Hindawi Publishing Corporation
Journal of Automated Methods and Management in Chemistry
Volume 2008, Article ID 586310, 11 pages
doi:10.1155/2008/586310

Research Article

Flow Injection Potentiometric Assay of Hexoprenaline in Its Pure State, Pharmaceutical Preparations, and Biological Samples

Rasha M. El-Nashar[1, 2]

[1] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Biotechnology, The German University in Cairo, Main Entrance of El Taghamoo El Khamess, New Cairo City, Cairo, Egypt*
[2] *Department of Chemistry, Faculty of Science, Cairo University, Giza, Egypt*

Correspondence should be addressed to Rasha M. El-Nashar, rasha.elnashar@guc.edu.eg

Received 28 October 2007; Accepted 07 April 2008

Recommended by Peter Stockwell

Different hexoprenaline (Hx_2SO_4) conventional and coated wire electrodes were constructed and evaluated. Membranes were based on hexoprenalinium phosphotungstate (Hx-PTA) and hexporenalinium phosphomolybdate (Hx-PMA). The electrodes were fully characterized in terms of their composition, response time, life span, pH, and temperature and then were applied to the potentiometric determination of the hexoprenalinium ion in its pure state, pharmaceutical preparations, and biological samples, urine and plasma, under batch and flow injection conditions. The selectivity of the electrodes towards many inorganic cations, sugars, amino acids, and some other brochodilatures of close chemical composition was also tested.

1. INTRODUCTION

Hexoprenaline sulphate, N,N′-Hexamethylene bis [2-amino-1-(3,4-dihydroxy-phenyl)ethanol] sulphate [1], [CAS-32266-10-7], is a selective β-sympathomimetic agent which has a double action. It can be used as a bronchospasmatic agent that can reduce bronchial secretion and promotes the efficiency of the bronchial epithelium as well as being used as a sympathomimetic agent that relaxes the uterus through decreasing or arresting both the frequency and intensity of the uterine contraction, thus inhibiting both the spontaneous and the oxytocin-induced labour [2].

A limited number of methods are available in literature for the determination and assay of hexoprenaline in its pure state or pharmaceutical preparations including colorimetric determination using $NaNO_2$ in alkaline medium [3] and HPLC [4].

Ion-selective electrodes have been increasingly used for quantitative measurement of drugs. Potentiometric methods based on this technique are simple, rapid, and offer enough selectivity towards the drugs in the presence of various pharmaceutical excipients [5, 6]. Yet, there is no literature data available about the use of ion-selective electrodes for the determination of hexoprenaline.

In this study, plastic membrane electrodes (conventional) for Hx-cation have been constructed based on the incorporation of either Hx-PTA or Hx-PMA ion exchanger in polyvinyl chloride (PVC) membranes plasticized with dioctylphthalate (DOP). The electrodes were fully characterized in batch conditions and then used for the determination of Hx-cation in its pure state, pharmaceutical preparation, and biological samples, both in batch and flow injection (FIA) techniques, which are considered to be a very efficient way of automation and improving the performance of the characteristics of ion-selective electrodes. In flow analysis, a high-sampling rate can be attained within a short period of time besides being able to handle micro volumes of different concentrations with equivalent accuracy and precision to batch conditions. Also, coated graphite, silver, copper, and platinum wire electrodes were also constructed and applied to assay the above-mentioned samples under both conditions (batch and FIA).

2. EXPERIMENTAL

2.1. Reagents and materials

All chemicals used for preparation of solutions were of analytical grade. Doubly distilled water was used for preparing solutions and as a flow stream in FIA measurements. The carrier and reagent solutions were degassed by means of vacuum-suction pump. Sample solutions used for injections were freshly prepared prior to measurements. Pure grade hexoprenaline sulphate and its pharmaceutical preparations (Asmadol, tablets 0.5 mg/tablet and Gynipral, 0.5 mg/tablet) were provided by the Arab Drug Company, ADCo, Egypt.

2.2. Apparatus

The potentiometric measurements in batch mode were carried out with a Schott-Gerate CG 820 pH-meter (Hofheim, Germany) and WTW microprocessor pH/ion meter pMX 2000 (Weilheim, Germany). A Techne circulator thermostat Model C-100 (Cambridge, England) was used to control the temperature of the test solutions. A WTW packed saturated Calomel (SCE) was used as an external reference electrode. The electrochemical system may be represented as follows.

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

The flow injection setup was composed of a 4-channel peristaltic pump (Ismatec, ISM 827), (Zurich, Switzerland), injection valve model 5020 with exchangeable sample loop from Rheodyne (Cotati,California, USA). The working electrodes were connected to WTW microprocessor pH/ion meter pMX 2000 (Weilheim, Germany) and interfaced to a strip chart recorder Model BD111 from Kipp and Zonn (Deflt, Netherlands).

A wall-jet cell, providing low-dead volume, fast response, good wash characteristics, ease of construction, and compatibility with electrodes of various shapes and sizes, was used in flow measurements where a Perspex cup with axially positioned inlet polypropylene tubing is mounted at the sensing surface of the electrode body. The optimized distance between nozzle and the sensing surface of the electrode was 5 mm; this provides the minimum thickness of the diffusion layer and consequently a fast response [7]. The ion-selective electrode with flow cup, reference electrode (SCE), and the outlet tube were placed in a beaker, where the level of solution was kept 1 cm above the electrode surface. This arrangement shields the membrane from the solution in the beaker, and the electrode response is, therefore, determined solely by the carrier stream. Any disturbance in the flow pattern, for example, a short interruption of the liquid flow or a strong convection current within the beaker immediately results in erratic performance owing to backmixing [8]. Figure 1 represents a schematic diagram of the flow injection system used in measurements.

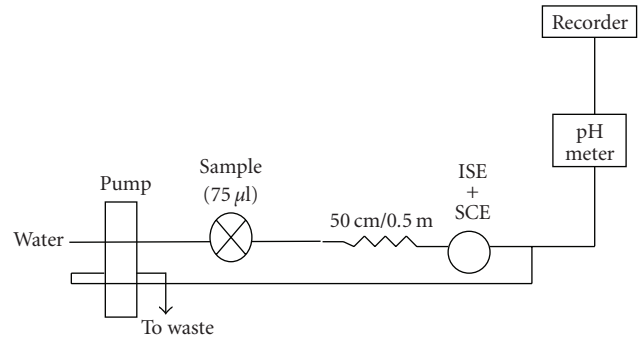

Figure 1: Schematic diagram of the flow injection system used in measurements.

