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Research Article

Developing a Potentiometric Ractopamine-Selective Electrode and Its Application

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Abstract

In this study, a potentiometric ion-selective electrode (ISE) was developed in order to quantify ractopamine. Ractopamine is a phenylethanolamine with ß2-adrenergic agonist properties; was in fact developed for treating respiratory diseases; and is used as tocolytic, bronchodilator and heart tonic in veterinary and human medicine (1). Ion-pair of Ractopamine-Tetraphenylborate was synthesized. The ion pair that was synthesized was employed as ionophore in the structure of electrode membranes. For the optimization of the membranes, PVC membrane ion-selective electrodes were produced in various compositions using the synthesized ion pairs and potentiometric performance characteristics of these electrodes were explored. It was determined that the electrode with the composition of 5% Rac-TPB+32%PVC+62.6%DBF+0.4% KTpClPB (Potassium tetrakis (4-chlorophenyl borate) demonstrated the best potentiometric performance characteristics. The linear range, slope, limit of quantification, pH range, and response time of this electrode were determined to be 1.0×10^{-5}-1.0×10^{-1} M, 51.0 mV/decade, 1.0×10^{-5} M, 3.0-5.0, and< 5s, respectively. The electrode has demonstrated a highlyrepeatable potentiometric response. It was applied in pigswill using ractopamine selective electrode.

Keywords: Ion-selective electrode, quantification of ractopamine, PVC membrane electrode

Potansiyometrik Raktopamin Seçici Elektrot Geliştirilmesi ve Uygulaması

Öz

Bu çalışmada, raktopamin miktarını belirlemek için potansiyometrik iyon seçici bir elektrot (ISE) geliştirildi. Raktopamin, aslında solunum yolu hastalıklarının tedavisi için geliştirilmiş ve veterinerlikte ve insan tıbbında tokolitik, bronkodilatör ve kalp tonik olarak kullanılmakta olan ß2-adrenerjik agonist özelliklere sahip bir feniletanolamindir (1). Raktopamin-Tetrafenilborat iyon çifti sentezlendi. Sentezlenen iyon çifti, elektrot membranının yapısında iyonofor olarak kullanıldı. Membranların optimizasyonu için, PVC membran iyon seçici elektrotları, sentezlenen iyon çiftleri kullanılarak çeşitli bileşimlerde üretildi ve bu elektrotların potansiyometrik performans özellikleri araştırıldı. % 5 Rak-TPB +% 32 PVC +% 62,6 DBF +% 0,4 KTpClPB (Potasyum tetrakis (4-klorofenil borat) bileşimine sahip olan elektrodun en iyi potansiyometrik performans özelliklerini gösterdiği tespit edildi. Bu elektrodun doğrusal çalışma aralığı, eğimi, tayin limiti, pH aralığı ve cevap zamanı aralığı sırasıyla 1.0 x 10^{-5}-1.0 x 10^{-1} M; 51.0 mV; 1.0 x 10^{-5} M, 3.0-5.0 ve <5s olarak belirlendi. Elektrot son derece tekrarlanabilir bir potansiyometrik cevap gösterdi. Raktopamin seçici elektrodu domuz yemine uygulandı.

