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ARTICLE

Innovative Potentiometric Quantification of Flutamide Using Novel Ion-Selective Electrodes with Sulfonphthalein Dyes

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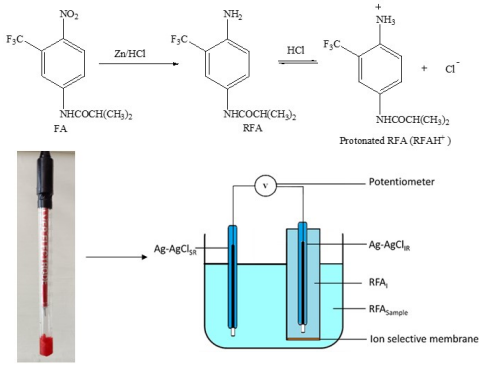
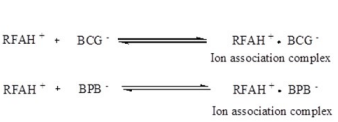
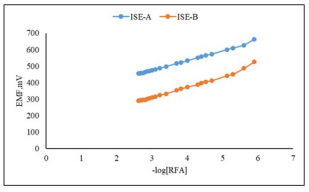




Ion selective electrode constructed using ion association complex

Schematic diagram of electrochemical cell constructed



Calibration curves of EMF v/s -log [RFA] using ISE-A and ISE-B

Two potentiometric sensors, referred to as ion-selective electrodes ISE-A and ISE-B, were constructed and validated for the quantification of flutamide (FA) in drug formulations and extended to the analysis of human urine samples infused with drugs. Bromocresol green and bromophenol blue were used as ion-pair complexing agents in the construction of ISE-A and ISE-B, respectively. Dibutyl phthalate served as a plasticizer within a polyvinyl chloride matrix, with tetrahydrofuran as the solvent. The methods demonstrated linearity over the ranges of 2.5 x 10^{-6} to 2 x 10^{-3} M and 5 x 10^{-6} to 1.8 x 10^{-3} M for ISE-A and ISE-B, respectively. The Nernstian slop of 58.8 ± 0.96 and 62.11 ± 0.83 were exhibited by the proposed methods using ISE-A and ISE-B, respectively, within a response time of 25 seconds and were effective for the analysis of FA over a period of 52 days within a pH range of 1.0 to 3.8. The coefficient of determination (R^2) was very close to one, indicating an excellent fit between the actual and measured data. For ISE-A, the computed limits of detection and quantification were 0.82 x 10^{-6} M and 2.45 x 10^{-6} M, respectively, and 1.64 x 10^{-6} M and 4.86 x 10^{-6} M for ISE-B. The ICH recommendations were followed during the validation process, which evaluated the methods' robustness, ruggedness, sensitivity, accuracy, precision, and selectivity.

Keywords: drug, quality control, ion association complex, electrochemical cell, membrane

INTRODUCTION

Flutamide (FA) is a synthetic, nonsteroidal antiandrogen drug, also known by its chemical name 2-methyl-N-[4-nitro-3-(trifluoromethyl)phenyl]propanamide (Figure 1). It is frequently used to treat hirsutism, acne,[1] and polycystic ovary syndrome (PCOS),[2] in addition to prostate cancer.[3,4] The primary antiandrogenic effects of FA are attributed to its active metabolite, 2-hydroxy-flutamide, which acts on target tissues