2.3. Preparation of the ion exchangers

The ion exchangers, hexoprenalinium-phosphotungstate [Hx_3-PMA], (buff powder), and hexoprenalinium-phosphomolybdate [Hx_3-PMA], (yellowish green powder), were prepared by the addition of 150 cm^3 of 10^{-2} M hexoprenaline sulphate (Hx_2SO_4) solution to 100 cm^3 of 10^{-2} M of each of phosphotungstic acid or phosphomolybdic acid, respectively. The precipitates were filtered, washed thoroughly with distilled water until sulphate free and then air dried. The chemical composition of the precipitates was identified and confirmed by elemental analysis (C, H, N, S) at the microanalysis unit, Cairo University, Egypt.

2.4. Preparation of conventional and coated wire electrodes

The conventional-type electrodes were constructed as previously described [9]. The membrane composition was studied by varying the percentages (w/w) of the ion exchanger(s), PVC, and DOP until optimum composition that exhibits the best performance characteristics, and Nernestian behavior is reached. A 5 mm diameter disc was cut out from the prepared membrane and attached to a Phillip's body filled with a solution that is 10^{-2} M with respect to both NaCl and Hx_2SO_4 and preconditioned by soaking in 10^{-3} M Hx_2SO_4 solution.

The coated wire electrodes were prepared using a graphite rode and platinum, pure silver and pure copper wires, 15 cm length and 5 mm diameter each. One of the two ends of the rode or wire is used for connection while the other, about one cm length, is dipped in a solution of the same optimum membrane composition used for the conventional electrode, which was previously mixed and the solvent was evaporated slowly until an oily concentrated mixture is formed. Two drops of this mixture are then introduced and spread on the surface of the solid electrode and then is kept to dry at room temperature for about 24 hours.

2.5. Potentiometric determination of Hx_2SO_4

In batch measurements, Hx_2SO_4 has been determined potentiometrically using the investigated electrodes by both the interpolation and standard additions methods. In the latter, small portions (0.1 cm^3) of standard 10^{-2} M Hx_2SO_4 solution were added to 50 cm^3 water-containing concentrations (10^{-6} – 10^{-2} M Hx_2SO_4 covering the range (5.186–518.600 mg) of pure compound or their equivalents from

pharmaceutical preparations. The change in mV readings was recorded after each addition and used to calculate the concentration of Hx_2SO_4 sample solution using the following [10]:

$$C_x = C_s \left(\frac{Vs}{V_x + V_s}\right)\left(10^{n(\Delta E/s)} - \frac{V_x}{V_x + V}\right)^{-1}, \quad (1)$$

where C_x and V_x are the concentration and the volume of the unknown, respectively, C_s and V_s the concentration and the volume of the standard, respectively, S the slope of the calibration graph, and ΔE is the change in millivolt due to the addition of the standard.

For sampling of tablets, (Asmadol, tablets 0.5 mg/tablet, and Gynipral, 0.5 mg/tablet), 20 tablets were ground together and appropriate weights of each were taken as samples. The required amount (5.186–518.600 mg) was dissolved in 0.1 M HCl, (about 0.1 cm^3/mg tablets) and completed to 50 cm^3 with distilled water, and then the standard additions technique was applied as described above.

For preparation of biological samples (plasma and urine), fresh whole blood was centrifuged at 3000 rpm for 10 minutes. The supernatant was transferred to a test tube and used as blank plasma. Different amounts of Hx_2SO_4 (5.186–518.600 mg) and 1 mL plasma or 5 mL urine were transferred to a 100 mL volumetric flask and completed to the mark with 0.01 M HCl; these solutions were measured using the standard additions method.

In FIA, the peak heights obtained by a series of solutions of tablets or biological fluids were compared to those obtained from a standard series of solutions prepared using the pure drug and then used for calculating the recovery percentages of Hx_2SO_4 in tablets and biological samples.

3. RESULTS AND DISCUSSION

3.1. Optimization of the ISE response in batch conditions

3.1.1. Composition of the membranes

Several membrane compositions were investigated in which the content of ion exchanger ranged from 1.0 to 20.0% of Hx-PTA and Hx-PMA. For each composition, the electrodes were repeatedly prepared four times. The preparation process was highly reproducible as revealed from the low relative standard deviation (RSD) values of the slopes obtained employing the prepared membranes (the mean RSD was about 0.98%).

The best performances were obtained by using compositions containing 10.0% Hx-PTA, 45.0% PVC, and 45.0% DOP; or 5.0% Hx-PMA, 47.5% PVC, and 47.5% DOP for Hx-PTA and Hx-PMA electrodes, respectively. These compositions were also used to prepare the coated wire electrodes. The usable concentration range for the prepared electrodes was 5.5×10^{-6}–1.0×10^{-2} M.

3.1.2. Effect of soaking

Any plastic membrane electrode needs a preconditioning by soaking in the respective ion solution before use. This process activates the formation of a thin gel layer at the membrane surface at which the exchange process can take place. The time of preconditioning varies according to the physical properties of the membranes and depends on diffusion and equilibrium at the interface of the membrane and the soaking solution. Fast establishment of equilibrium is certainly a sufficient condition for fast response [11]. In this work, the presoak time ranged from 1/2 up to 2 hours depending on the membrane nature. For the coated wire electrodes, the electrodes were left to dry in air for 24 hours before use to stabilize the formed membrane layer.

Nevertheless, the continuous soaking of the electrodes in 10^{-3} M Hx_2SO_4 solution affects negatively their response to the hexoprenalinium cation, which can be attributed to leaching of the active ingredients [ion exchanger(s) and solvent mediator] to the bathing solution [12].

For the conventional-type electrodes, it was noticed that the slopes of the calibration graphs obtained using the preconditioned electrodes remained almost constant for 10 days and then to decrease gradually to 50.0 mV per concentration decade after 6 weeks and reaching about 45.0 mV per concentration decade after 8 and 9 weeks of continuous soaking for Hx-PTA and Hx-PMA electrodes, respectively.

For the coated graphite electrodes, the slopes of the calibration graphs were constant for the first 5 days and then decrease to about 50.0 mV per concentration decade after 7 and 6 days and reaching 45.0 mV per concentration decade after 15 and 12 days for Hx-PTA and Hx-PMA electrodes, respectively. For the coated copper electrodes, the slopes were constant for the first 5 days and decreased gradually reaching 50.0 mV per concentration decade after 7 and 9 days reaching 45.0 mV per concentration decade after 10 and 11 days for Hx-PTA and Hx-PMA electrodes, respectively. For the coated silver electrodes, the slopes were constant for the first 2 days and decrease gradually reaching 50.0 mV per concentration decade after 4 and 6 days reaching 45.0 mV per concentration decade after 7 days for Hx-PTA and Hx-PMA electrodes, respectively. For the coated platinum electrodes, the life span was limited to only 48 hours of continuous soaking for the two electrodes.