Anahtar kelimeler: İyon seçici elektrot, raktopamin tayini, PVC membran elektrot

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1. Introduction

Ractopamine is a phenylethanolamine with ß2-adrenergic agonist properties and it was in fact developed for treating respiratory diseases. It is used as tocolytic, bronchodilator and heart tonic in veterinary and human medicine (Lu et al., 2012). This compound is illegally administered as a nutrient in the livestock industry in China in order to direct nutrients from fat accumulation to muscle growth (Dong at al., 2012). But, it poses a potential risk such as drug residues that accumulated in animal tissues, muscle tremors, vomiting, nervousness, and heart palpitation in terms of consumer health (Bolera et al., 2012; Halsey et al., 2011). Therefore, in many countries, ractopamine is not licensed for animal production. Ractopamine residue in pork products may pose a health risk for people with asthma cardiovascular. Use of ractopamine in pigs is banned by many countries such as China, Japan, and the European Union. The maximum residue limit of ractopamine in pig liver is definitely 0.15 µg / g or less. However, the toxic effects and potential hazards of beta-agonists including cardiac palpitation, tachycardia, nervousness, muscle tremors, and confusion have been reported in many studies (Brambilla et al., 2000; Von Haehling et al., 2009). To date, various analytical methods were developed to detect ractopamine in animal feeds, animal tissues, and body fluids. Liquid chromatography-mass spectrometry (Blanca et al., 2005), gas chromatography-mass spectrometry (He et al., 2007), immunoassay (Pleadin et al., 2012; Shen et al., 2007), high-performance liquid chromatography (Turberg et al., 1996), and capillary electrophoresis (Wang et al., 2010) are some of those methods. Because ractopamine comprises a phenolic hydroxyl group, it is active electrochemically and it can be oxidized on an electrode surface. Therefore, because of its benefits of low instrumental cost and rapid analyzing, for detecting ractopamine, electrochemical methods can be employed. However, as these methods generally require complex procedures, expensive instruments, expensive materials, and high-cost equipment, they are not very suitable for the analysis of environmental samples at high amounts and this makes on-site and real-time detection difficult. Potentiometry is preferred because of its advantages such as simplicity, cheapness, providing rapid and reliable results, being miniaturized, and not requiring specialist technicians. We reviewed the literature and saw that there is no ractopamine-selective potentiometric sensor that was developed to date.

In the study, it was aimed to develop a ractopamine-selective sensor using all-solid-state contact PVC membrane potentiometric sensor technology. In order to use different ion pairs of ractopamine as ionophore substance in its structure, firstly ion pair type that provides the best potentiometric characteristics in the electrode membrane by synthesizing different ion pairs to be employed as an active ingredient in the structure of the PVC membrane ion-selective electrode membrane. The optimum membrane composition that exhibited the best potentiometric performance characteristics was investigated and the potentiometric performance characteristics for the electrode with this membrane composition (Linear range, limit of quantification, selectivity, response time, pH range, lifetime, repeatability, sensitivity) were determined. Finally, an application was performed for quantification of ractopamine on pig sample using the produced electrode.

2. Materials and Method

2.1. Reagents

Tetrahydrofuran (THF), high molecular weight poly (vinyl chloride) (PVC), o-nitrophenyloctyl ether (NPOE), dibutyl phthalate (DBP), dioctyl phthalate (DOP), dioctylsebacate (DOS), sodium tetraphenylborate (NaTPB), ractopamine hydrochloride (Rac-HCl), potassium tetrakis (4- chlorophenyl) borate (KTpClPB), and graphite powder (<20 µm) were procured from Sigma-Aldrich. Epoxy (TP3100) and hardener (Desmodur RFE) are used in the preparation of conductive solid contact, and they were bought from Denlaks (Turkey) and Bayer AG (Germany), respectively. Pigswill was obtained from a company in Turkey.

The analytical grade nitrate salts of the respective cations were used in the preparation of all the standard and stock solutions. In the study, deionized water with a resistivity of 18.3 MΩ.cm was used when the aqueous solutions were being prepared.

2.2. Instruments

A computer-controlled laboratory- made high- input impedance multi-channel potentiometric measurement system was employed for the performing of potentiometric measurements. Through out the potentiometric measurements, a saturated silver/silverchloride reference electrode (Gamry) was employed. A Starter 3100 model benchtoppHmeter (Ohaus Corporation) was used for the measurement of pH values.