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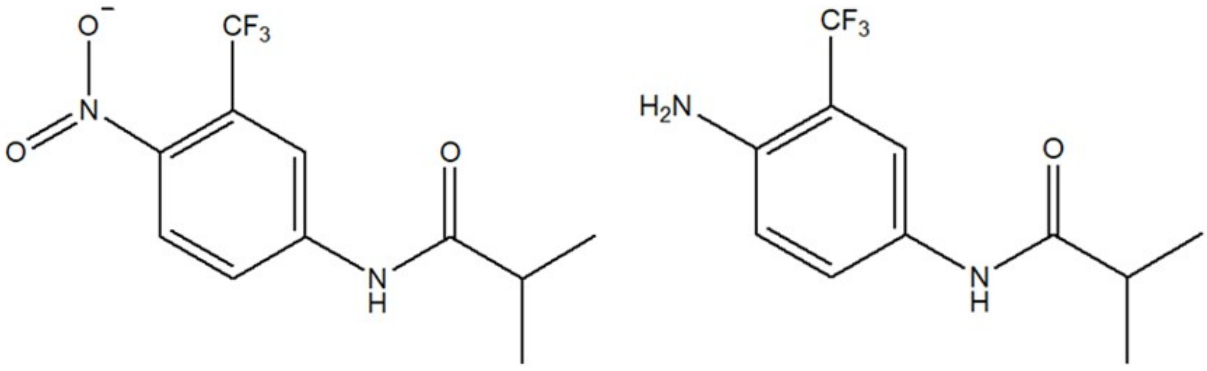
including as hair follicles, the prostate, the testes and the skin by binding to and inhibiting intracellular androgen receptors.[5–7]

A luteinizing hormone-releasing hormone (LH-RH) agonist combined with 375 mg of FA per day is recommended by pilot research on maximal androgen-depletion therapy for advanced prostate cancer.[8] Severe adverse effects, on the other hand, might result from overusing FA. These include methemoglobinemia, hot flashes, sleepiness, liver failure, enlarged male breasts, vomiting, blood in the urine, nausea, and rectal bleeding.[9] It is therefore essential to develop a rapid and accurate method for the quantification of flutamide in drugs and body fluids.

Despite the extensive applicability of electroanalytical techniques in quantifying therapeutic compounds, their usage remains limited. However, potentiometry, which employs selective electroanalytical sensors, offers significant benefits for quantifying the medicinal substances like FA. It is favoured for its ease of use, affordability, and the fact that it does not necessitate highly skilled personnel.

A comprehensive review of the literature on analytical methods for quantifying FA in pharmaceuticals reveals numerous techniques beyond the chromatographic approaches outlined in the European Pharmacopoeia (EP)[10] and United States Pharmacopeia (USP).[11] These include titrimetry,[12] spectrophotometry,[3,12–20] spectrofluorometry,[21,22] HPLC(High Performance Liquid Chromatography), and HPTLC (High-performance thin-layer chromatography),[23–28] ESI-MS (Electrospray ionization mass spectrometry) coupled with Soxhlet extraction,[29] flow injection analysis,[30] voltammetry,[4,9,31–39] differential pulse polarography,[40] and a capture-on-paper approach.[41] Biological samples, medicines, and pure forms of FA and its metabolites have been quantified by researchers using these protocols.

The mentioned chromatographic and voltametric procedures require advanced equipment and highly trained personnel, which are beyond the reach of many laboratories. Thus, for the first time, two simple, selective and economical sensors (Ion selective electrode (ISE)-A and -B) have been created to quantify FA. Ion pair complexes of reduced FA (RFA; Figure 1b) with sulfonthalein dyes, namely Bromocresol green (BCG) and Bromophenol blue (BPB), are used in these ISEs.



(a) (b)

Figure 1. (a) Structure of 2-methyl-N-[4-nitro-3-(trifluoromethyl)phenyl]propanamide (FA) and (b) structure of RFA (Reduced flutamide).

MATERIALS AND METHODS

Instruments

A pH meter from Elico (Mumbai, India) was employed to measure the pH values of the solutions, while a digital dual-channel potentiometer from PICO (Chennai-32, India) was employed to record the potentials. Ag/AgCl electrodes were used in the construction of ISEs as well as electrochemical cell.

Chemical reagents

This investigation was done by making use of chemical reagents and materials particularly of analytical reagent grade. The 99.8% pure FA was generously supplied by Cipla, India Ltd. Cytomid tablets (Cipla) each containing 250 mg of FA were procured locally. Whatman No. 42 filter paper was one of the materials used, along with ion-pair associating agents like sodium tetraphenylborate (NaTPB), BCG, and BPB, plasticizers such as nitrophenyl octyl ether (NPOE), dibutyl sebacate (DBS) and dibutyl phthalate (DBP), ionophore such as beta cyclodextrin (β-CD), the matrix material poly vinyl chloride (PVC) and solvents like tetrahydrofuran (THF) and chloroform ($CHCl_3$). For the research, zinc dust, methanol (CH_3OH), potassium chloride (KCl) and concentrated hydrochloric acid (HCl) were purchased from Merck India Ltd. Urine sample was collected from a healthy men volunteer and analysed. The Ethical Committee (ECR/387/Inst/KA/2013/RR-19) approval (Letter No. JSS/MC/PA/5659/2024-25, dated 6-11-2024) has been obtained for pursuing the urine analysis for this research work.