This variation in properties is highly related to the nature of the membranes and their adherence and interaction with the different supporting electrodes. Also, it can be correlated with the diffusion and partition coefficients of the ion exchangers and the plasticizer [13–16].

It was noted that in all cases electrodes which had been kept dry in a closed vessel and stored in a refrigerator showed nearly constant slope values and the same response properties extending to several months. Table 1 shows the response characteristics of the prepared electrodes.

3.1.3. Effect of temperature of the test solution

Calibration graphs [electrode potential (E_{elec}) versus pHx] were constructed at different test solution temperatures (25, 30, 40, 50, 60, and 70°C) for all the electrodes. For the determination of the isothermal coefficients (dE°/dt) of the electrodes, the standard electrode potentials (E°) against

Table 1: Response characteristics of the electrodes under investigation.

Electrode	Min Presoak time (h)	Slope mV/concentration decade	Response time (s)	Lifespan
Hx-PTA conventional (I)	1/2	58.7	30	8 weeks
Hx-PTA graphite (II)	1.0	57.3	25	2 weeks
Hx-PTA Copper (III)	1.0	57.0	20	10 days
Hx-PTA Silver (IV)	1.5	56.4	20	7 days
Hx-PTA Platinum (V)	1.5	55.0	20	48 hours
Hx-PMA Conventional (I)	1.0	59.3	30	9 weeks
Hx-PMA graphite (II)	2.0	58.5	25	12 days
Hx-PMA Copper (III)	1.5	58.0	20	11 days
Hx-PMA Silver (IV)	1.5	55.4	20	7 days
Hx-PMA Platinum (V)	1.5	55.0	20	48 hrs

normal hydrogen electrode, at the different temperatures, were obtained from the calibration graphs as the intercepts at pHx = 0 (after subtracting the corresponding values of the standard electrode potential of the Calomel at these temperatures) and plotted versus $(t - 25)$, where t is the temperature of the test solution in °C (Figure 2). A straight line plot is obtained according to the following [17]:

$$E^\circ = E^\circ_{(25)} + \left(\frac{dE^\circ}{dt}\right)(t - 25). \quad (2)$$

The slopes of the straight lines obtained represent the isothermal coefficients of the electrodes which were found to be 7.6×10^{-4} and 1.1×10^{-4} V/°C for Hx-PTA and Hx-PMA, respectively. These slopes were compared with the theoretical values at the given temperature and found to be in a good agreement to them revealing a fairly high thermal stability of the electrodes within the investigated temperature ranges. The investigated electrodes were found to be usable up to temperatures reaching 70°C without any deviation from the theoretical Nernstian behavior at these temperatures.

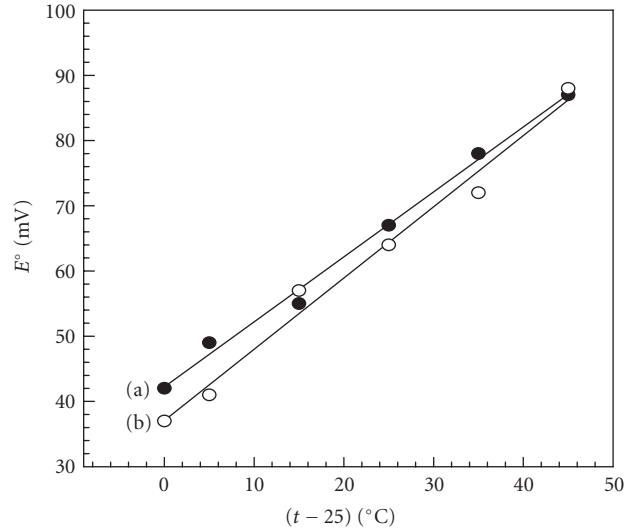

Figure 2: Variation of E° of Hx-PTA (a) and Hx-PMA (b) graphite electrodes with temperature.

3.2. Optimization of FIA response

There are many important variables that affect the response of an ion-selective electrode on operation in FIA conditions. These factors should be studied and taken into consideration on designing the flow system, the most important of which are dispersion coefficient, sample volume, flow rate, and carrier composition.

3.2.1. Dispersion coefficient

Dispersion coefficient (D) is one of the most important factors to be taken into consideration on constructing a FIA system because it shows how much the original sample solution is diluted on its way towards the sensor, and how much time has elapsed between the sample injection and readout.

In case of potentiometric detection using ISE, limited dispersion, (D = 1–3) is preferable because the original composition of the sample should be measured and the sample should only be diluted to the detection limit of the ISE [18, 19]. The dispersion coefficient, determined by measuring the ratio between the peak height obtained at steady state conditions (where the sample acts as carrier stream) and at the state of maximum peak height, maximum dispersion, (where the sample is injected in the carrier stream), was found to be 1.2 and this value is affected by many parameters as sample volume, flow rate, and channel geometry.

3.2.2. Sample volume

Samples of different volumes (20.0, 37.5, 75.0, 150.0, 340.0, and 500.0 μl) were injected; changing the injected sample volume is a powerful way to affect the dispersion process. An increase in the peak height, sensitivity of measurement, and longer residence time of the sample at the electrode surface can be achieved by increasing the volume of the injected sample solution, but this requires longer time to reach a steady state after each injection and higher sample consumption [20]. A sample loop of size 75.0 μl was used

through out this work giving about 95% of the maximum peak height obtained by a 500.0 μl loop but with a shorter time to reach the baseline and of course less consumption of reagents.

3.2.3. Flow rate

The dependence of the peak heights and time required to recover the baseline on flow rate was studied where the response of the electrodes under investigation to a solution, that is, 1.0×10^{-2} M Hx_2SO_4, was studied at different flow rates (4.15, 5.35, 7.50, 9.70, 12.50, 17.85, 23.25, 25.00, 27.00, and 30.0 mL/min).

With constant injection volume, the residence time of the sample is inversely proportional to the flow rate [21]. Therefore, low flow rate would seem most likely to produce a steady state signal but will also lead to increased response time due to increased residence time of the sample at the active membrane surface (thicker boundary layers).