2.3. Preparation of the Standard Solutions

The standard solutions used in the measurements were prepared using analytical grade substances. Firstly, standard solutions of each type were prepared in pH=4 Acetate buffer and 0.1 M concentration. Then, the standard solutions of these solutions at required concentrations in the study were prepared by diluting the initially prepared standard solutions with 0.1 M concentration using pH=4 Acetate buffer.

2.4. Preparation of the Buffer Solutions

It was prepared as follows: 57.2 mL Acetic acid and 23.65 g Sodium acetate were dissolved in deionized water, adjusted to pH 4 and made up to 1000 mL with deionized water.

2.5. Synthesizing Maprotiline- Tetraphenylborate Ion-Pair

Ractopamine-Tetraphenyl Borate (Rac-TPB) ion pair: The solution of 2 mL 10^{-2} M sodium tetraphenylborate was added graduallyto the 2 mL of the solution that contained 10^{-2} M RactopamineHCl and was stirred continuously. The formed bright white-colored precipitate was centrifuged until it was decontaminated and then, it was left to dry in dark under room conditions. After it was dried, it was used directly as ionophore.

2.6. Preparation of theElectrodes

In the study, the Rac-TPB ion pair, which ractopamine formed with Sodium tetraphenylborate, was used in the structure of membrane as ionophore substance at different rates and Rac-selective electrode was formed in this way. Electrode production takes place in two stages in general. The first stage consists of the preparation of the solid contacts that form the surface on which the membrane will be coated, and the second stage consists of the preparation of the membrane cocktails that contained Rac-TPB ion pairs and coating the solid contact surfaces with it. The solid contacts were prepared by submerging of one of the open ends of a copper wire into a mixture that contained 50% (a/a) graphite, 35% epoxy (a/a), and 15% (a/a) hardener and was homogenized, and then its drying under room conditions for a period of 1 night. All the PVC membranes constantly contain 32% PVC. The other components of the PVC membranes comprised of plasticizers at different types, ion pairs, and sometimes ionizer component. The membranes were prepared by solving of total membrane mass of 100 mg in 2 mL THF. The solid contact surfaces were coated by being submerged into the prepared PVC membrane cocktails for five times and were left to dry under room conditions for 24 hours. After the electrode membranes were dried, the electrodes were submerged into the 10^{-2} M RactopamineHCl containing the standard solution, which was prepared in pH=4 Acetate buffer, for 12 hours and they were conditioned and made ready for measurement. The electrodes were preserved in dark under laboratory conditions while they were not employed. Prior to starting each measurement, the electrodes were kept in the conditioning solution for half an hour.

2.7. PotentiometricMeasurements

The EMF valuesweremeasured at roomtemperature (20±2) °C usingthefollowingelectrochemicalcellassembly:

Ag/AgCl RE || RAC solution | PVC membrane | conductivesolidcontact | Cu wire

The reference and indicator electrode were submerged in 25 mL of the test solutions, which were mixed at a constant rate, and the potentiometric data were taken in this way. Before starting each measurement, the indicator and reference electrodes were cleansed using deionized water and then they were dabbed using a smooth paper that is capable of adsorbing.

3. Findings

3.1. Investigation of the Optimum Membrane Composition

In PVC membrane ion-selective electrodes, ionophore, plasticizer, ionizer, and PVC ratio, and also types of plasticizers and ionizers are important factors that determine the potentiometric performance characteristics of an electrode. Therefore, in order to detect the electrode that displays the best potentiometric performance characteristics, different membrane compositions were prepared by changing these parameters for the Rac-TPB ion pair, and the potentiometric performance characteristics (slope, the limit of quantification, linear range, and R^2 value for the calibration curve) of the electrodes that were prepared using these membranes were investigated. The compositions of 5 electrode membrane prepared using the Rac-TPB ion-pair were given in Table-1.