The solutions of silver nitrate, ammonium chloride, sodium hydroxide, sodium carbonate (Na_2CO_3), calcium carbonate, cobalt chloride, zinc sulphate, oxalic acid, formic acid, citric acid, benzoic acid, salicylic acid tartaric acid, phthalic acid, boric acid, talc, glycine and ammonium oxalate (S.D. Fine Chem) were all prepared with a strength of 1 M each.

Experimental procedures

Preparation of 0.005 M standard solution of RFA

In a 100 mL beaker, 20 mL of 1:1 HCl solution was used to dissolve 138.1 mg of FA, which is equivalent to 123.1 mg of RFA. To this, 3 g of zinc dust was added and thoroughly mixed for about half an hour. After that, the mixture was filtered through Whatman No.42 filter paper and washed with distilled water. To make a 0.005 M RFA solution, the filtrate was collected in a 100 mL standard flask and the solution was diluted with distilled water to the appropriate level.

Preparation of RFA-BCG and RFA-BPB ion associate complexes

Two samples, each containing 22.44 mg of FA, were accurately weighed and placed in separate 100 mL beakers, corresponding to 20 mg of RFA. To each beaker, 1 g of zinc dust and 10 mL of 1:1 HCl were added, followed by thorough stirring for about 30 minutes. Whatman No. 42 filter paper was used to filter the resultant RFA into two separating flasks and traces were washed with distilled water.

The two separating flasks containing the filtered RFA and 56.54 mg of BCG, the filtered RFA and 54.27 mg of BPB were treated with 25 mL of $CHCl_3$ each, manually shaken well for about 2 minutes, and set aside for layer separation. The organic layer from each flask was collected into different 250 mL beakers. Each separating flask was then treated with another 25 mL of fresh $CHCl_3$, and the shaking and separation process was repeated to collect the organic layer. This process was performed three times. The organic solvent was then evaporated to dryness to obtain the RFA-BCG and RFA-BPB ion associate complex residues, respectively.

Construction of ISEs

In a 100 mL beaker, 10 mL of THF was used to dissolve 20 mg of the RFA-BCG ion association complex, 75 mg of DBP as a plasticizer, and 250 mg of PVC as a matrix material in order to create the ISEs. Another 100 mL beaker was filled with 10 mL of THF, 20 mg of the RFA-BPB ion association complex, 50 mg of DBP, and 200 mg of PVC. The resulting solutions were carefully poured into two different petri dishes, each with diameter of 5 cm and kept aside to dry for about 24 hours at room temperature. Following their formation, the thin membranes were bonded with THF to the ends of two distinct plastic tubes and allowed to dry at ambient temperature for another 24 hours.

To each tube with the attached membrane sensors, 4 mL of a 0.005 M RFA solution and 1 mL of a 1 M KCl solution were added. Ag-AgCl electrodes as such were immersed in these solutions to construct ISE-A and ISE-B, respectively. The electrodes were soaked in a 0.005 M RFA solution for about 4 hours before being used to measure the potential of analyte solutions containing RFA.

Procedure for the construction of calibration curve / FA analysis

With the aid of a micro burette, an appropriate volume of aliquot of 0.005 M RFA standard solutions was taken in two sets of 25 mL standard flasks. These sets of solutions range in RFA concentration from 1.25 x 10^{-6} to 2.4 x 10^{-3} M. After adding 1:1 HCl to each solution to make a total volume of 5 mL, the pH was brought to around 3 by adding 2 M Na_2CO_3 solution. After adding distilled water to each flask as needed, the volume was adjusted and properly mixed. One set of solutions' potentials was measured using ISE-A, and the other set's potentials were measured using ISE-B. Ag-AgCl reference electrodes were utilized with ISE-A and ISE-B. To create calibration curves, the observed EMF (Electromotive force; the potential of the solutions measured using the proposed electrochemical cell) was plotted against the -log [RFA]. Regression equations were computed using the gathered data to ascertain the RFA concentrations in unknown samples using these curves.