It was found that, as the flow rate increased, the peaks become higher and narrower until a flow rate of 23.25 mL/min, where the peaks obtained at higher flow rates are nearly the same. A flow rate of 7.50 mL/min, which led to 95% of the maximum peak height obtained by higher flow rates, was used for Hx-conventional-type electrodes, while a flow rate of 5.35 mL/min was used for the Hx-coated wire electrodes offering a higher residence time at the surface of the metallic electrodes.

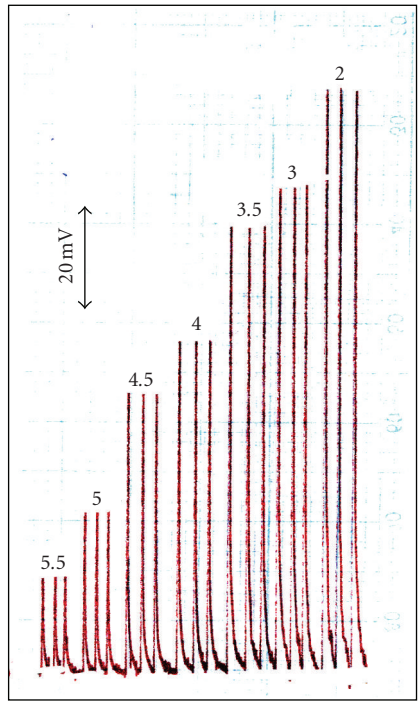

Figure 3: Recordings obtained for hexoprenaline solution having pHx = 5.5–2.0 using Hx-PTA conventional electrode at optimum FIA conditions.

3.2.4. Carrier composition

The composition of the carrier should be as similar as possible to that of samples; this is highly advantageous for baseline stability, response time, and characteristics [22]. The baseline attainment for the studied electrodes takes a very short time.

A two-line configuration FIA system was used to study the effect of pH and addition of main ion carrier in case of any need for baseline stabilization. It was found that the addition of a small concentration of the studied drugs (1.0×10^{-3}–1.0×10^{-5} M) as a carrier stream in the second channel of the flow system did not affect this stabilization time but only led to a small increase in the peak heights and higher consumption of reagents, that is, why only water was used as a carrier in a single-channel manifold FIA system throughout the present work.

3.2.5. Electrode response in FIA

In potentiometric detection, the electrode potential depends on the activity of the main sensed ion. This can be considered as a principle advantage of this method; also in flow measurements, the dependence is semilogarithmic over a wide analyte activity range according to the Nickolsky-Eisenman equation, but the main unfavorable feature of this detection is the slow response of the electrode potential to concentration change, and this is pronounced when low concentrations are measured which in turn depends on the state of the membrane surface at the interface with the measured solution [23]. This slow response is a quite good reason for the super-Nernstian sensitivities obtained in FIA measurements using the investigated electrodes at different flow rates.

An increase in the slope of the calibration plots in FIA was observed compared to batch measurements, where potential is measured in conditions very close to the equilibrium at membrane solution interface [24]. The slopes of the calibration graphs ranged from 59.7 to 77.0 mV/concentration decade for the studied electrodes. The variation of calibration graph slope with flow rate is shown in Table 2. Figure 3 is a representative for the recording obtained on measuring solutions having pHx of 5.5–2.0 using Hx-PTA conventional electrode at optimum FIA conditions.

3.3. Effect of pH

For batch measurements, the effect of pH on the potential readings of the electrodes was studied by varying the pH of the test solution of different concentrations (1.0×10^{-4}, 1.0×10^{-3}, and 1.0×10^{-2} M) of the pharmaceutical compounds gradually and measuring the respective potential readings. For FIA measurements, the effect of pH of the test solution on the electrode potentials was studied by preparing a series of solutions of concentration, that is, 1.0×10^{-4}, 1.0×10^{-3}, and 1.0×10^{-2} M of the drug and pH ranging from 1.0–10.0 which were then injected in the flow stream and the peak

Table 2: Variation of the slopes of the calibration graphs (mV/concentration decade) with flow rate.

Flow rate	4.15	5.35	7.50	9.70	12.50	17.85	23.25	25.00	27.00	30.00
Electrode	Slope (mV/ concentration decade)									
Hx-PTA (I)	63.1	64.5	**65.4***	68.2	69.7	72.4	75.4	75.9	76.3	77.0
Hx-PTA (II)	61.0	**61.9***	62.7	64.5	66.8	68.2	69.6	70.4	71.2	72.6
Hx-PTA (III)	62.5	**64.1***	64.9	65.8	66.3	67.2	68.4	69.3	70.3	71.2
Hx-PTA (IV)	61.5	**62.7***	63.5	64.9	66.7	67.3	68.2	69.0	69.2	70.3
Hx-PTA (V)	63.3	**64.5***	66.2	67.5	68.3	69.7	70.4	71.2	71.9	72.3
Hx-PTA (I)	59.7	60.4	**61.8***	63.0	64.7	66.2	67.1	67.8	68.0	69.3
Hx-PTA (II)	61.4	**63.2***	64.5	65.7	67.1	68.4	69.5	69.8	70.2	70.7
Hx-PTA (III)	62.5	**63.7***	64.6	65.9	67.2	68.3	69.8	70.3	70.9	70.6
Hx-PTA (IV)	61.3	**62.4***	63.5	65.0	66.2	67.8	68.9	70.4	71.5	73.0
Hx-PTA (V)	62.0	**63.1***	63.9	65.7	66.8	68.1	69.2	70.3	70.9	71.6

*Slop at flow rate selected as optimum working condition.

heights, representing variation of potential response with pH were recorded.

In both conditions, the change in pH does not affect the potential readings or peak heights, in FIA conditions, within the pH range 2.5–8.0. In this range, the electrodes can be used safely for the respective determination. At pH values lower than this range, the potential readings and the peak heights decrease gradually with pH which may be due to the penetration of the hydronium ion into the membrane gel layer. While at pH higher than the given ranges, the potential readings and the peak heights decrease gradually which can be attributed to the formation of the free base of the drug and disappearance of the protonated species [25]. Figure 4 is a representative graph for the effect of pH on the recordings of a solution, that is, 1.0×10^{-3} M hexoprenaline measured using the five Hx-PTA electrodes under optimum FIA conditions.