Table 1. The compositions of the electrode membranes that were prepared using Rac-TPB ion pair as ionophore

	Membrane Composition (% a/a), m					
Electrode No.	PVC	NPOE	DBF	DOS	Rac-TPB	KT_pCIPB
A1	32.0	-	62.6	-	5.0	0.4
A2	32.0	-	62.0	-	5.0	1.0
A3	32.0	-	62.0	-	5.0	1.0
A4	32.0	-	62.7	-	5.0	0.3
A5	32.0	-	62.5	-	5.0	0.5

To carry out the optimization of the membranes, PVC membrane ion-selective electrodes were produced in various compositions using the synthesized Ractopamine-Tetraphenylborate ion pair and then potentiometric performance characteristics of these electrodes were explored. It was determined that the best potentiometric performance characteristics belonged to the electrode with the composition of 5% Rac-TPB + 32% PVC + 62.6% DBF + 0.4% KTpClPB (Potassium tetrakis (4-chlorophenyl borate).

To determine the potentiometric performance characteristics of the developed electrodes, ractopamine solutions of 1.0×10^{-1} M – 1.0×10^{-5} M were made ready in pH=4 acetate buffer. Slope, linear range, and R^2 values of each electrode from the calibration graphs obtained with the recorded potential values were given in Table 2.

Table 2. Potentiometric performance characteristics of the PVC membrane Ractopamine-selective electrode that was prepared using Rac-TPB ion pair

Electrode No.	Slope, mV/decade	Limit of Quantification, M	Linear Range, M	R^2
A-1	51.0	1.0×10^{-5}	1.0×10^{-5}-1.0×10^{-1}	0.9948
A-2	37.1	1.0×10^{-5}	1.0×10^{-4}-1.0×10^{-1}	0.9711
A-3	34.3	1.0×10^{-5}	1.0×10^{-4}-1.0×10^{-1}	0.9790
A-4	33.4	1.0×10^{-5}	1.0×10^{-4}-1.0×10^{-1}	0.9870
A-5	48.7	1.0×10^{-5}	1.0×10^{-4}-1.0×10^{-1}	0.9850

As can be understood from Table 2, the electrode A-1 has the best membrane composition as it has a high slope, low limit of quantification, wide linear range, and high R^2 value. After this stage, potentiometric performance characteristics of the electrode A-1 will be examined. The potentiometric behavior and the relevant calibration graph obtained from the calibration of 1.0×10^{-1} M – 1.0×10^{-5} M ractopamine solutions for the electrode A-1 were given in Figure 1 and Figure 2, respectively.

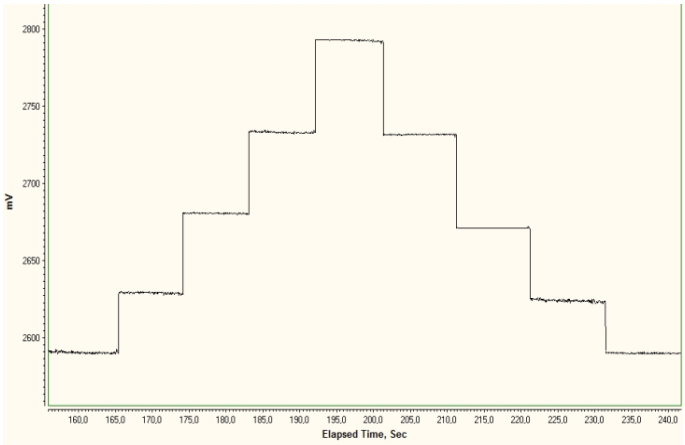

Figure1. Potential values of theractopamine solutions of A-1 Rac-selective electrode at different concentrations(1) 10^{-1} M(2) 10^{-2} M (3) 10^{-3} M (4) 10^{-4} M (5) 10^{-5} M