Procedure for the analysis of tablet

We roughly weighed ten Cytomid-250 tablets (UCB Pharma Ltd. Mumbai, India) and finely powdered. After accurately weighing a 416.3 mg of this powder (equivalent to 138.1 mg of FA), it was transferred into a 100 mL beaker and dissolved in 25 mL of CH_3OH. The solution was filtered using Whatman No. 42 filter paper to get a filtrate containing FA and then evaporated to dryness on a water bath. 20 mL of 1:1 HCl was added to the beaker containing FA once it had cooled to room temperature. After adding 3 g of zinc dust, the liquid was vigorously agitated for almost half an hour. Whatman No. 42 filter paper was used to filter the mixture, and distilled water was used to wash it. A tablet extract with a concentration of 0.005 M RFA was produced by collecting the filtrate in a 100 mL standard flask and adjusting the volume to the appropriate level with distilled water. The aliquots of tablet extract were then subjected to the technique mentioned for the FA analysis.

Procedure for spiked urine sample analysis

A 1 mL of urine sample collected from a healthy man was spiked with 2 mL of 0.005 M RFA, acidified with 1 mL of 1:1 HCl, the pH was brought to around 3 by adding 2 M Na_2CO_3 and total volume was adjusted to 25 cm^3 using distilled water. The potential of the resultant solution was measured by using proposed sensors. The concentration of FA was then subsequently determined.

Interference study and Sensor's Selectivity Coefficient ($K_{RFA.I}$) determination

The chemical interaction between the analyte and the membrane's active sites produces the membrane potential (E_m). The influence of the chemical process on the signal means that the selectivity of the membrane needs to be guaranteed for a single species. E_m is essentially proportional to the ionic strength that can interact with the active sites of the membrane. Equation 1 is the generalized form of the Nernst equation, which takes an interferent (I) into account.

$$E_{cell} = K + \frac{0.05916}{Z_{RFA}} \log\left([RFA] + K_{RFA.I}[I]^{Z_{RFA}/Z_I}\right) \qquad \text{Equation 1}$$

The charges on RFA and I are denoted by Z_{RFA} and Z_I, respectively. K is a constant that encompasses liquid junctions, reference electrodes and membrane potentials. K stands for a constant that includes the analyte concentration in the internal solution, the potentials of the reference electrodes, any liquid junction potentials, and the asymmetry potential. $K_{RFA.I}$ is the coefficient of selectivity. Equation 2 was used to determine the $K_{RFA.I}$ for each interferent.[42,43]

$$K_{RFA.I} = \frac{[RFA]_E}{[I]_E^{Z_{RFA}/Z_I}} = \frac{[RFA]_{Int}}{[I]_{Add}^{Z_{RFA}/Z_I}} \qquad \text{Equation 2}$$