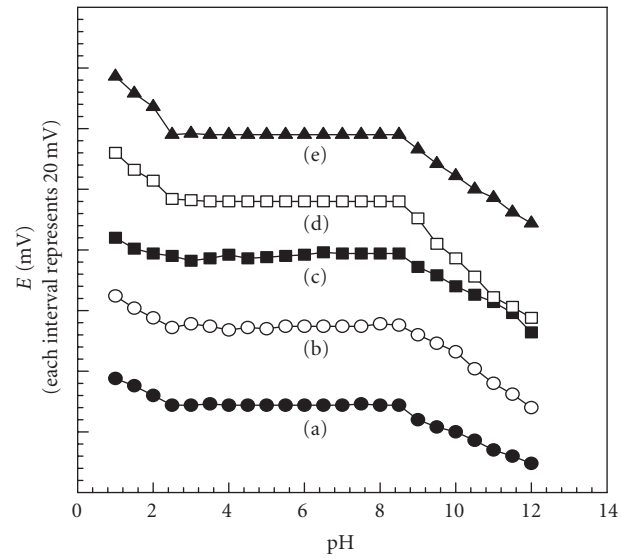

Figure 4: Effect of pH of the test solution of concentration 1×10^{-3} M hexoprenaline on the potential response of a-Hx-PTA(I), b-Hx-PTA(II), c-Hx-PTA(III), d-HX-PTA(IV), and e-Hx-PTA(V) electrodes at optimum FIA conditions.

3.4. Selectivity of the electrodes

The selectivity coefficient $K^{pot}_{Drug,J^{Z+}}$ is the main source of information concerning interferences on the electrode response. In analytical applications, the coefficients must be very small so that the electrode exhibits a Nernstian dependence on the primary ion over a wide concentration range.

The response of the electrodes towards different substances and ionic species such as inorganic cations, amino acids, sugars, and bronchodilators pharmaceutical compounds of close chemical structure to hexoprenaline such as salbutamol, terbutaline, and orciprenaline sulphates was checked in both batch and FIA conditions, and the values of the selectivity coefficients, shown in Table 3, were used to evaluate their degree of interference.

For many years, the method of determination of selectivity of membrane electrodes in potentiometric measurements was a subject of discussion in the analytical literature [26–28]. In ideal case, this should reflect the physicochemistry of the processes involved in the formation of membrane potential in the presence of the main sensed ion and interferent.

In batch measurements, the separate solution method [29] was used mainly to determine the selectivity coefficient value. Although there are many restrictions that must be taken into consideration on using this method in the determination of the selectivity coefficients specially in case of solutions of differently charged ions, it is still the simplest way to show whether interference takes place or not and is used to perform measurements in important biological samples as blood [17] as it is simple and easy to perform. A considerably high concentration of the interferent ion is used 1.0×10^{-2} M to ensure that there is no interference if lower concentrations than this are present. The mixed solution method [30] was used only as a confirmation when the $K^{pot}_{Drug,J^{Z+}}$ is less than 3.5 as it is time-consuming due to the need of preparation of many solutions and performing many steps.

Table 3: Selectivity coefficients and tolerance values for the Hx conventional-type electrodes.

	Hx-PTA			Hx-PMA		
	Batch		FIA	Batch		FIA
Interferent	SSM*	MSM**		SSM*	MSM**	
Na^+	3.42	4.62	5.23	3.18	4.35	4.98
K^+	2.98	3.68	5.75	3.34	4.82	5.32
Mg^{2+}	4.75	—	6.21	5.45	—	6.74
Ca^{2+}	5.32	—	5.98	5.87	—	6.48
Cr^{3+}	3.89	—	4.96	4.62	—	6.08
Fe^{3+}	4.21	—	5.66	4.51	—	6.14
Al^{3+}	6.83	—	7.55	5.29	—	6.59
Theronine	—	5.95	8.20	—	5.74	6.79
Leucine	—	5.45	8.97	—	6.32	7.86
Valine	—	7.62	9.32	—	5.87	7.36
Glycine	—	5.96	6.87	—	6.35	9.42
Alanine	—	8.62	10.75	—	7.44	9.87
Salbutamol	—	7.65	10.94	—	5.98	10.24
Orciprenaline	—	6.85	10.44	—	6.47	10.53
Terbutiline	—	6.77	9.87	—	6.85	10.42
Urea	—	6.50	8.09	—	7.12	10.88
Lactose	—	4.98	6.42	—	4.68	7.82
Glucose	—	4.63	7.01	—	4.79	6.89
Maltose	—	5.01	6.85	—	5.13	8.44
Fructose	—	5.23	7.35	—	5.58	7.96
Saccharose	—	4.99	7.12	—	5.62	8.90
Gum xanthine	—	6.32	7.93	—	5.78	7.38

*SSM: separate solution method.
**MSM: mixed solution method.

Table 4: Determination of hexoprenaline employing the different Hx-PTA electrodes applying the standard additions method under batch conditions.

		Hx-PTA (I)		Hx-PTA (II)		Hx-PTA (III)		Hx-PTA (IV)		Hx-PTA (V)	
Sample	Taken [(a)]/mg	Recovery (%)	RSD[(b)] (%)	Recovery (%)	RSD[(b)] (%)	Recovery (%)	RSD[(b)] (%)	Recovery (%)	RSD[(b)] (%)	Recovery (%)	RSD[(b)] (%)
Pure solutions	5.186	98.7	0.19	99.5	0.15	99.7	0.25	99.2	0.39	98.7	0.24
	51.860	99.5	0.15	99.7	0.21	99.4	0.29	98.6	0.24	98.3	0.35
	518.60	99.8	0.19	100.2	0.35	99.9	0.31	99.5	0.35	99.0	0.18
Asmadol®	5.186	98.8	0.09	98.5	0.30	97.8	0.24	99.6	0.28	100.0	0.31
	51.860	100.2	0.36	99.3	0.19	98.4	0.19	100.0	0.44	100.6	0.46
	518.60	101.4	0.24	99.9	0.28	99.3	0.35	100.5	0.37	101.6	0.52
Gynipral®	5.186	98.5	0.18	99.2	0.37	98.6	0.41	100.1	0.19	99.6	0.22
	51.860	100.5	0.16	100.1	0.19	99.8	0.33	99.8	0.25	98.8	0.36
	518.60	99.9	0.21	100.9	0.26	100.4	0.27	99.4	0.42	100.4	0.48
Urine	5.186	97.5	0.15	100.0	0.37	100.2	0.22	99.6	0.17	98.9	0.21
	51.860	98.6	0.19	100.6	0.42	100.1	0.34	100.2	0.26	97.6	0.38
	518.60	99.8	0.23	101.2	0.36	101.6	0.16	101.5	0.37	99.4	0.29
Plasma	5.186	98.0	0.41	99.3	0.29	98.6	0.33	99.4	0.36	97.5	0.36
	51.860	98.6	0.25	98.7	0.17	99.2	0.24	98.5	0.45	98.3	0.45
	518.60	98.8	0.22	99.6	0.23	100.4	0.44	97.3	0.38	98.6	0.40

[(a)]Taken mg per 50 mL.
[(b)]Five determinations.