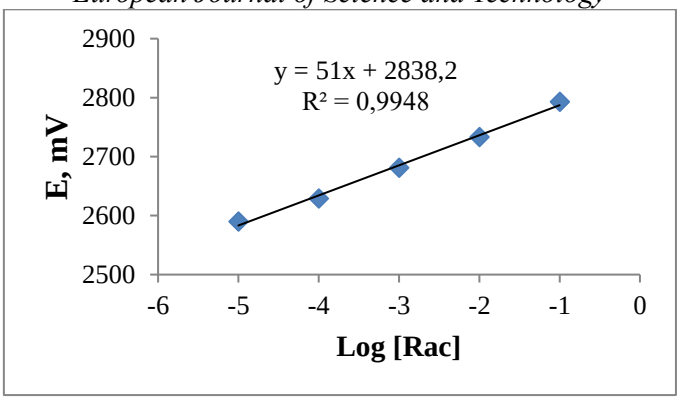

Figure 2. Calibration graph of the A-1 Rac-selective electrode

3.2. Determining the response time of the Ractopamine-selective electrode

The equilibration time that the electrode had was determined in the 10^{-3} M and 10^{-2} M ractopamine solutions that were prepared to determine the response time of the electrode. While the solution was being mixed at a constant speed, the times required for the potential to become stable were written down (t_{95}). The mean time during which the potentials reached stable position was set as the response time of the electrode. The equilibration time of the electrode in the 10^{-3} M and 10^{-2} M ractopamine solutions is seen in Figure 3. It was found that the mean response time of the electrode was < 5 sec.

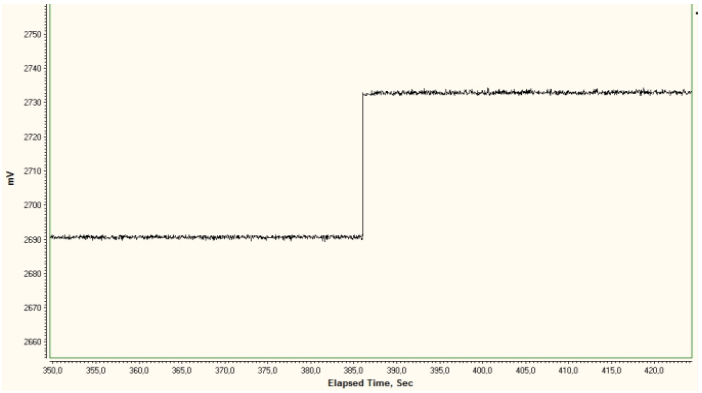

Figure 3. Response time of the A-1 Rac-selective electrode for 10^{-2} M-10^{-3} M RacHCl solutions

3.3. Determining the response time of the Ractopamine-selective electrode

Measurements of the A1 (5% Rac-TPB + 32% PVC + 62.6% DBF + 0.4% İonic) electrode were taken consecutively in 10^{-2} M and 10^{-3} M ractopamine solutions to determine the repeatability of the ractopamine-selective electrode. Potential measurements and repeatability graph for the electrode A1 were given in Table 3 and Figure 4, respectively.

