaceutical preparation (Vasorin in ampules) by the conductometric technique.

In addition, the proposed method was compared to some other potentiometric methods used for the determination of papaverine. The results of comparison are given in Table 9. The electrodes proposed in this study show a wide range of selectivity with respect to a large number of inorganic cations, amino acid and sugars when compared with the two other methods.

3.9. Solubility products of Pap – PTA and Pap – PMA ion associates. The determination of the solubility product of ion associates is important, since the reciprocal value is ap-

TABLE 9. Comparison Between the Present Method and Other Referenced Methods Used for Potentiometric Determination of Papaverine Hydrochloride

Method	Usable concentration range, mole/liter	pH	Recovery%
Method 1	$1.0 \times 10^{-5} - 2.5 \times 10^{-3}$	1.0 – 6.0	92.50 – 100.60%
Method 2	$1.0 \times 10^{-5} - 5.0 \times 10^{-2}$	3.5 – 5.5	97.0%
Method 3	$1.0 \times 10^{-5} - 1.0 \times 10^{-3}$	–	–

Method 1 the present work (results of potentiometric titrations under batch conditions).
Method 2 determination of papaverine by liquid membrane ion-selective electrode [8].
Method 3 determination of papaverine and ethaverine using ion-selective electrode based on ion associates of papaveine and ethaverine with tetraphenylborate [9].

proximately equal to the rate constant of the formation of ion associates, which is tightly related to the degree of hydrophobicity of these species. As the hydrophobicity increases, the process of leaching to the aqueous bathing solution (which is the main factor determining the lifetime of the electrode membrane) decreases. The solubility products of Pap – PTA and Pap-PMA were determined as described in the experimental part. The corresponding values are 4.78×10^{-9} and 3.24×10^{-9} for Pap – PTA and Pap – PMA, respectively. These values are indicative of a very low solubility of both ion associates (3.64×10^{-3} and 1.20×10^{-3} mole/liter for Pap – PTA and Pap – PMA, respectively). Accordingly, the rate constants for the Pap_3 – PTA and Pap_3 – PMA reactions are 2.09×10^8 and 3.08×10^8, respectively, revealing that the degree of completeness of these reactions exceeds 99.9%. In the above equilibria, the solubility of undissociated ion pairs in water (i.e., the intrinsic solubility) is ignored, since it provides a negligibly small contribution to the total solubility. The ion associates are sparingly soluble in water, and their saturated solutions are very dilute [42].

4. CONCLUSIONS

The results of application of the new electrodes to the determination of papaverine hydrochloride in the parent substance and the pharmaceutical preparation (Vasorin in ampules) showed that the proposed method is characterized by high precision and repeatability in comparison to the official method. In addition, the proposed technique is simple, rapid, highly sensitive, selective, and inexpensive, and does not require any sophisticated instruments and unavailable reagents. So, it can be used for routine analysis and verification in quality control and quality assurance during manufacture of papaverine and related pharmaceuticals, as well as for checking the stability of these drugs on storage. In addition, FIA conditions shorten the time needed for the determination and extend the limits of determination to higher concentrations of the drug in both parent substance and related pharmaceutical preparations.

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