Table 5: Determination of hexoprenaline in plasma and urine employing the different Hx-PTA and Hx-PMA electrodes under FIA conditions.

	Urine						Plasma					
Taken	5.186 mg		51.860 mg		518.600 mg		5.186 mg		51.860 mg		518.600 mg	
Recovery*	A	B	A	B	A	B	A	B	A	B	A	B
Hx-PTA (I)	85.3	98.2	88.6	99.5	90.5	100.4	84.6	99.7	87.6	100.2	95.4	100.8
Hx-PTA (II)	84.1	98.3	89.1	100.3	92.3	100.7	85.2	98.5	86.9	101.4	96.8	99.7
Hx-PTA (III)	83.7	98.7	88.2	98.9	94.3	101.2	83.2	100.8	88.9	100.5	97.0	102.6
Hx-PTA (IV)	84.2	99.2	89.4	100.5	95.4	99.9	83.9	100.0	90.1	100.9	95.5	101.5
Hx-PTA (V)	85.4	98.4	92.3	101.2	93.8	98.9	84.7	101.2	95.4	100.4	92.8	100.7
Hx-PMA (I)	86.3	99.9	90.1	100.3	96.2	100.2	83.9	100.7	93.1	99.9	97.4	99.6
Hx-PMA (II)	83.7	100.2	89.9	98.6	94.7	99.6	86.1	99.6	89.8	100.1	96.1	98.9
Hx-PMA (III)	84.6	100.5	93.2	100.8	93.8	101.3	88.6	98.5	90.7	99.8	95.4	100.0
Hx-PMA (IV)	88.0	98.6	91.6	102.1	96.5	100.5	85.9	100.4	92.3	101.3	93.8	101.4
Hx-PMA (V)	86.9	99.3	90.4	101.3	97.0	100.2	86.2	100.9	94.3	100.2	95.3	100.6

A: recovery at optimum flow rate (7.50 mL/min for conventional electrodes, 5.35 mL/min for coated wire electrodes).
B: recovery at flow rate 2.50 mL/min.
*Average of three determinations.

The matched potential method [31] was used to determine the tolerance values of neutral interferents such as amino acids, sugars and to determine the selectivity coefficient values for all the studied ions in FIA conditions. It was shown earlier for solid state membrane electrodes that the apparent (or conditional) selectivity coefficient measured in transient flow injection conditions may differ significantly from that measured in batch conditions [32–34]. This is interpreted by a difference in time of interaction of interferent with the membrane surface and the rate at which the exchange process at the membrane surface takes place which increases with increase of interaction of interferent with membrane in comparison to the main sensed ion. This is in good agreement with earlier results that interferences caused by ions that have exchange reaction constants greater than unity are governed to a most significant degree by diffusion processes [35]. Therefore, in FIA measurements, where sample remains in contact with the electrode for a short period of time, the apparent selectivity of the membrane should be different from that found in batch conditions. The electrodes exhibit good tolerance towards sugars and amino acids as the presence of these species up to 4–11 folds did not affect significantly the potential reading. This indicates that the presence of these species is tolerated to a very high extent. This high tolerance is mainly attributed to the differences in polarity and lipophilic nature of their molecules relative to those of the drug under investigation.

The selectivity coefficients of the electrodes (shown in Table 3) reflect a very high selectivity of the investigated electrodes for their respective drug even in the presence of some bronchodilators of nearly very close structure to that of hexoprenaline.

3.5. Analytical applications

Several methods are applied for quantitative analysis using ion-selective electrodes in batch and FIA conditions: (i) in batch conditions, direct calculation of the concentration applying Nernst equation, (ii) in FIA, peak height comparison, and (iii) in both conditions, standard additions method, which is frequently applied in FIA conditions as the large consumption of reagents and time will be the mean trouble as we need to prepare many series of solutions to make just one measurement. So, it was only applied for the assay of biological samples (urine and plasma) to overcome the matrix effect of these samples.

The results of the standard additions method under batch conditions were found to be in good agreement with those obtained from the official method (which involves the spectrometric measurement in 0.1 M HCl at 250 nm) [1]. The mean recovery of the amounts taken using the conventional and coated wire electrodes is comparable and ranged from 97.5 to 102.4% with RSD = 0.12–0.65%. Representative results of application of the standard additions method in batch conditions using Hx-PTA electrodes are shown in Table 4.

As for FIA conditions, the results obtained from peak heights comparison method at optimum flow rates for pure solutions and the pharmaceutical preparations were in agreement to those obtained under batch conditions and that of the official method and the recovery values ranged from 97.0 to 101.5% with RSD = 0.12–0.74%.

As for urine and plasma samples, the results obtained at optimum flow rates were much lower than under batch conditions. On using higher flow rates reaching 23.5 mL/min, the recovery values were getting worse and this proves that the sample needs a much longer time in contact with the electrode surface besides a longer time of washing to remove matrix components from the electrode surface.

Results comparable to batch conditions were only obtained when the flow was lowered to 2.5 mL/min, although this decreased the sampling rate, but the recovery values were improved from 83.2 to 97.4% with RSD 0.18–0.78%, at optimum flow rate, to reach 98.0–102.6% with RSD 0.24–0.65%, at flow rate 2.5 mL/min. Results of the recovery

Table 6: Statistical treatment of data obtained for the determination of Hexoprenaline using Hx conventional electrodes in comparison with the official method.