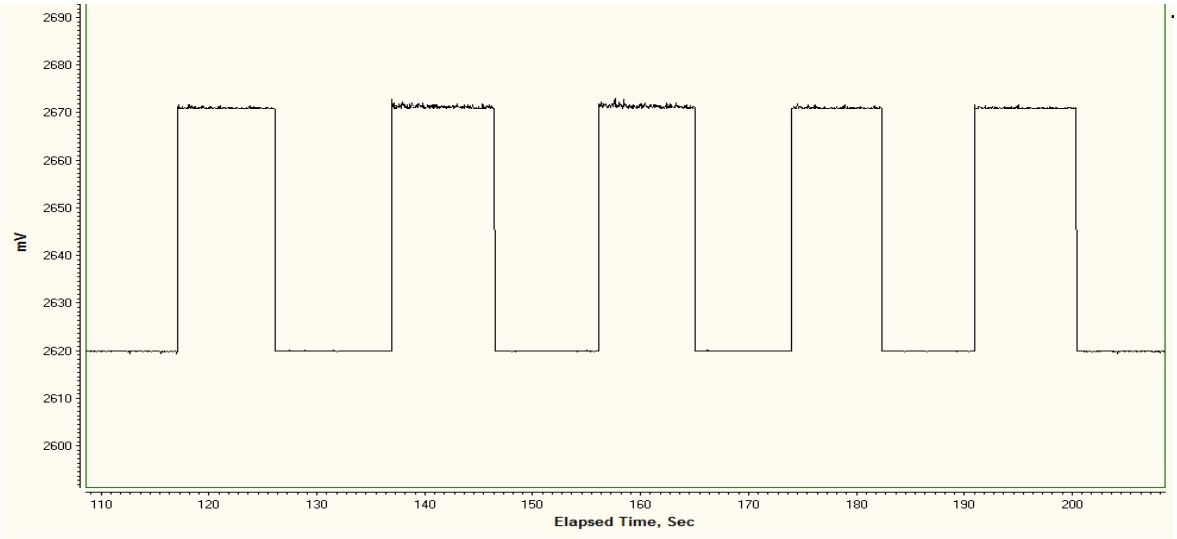

Figure 4. Repeatability of the A-1 Rac-selective electrode in 10^{-3} M and 10^{-2} M solutions

Table 3. Repeatability of the A-1 electrode in 10^{-2} M, 10^{-3} M ractopamine solutions

Ractopamine Concentration (mol/L)	Potential, mV					X±S
10^{-2}	2671	2672	2672	2671	2671	2671.4±0.55
10^{-3}	2620	2620	2620	2620	2620	2620.0±0

3.4. Determining the pH range of the Ractopamine-selective electrode

For determining the pH range of the ractopamine-selective electrode, the reference electrode, A-1 ractopamine-selective electrode, and pH meter were simultaneously submerged into the 25 mL 10^{-2} M and 10^{-3} M ractopamine solutions. 10^{-2} M HCl and 10^{-2} M NaOH were slowly added to the ractopamine solution and pH value of the solution was altered in this way. Each time acid and base were included to the solution, the pH value of the solution and its potential values taken from potentiometric cell were noted. The obtained potential values corresponding to the read pH values were plotted on the graph. The obtained graph was given in Figure 5. As one can see in the graph, the potentiometric response of the electrode is not affected by hydronium ion within the range of pH=3.0-5.0. But, it is seen that electrode potentials start to decrease in increasing pH values.It is thought that this is because of the decrease in the amount of ractopamine, which was protonated in the increasing pH values.

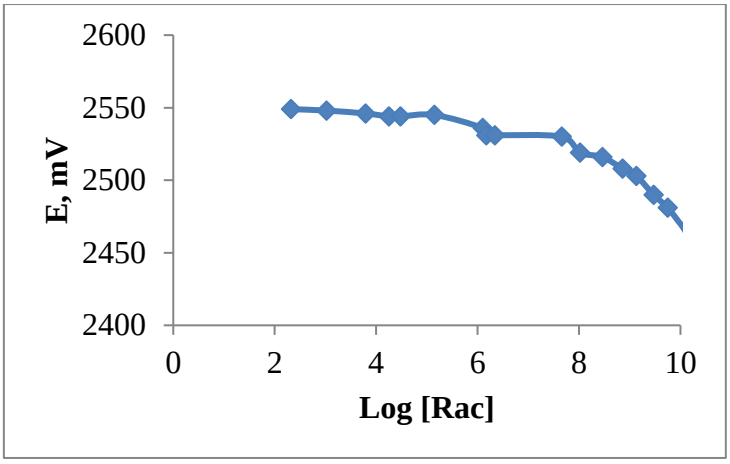

Figure 5. pH range of the Rac-selective electrode for 10^{-3} M Ractopamine solution

3.5. Determining the Selectivity of the Rac-Selective Electrode

For determining the effects of some common alkali metals, alkaline earth metals, and heavy metals on the response of the ractopamine-selective electrode, selectivity coefficients for these types were found by the separate solution method (E_A=E_B). In calculating the selectivity coefficients, ractopamine concentrations for the potential values read in 1.0×10^{-2} M solutions of interfering ions were detected by using calibration curve.Concentration value, which corresponded to the potential value of the interfering ion for 1.0×10^{-2} M concentration in the calibration curve obtained for ractopamine, was placed in the selectivity coefficient equation and the selectivity coefficients of the electrode were determined for each interfering cation and organic molecule. The selectivity coefficients were inspected carefully and it was seen that the electrode was very selective for the measured types. Potentiometric behavior of the ractopamine-selective electrode for ractopamine and interfering types was given in Figure 6. Selectivity coefficients of the ractopamine-selective electrode that were found for some types by the separate solution method were given in Table 4.

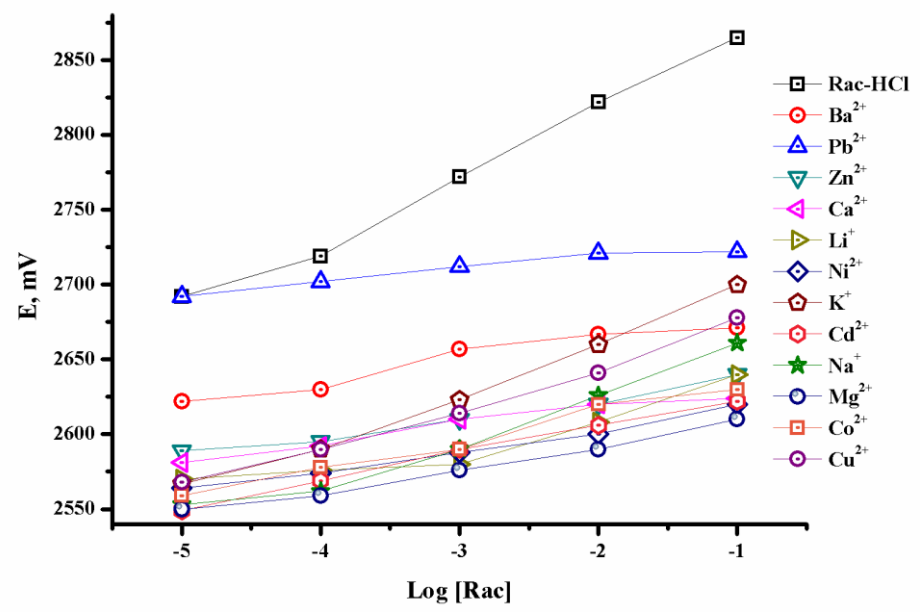

Figure 6. Potentiometric behavior of the ractopamine selective electrode for ractopamine and some interfering types

Table 4. Selectivity coefficients of the ractopamine-selective electrode found by using the separate solution method for some types

No.	Type	$K_{A,B}$	log $K_{A,B}$
1	Ba^{2+}	4.13×10^{-5}	-4.38
2	Pb^{2+}	6.6×10^{-4}	-3.18
3	Zn^{2+}	3.71×10^{-6}	-5.43
4	Ca^{2+}	3.71×10^{-6}	-5.43
5	Li^{+}	2.0×10^{-5}	-4.7
6	Ni^{2+}	1.3×10^{-6}	-5.9
7	K^{+}	2.9×10^{-4}	-3.5
8	Cd^{2+}	1.8×10^{-6}	-5.7
9	Na^{+}	5.1×10^{-5}	-4.3
10	Mg^{2+}	7.9×10^{-7}	-6.1
11	Co^{2+}	3.7×10^{-6}	-5.4
12	Cu^{2+}	1.1×10^{-5}	-4.96

3.6. Determining the Lifetime of the Rac-Selective Electrode

For determining the lifetime of the rac-selective electrode, the ractopamine-selective electrode was used to take the measurements. The measurements were carried out in certain days in the concentration range of 1.0×10^{-5}-1.0×10^{-1} M where the electrode A1 worked linearly. The electrode was conditioned in 1.0×10^{-2} M ractopamine solution before the measurements for half an hour each time. The electrode was preserved in room conditions and in a closed and dark medium when not in use. The slope values, obtained by the correspondence of the results of the measurements with the days of the measurements, were plotted on the graph and the graph in Figure 7 was obtained.