	Hx-PTA		Hx-PMA	
	Batch	FIA	Batch	FIA
Pure solutions				
X ± S.E.*	100.23 ± 0.85	99.85 ± 0.74	99.80 ± 0.93	101.27 ± 0.21
Relative error (%)	0.13	0.15	0.07	0.22
$F^{3,3}$ value (9.27)**	2.56	2.98	3.45	4.21
Slope of regression line	0.998	0.986	0.995	0.978
Intercept of regression line	−0.087	−0.073	0.045	−0.062
Asmadol®				
X ± S.E.*	100.54 ± 0.23	100.75 ± 0.74	99.63 ± 0.44	100.82 ± 0.50
Relative error (%)	0.05	0.13	0.09	0.24
$F^{3,3}$ value (9.27)**	3.78	4.12	2.68	2.49
Slope of regression line	0.999	0.988	0.986	0.993
Intercept of reg. line	0.012	−0.023	−0.031	0.009
Gynipral®				
X ± S.E.*	100.81 ± 0.34	100.25 ± 0.62	100.26 ± 0.48	101.2 ± 0.21
Relative error (%)	0.14	0.52	0.18	0.31
$F^{3,3}$ value (9.27)**	4.57	3.51	2.88	3.69
Slope of regression line	0.992	0.989	0.996	0.985
Intercept of regression line	−0.091	0.036	0.018	0.042
Urine				
X ± S.E.*	100.54 ± 0.41	99.48 ± 0.56	100.56 ± 0.45	100.12 ± 0.34
Relative error (%)	0.09	0.12	0.24	0.06
$F^{3,3}$ value (9.27)**	3.74	3.92	4.53	5.26
Slope of regression line	0.991	0.984	0.975	0.994
Intercept of regression line	0.029	−0.015	−0.026	−0.035
Plasma				
X ± S.E.*	100.2 ± 0.85	99.8 ± 0.74	99.8 ± 0.93	100.36 ± 0.54
Relative error (%)	0.18	0.14	0.25	0.32
$F^{3,3}$ value (9.27)**	2.87	3.19	3.57	2.96
Slope of regression line	0.999	0.978	0.982	0.990
Intercept of regression line	−0.014	−0.026	0.034	0.091

X ± S.E.*: average ± standard error.
** One tailed critical F-value.
N.B.: for the official method (X ± S.E.) = 100.8 ± 0.65.

values obtained for urine and plasma samples on using the different Hx-electrodes (conventional and wire coated) at optimum flow-rate value and after lowering the flow rate to 2.5 mL/min are given in Table 5.

3.6. Statistical validation of the obtained results

The linearity of the presented electrodes was tested by measuring a series of different concentrations of pHx in the range 5.5–2.0 using the different Hx electrodes for 3 consecutive days, and these results were subjected to linear regression analysis (found versus taken), using sigma plot 10.0, in order to establish whether the investigated electrodes exhibit any fixed bias. The slopes and intercepts of the regression lines did not differ significantly from the ideal values, revealing the absence of a systematic error during the measurements within the investigated concentration range. The accuracy of the results, recovery values of 97.5–102.4% with RSD = 0.12–0.65%, tested using Students t-test [36], at confidence limit 99%, and the calculated values were much lower than the tabulated ones at that confidence limit. The repeatability (intraday) precision was tested by measuring a solution of concentration 1.0×10^{-2} M three times in the same day using the same electrode, while the intermediate (interday) precision was studied measuring a series of solution of pHx 5.5–2.0 for three consecutive days and in both cases F-test was applied to compare the obtained with those of the official method.

Table 6 represents some of the studied statistical parameters of data obtained for the determination of Hexoprenaline using Hx conventional electrodes in comparison with the official method. It is clear that presented electrodes are of a

comparable precision to the official method, and there is no significant difference between the mean values obtained by the two methods.

The limit of detection of the studied electrodes (LOD), defined as the Hx concentration corresponding to the intersection of the extrapolation of the linear part of the calibration curve, ranged from 6.3×10^{-6}to5.8×10^{-6} M, while the limit of quantification (LOQ), defined as the last point corresponding to the intersection of the liner part of the calibration curve, was found to be 5.0×10^{-6} M for the studied electrodes.

4. CONCLUSION

The present work offers two conventional and 8 coated wire electrodes for the determination of hexoprenaline sulphate in its pure state and pharmaceutical preparation in batch and FIA conditions. It is clear from the obtained data that, for all the determined drugs, the presented electrodes have the same usable concentration, temperature, and pH range, selectivity and can be applied to the determination of their respective drugs with nearly the same precision and accuracy, but it is noteworthy to mention that the lifespan of the wire coated electrodes much less than the conventional type, although such type of electrodes is much easier in construction.

The automation of the analysis by applying FIA lead to higher analysis rates (typically from 100 to 300 samples/hr compared to 5–10 samples/hr in batch conditions, enhanced response time) often less than 10–30 seconds between sample injection and detector response, compared to 5–10 minutes in case of batch conditions, much more rapid start up and shut down times besides the ease of monitoring many sorts of errors that may take place in batch conditions such as incorrect addition of reagents and mixing problems beside shorting the time and decreasing the amount of sample needed for analysis. All of the above-mentioned advantages of flow-injection automation make this application feasible and economic to be used for routine analysis and quality control.

The results obtained from the applications of the electrodes were compared with the official method for assaying the drug under investigation, and F-and t-tests were applied to compare the precision and mean values obtained, respectively, and the obtained values were much smaller than the tabulated ones.

Thus, it is clear that the presented electrodes are of high accuracy, precision, and selectivity compared to the official method beside being of low cost, fast response which results in high-sample measurements/hr, easy to apply without any steps of sample pretreatment or extraction, in case of biological samples, besides there is no need of complicated instruments; thus, the proposed electrodes can be very convenient for routine analysis of hexoprenaline in pharmaceutical preparations and biological samples.

REFERENCES

[1] A. C. Moffat, M. D. Osselton, and B. Widdop, Eds., *Clarke's Analysis of Drugs and Poisons*, Pharmaceutical Press, London, UK, 2006.

[2] W. Martindale, *The Extra Pharmacopoeia*, Pharmaceutical Press, London, UK, 29th edition, 1989.

[3] L. Gao and Y. Wu,*Chinese journal of pharmaceutical analysis (Fenxi- Zazhi)*, vol. 6, pp. 360–361, 1986.

[4] J. Traveset, V. Such, R. Gonzalo, and E. Gelpi, "High performance thin-layer chromatographic method for the quality control and stability assay of hexoprenaline," *Journal of High Resolution Chromatography*, vol. 4, no. 11, pp. 589–590, 1981.

[5] R. M. El-Nashar, N. T. Abdel-Ghani, and A. A. Bioumy, "Flow injection potentiometric determination of amitriptyline hydrochloride," *Microchemical Journal*, vol. 78, no. 2, pp. 107–113, 2004.

[6] N. T. Abdel-Ghani, R. M. El-Nashar, and A. A. Bioumy, "Flow injection potentiometric determination of dothiepin hydrochloride," *Analytical Letters*, vol. 37, no. 15, pp. 3237–3254, 2004.

[7] M. Trojanowicz, P. W. Alexander, and D. B. Hibbert, "Flow-injection analysis wit potentiometric detection for the speciation of fluoride and calcium," *Analytica Chimica Acta*, vol. 366, no. 1–3, pp. 23–33, 1998.

[8] W. Frenzel, "Enhanced performance of ion-selective electrodes in flow injection analysis: non-nernstian response, indirect determination, differential detection and modified reverse flow injection analysis," *The Analyst*, vol. 113, no. 7, pp. 1039–1046, 1988.