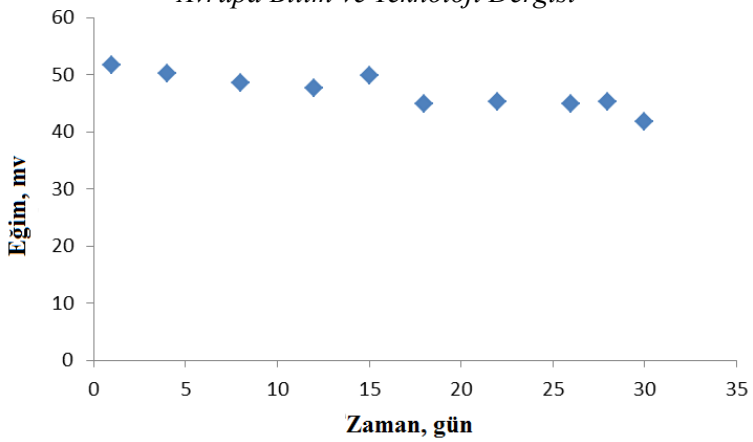

Figure 7. The lifetime of the ractopamine-selective electrode

The graph was examined and it was found that the lifetime of the electrode was 15 days.

3.7. Application of the Ractopamine-Selective Electrode to the Sample

After the potentiometric performance characteristics of the developed ractopamine-selective electrode were examined, the sample application of the electrode was carried out on pigswill. For this purpose, 1g pigswill was taken and dissolved in 100 mL pH=4 acetate buffer. The solution was centrifuged; the clear portion on the top of the solution was taken. Its 20 mL was taken and then its potentiometric measurement was taken. Ractopamine was added into the samples at certain concentrations using the recorded potential values from the calibration graph and standard addition method was employed here. The obtained results were given in Table 5.

Table 5. Application on pig sample with the A1 Rac-selective electrode

Added (mg/L)	**Found (mg/L)**	**Recovery (%)**
160,8	*158,8*	*98.7*
440	*418*	*95*
675	*675*	*100*

4. Discussions and Conclusion

Between the ractopamine-selective potentiometric electrodes prepared for the quantification of ractopamine, the optimum membrane composition was determined to be 5% Rac-TPB, 32% PVC, 62.6% DBF, and 0.4% KT_pCIPB. It was determined that the electrode demonstrated a highly selective potentiometric behavior for the commonly used anionic and cationic types. The developed electrode allows for the quantification of ractopamine directly, accurately, precisely, and in a reliable way in the complex matrix environments by this feature. The electrode has a response time of less than 5 sec and allows for rapid quantification of ractopamine. It was observed that the electrode could be used with no alteration in the slope within the linear range of the electrode for 15 days, the linear range decreased and its slope fell after 15 days. Therefore, the lifetime of the electrode was determined to be 15 days. Also, the developed electrode exhibited potentiometric behavior without being affected by the hydronium ion exchange of the solutions in the range of pH=3.0-5.0.

The developed electrode was used in the pigswill sample and standard addition method was used. As the developed electrode has advantages such as simplicity in preparation, low-cost, providing rapid, sensitive, and highly selective measurements, a wide linear range, and low limit of quantification, it has the potential to be used as an alternative to complex, more expensive, and time-consuming measurement techniques. Especially its shorter response time offers the potential to be used as a detector in automatic systems such as flow injection analysis.

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