[9] N. T. Abdel-Ghani, M. S. Rizk, and R. M. El-Nashar, "Salbutamol plastic membrane electrodes based on individual and mixed ion-exchangers of salbutamolium phosphotungstate and phosphomolybdate," *The Analyst*, vol. 125, no. 6, pp. 1129–1133, 2000.

[10] E. Baumann, "Trace fluoride determination with specific ion electrode," *Analytica Chimica Acta*, vol. 42, pp. 127–132, 1968.

[11] G. Eisenman,*Ion-Selective Electrodes*, Natural Bureau Standards, Spec. Publ., 314, U. S. Government Printing Office, Washington, DC, USA, 1969.

[12] E. Linder, K. Toth, and E. Pungor, *Dynamic Characteristics of Ion-Selective Electrodes*, CRS Press, Boca Raton, Fla, USA, 1988.

[13] V. Oesh and W. Simon, "Lifetime of neutral carrier based ion-selective liquid-membrane electrodes," *Analytical Chemistry*, vol. 52, no. 4, pp. 692–700, 1980.

[14] R. D. Armstrong and G. Horvai, "Properties of PVC based membranes used in ion-selective electrodes," *Electrochimica Acta*, vol. 35, no. 1, pp. 1–7, 1990.

[15] N. T. Abdel-Ghani, A. F. Shoukry, and R. M. El-Nashar, "Flow injection potentiometric determination of pipazethate hydrochloride," *The Analyst*, vol. 126, no. 1, pp. 79–85, 2001.

[16] A. F. Shoukry, S. S. Badawy, and Y. M. Issa, "Performance characteristics and regeneration of a tetraphenylboron(III) selective electrod," *Analytical Chemistry*, vol. 59, no. 8, pp. 1078–1081, 1987.

[17] U. Oesch, D. Ammann, and W. Simon, "Ion-selective membrane electrodes for clinical use," *Clinical Chemistry*, vol. 32, no. 8, pp. 1448–1459, 1986.

[18] J. Růžička and E. H. Hansen, "Flow injection analysis. Principles, applications and trends," *Analytica Chimica Acta*, vol. 114, pp. 19–44, 1980.

[19] M. Trojanowicz, *Flow Injection Analysis, Instrumentation and Applications*, World Scientific, Singapore, 2000.

[20] X. Yang, D. B. Hibbert, and P. W. Alexander, "Flow injection potentiometry by poly(vinyl chloride)-membrane electrodes with substituted azacrown ionophores for the determination of lead(II) and mercury(II) ions," *Analytica Chimica Acta*, vol. 372, no. 3, pp. 387–398, 1998.

[21] W. Frenzel and P. Brätter, "The fluoride ion-selective electrode in flow injection analysis, part 1. General Methodology," *Analytica Chimica Acta*, vol. 185, pp. 127–136, 1986.

[22] M. Trojanowicz and W. Frenzel, "Flow injection potentiometry for low level measurements in the presence of sensed ion in the carrier," *Fresenius' Zeitschrift für Analytische Chemie*, vol. 328, no. 8, pp. 653–656, 1987.

[23] W. E. Morf, E. Lindner, and W. Simon, "Theoretical treatment of the dynamic response of ion-selective membrane electrodes," *Analytical Chemistry*, vol. 47, no. 9, pp. 1596–1601, 1975.

[24] L. Ilcheva, M. Trojanowicz, and T. K. vel Krawczyk, "Effect of addition of main ion to carrier solution in potentiometric flow-injection measurements with solid state ion-selective electrodes," *Fresenius' Zeitschrift für Analytische Chemie*, vol. 328, no. 1-2, pp. 27–32, 1987.

[25] M. S. Rizk, N. T. Abdel-Ghani, and R. M. El-Nashar, "Construction and performance characteristics of terbutaline plastic membrane electrode in batch and FIA conditions," *Microchemical Journal*, vol. 70, no. 2, pp. 93–101, 2001.

[26] R. W. Burnett, A. K. Covington, N. Fogh-Andersen, et al., "Use of ion-selective electrodes for blood-electrolyte analysis. Recommendations for nomenclature, definitions and conventions," *Clinical Chemistry and Laboratory Medicine*, vol. 38, no. 4, pp. 363–370, 2000.

[27] K. Ren, "Selectivity problems of membrane ion-selective electrodes: a method alternative to the IUPAC recommendation and its application to the selectivity mechanism investigation," *Fresenius' Journal of Analytical Chemistry*, vol. 365, no. 5, pp. 389–397, 1999.

[28] G. Horvai, "The matched potential method, a generic approach to characterize the differential selectivity of chemical sensors," *Sensors and Actuators B*, vol. 43, no. 1–3, pp. 94–98, 1997.

[29] V. V. Cosofret and R. P. Buck, "Phenothiazine drug poly(vinyl chloride) matrix membrane electrodes and their use in pharmaceutical analysis," *The Analyst*, vol. 109, no. 10, pp. 1321–1325, 1984.

[30] E. Pungor and K. Tóth, "Selectivity of ion-specific membrane electrodes," *Analytica Chimica Acta*, vol. 47, no. 2, pp. 291–297, 1969.

[31] V. P. Y. Gadtzekpo and G. D. Christian, "Determination of selectivity coefficients of ion-selective electrodes by a matched-potential method," *Analytica Chimica Acta*, vol. 164, pp. 279–282, 1984.

[32] M. Trojanowicz and W. Matuszewski, "Potentiometric flow-injection determination of chloride," *Analytica Chimica Acta*, vol. 151, pp. 77–84, 1983.

[33] L. Ilcheva and K. Cammann, "Flow injection analysis of chloride in tap and sewage water using ion-selective electrode detection," *Fresenius' Zeitschrift für Analytische Chemie*, vol. 322, no. 3, pp. 323–326, 1985.

[34] D. E. Davey, D. E. Mulcahy, G. R. O'Connel, and R. S. C. Smart, "A flow-injection/XPS investigation of the effect of redox equilibria on the selectivity of an iodide-selective electrode," *Electroanalysis*, vol. 7, no. 5, pp. 461–470, 1995.

[35] L. K. Shipigun, O. V. Basanova, and Y. A. Zolotov, "Performance of solid-membrane cation-selective electrodes for flow-injection potentiometry," *Sensors and Actuators B*, vol. 10, no. 1, pp. 15–20, 1992.

[36] J. C. Miller and J. N. Miller, *Statistics and Chemometrics for Analytical Chemistry*, Ellis Horwood, Chichester, UK, 4th edition, 2001.