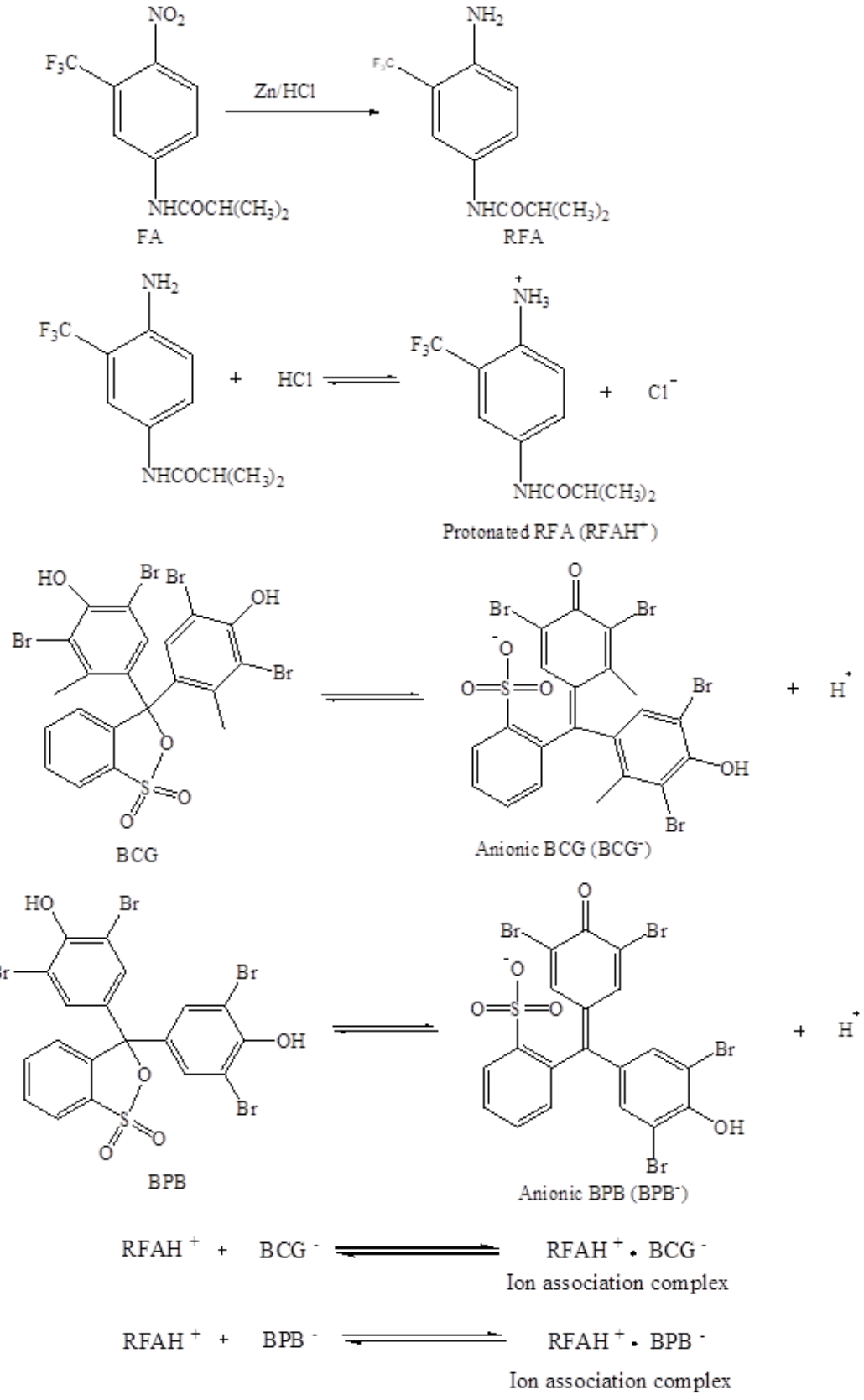
$[RFA]_E$ and $[I]_E$ stands for the RFA and interferent concentrations needed to produce the identical E_{Cell} values. The concentration of interferent added to or present in the RFA solution is denoted by $[I]_{add}$, while the concentration of RFA in the internal solution is denoted by $[RFA]_{Int}$.

The selectivity coefficient can be calculated by following the procedure: a series of solutions in 25 mL standard flasks were prepared by adding 2 mL of 0.005M pure RFA solution and 3 mL of 1:1 HCl to each flask. 1 mL of different interferent solutions of strength 1M was added to them, and they were thoroughly mixed for approximately two minutes. Each RFA solution containing interferents had its pH adjusted to about 3, and distilled water was added to each flask to fill it to the full capacity of 25 mL. After the mixtures were well homogenized, the two suggested ISEs were used to analyse them under the suggested protocol.

RESULT AND DISCUSSION

Method development

Two sulfonphthalein dyes, BCG and BPB, were employed to fabricate membrane sensors or ISEs for FA using DBP as a plasticizer and PVC as a matrix in THF. The pKa value of BCG and BPB is 4.9 and 4.1 respectively. These dyes are in monoanionic form in aqueous medium by losing the proton from hydroxyl group resulting in the formation of sulphonate ion at pH less than 3.8 and they will not get protonated in the mentioned pH range.[44-46] The ability of forming an organic solvent extractable ion associate complex with the drug is the prime factor for selecting these two dyes in the preparation of ISEs. Initially, the $-NO_2$ group of FA was reduced in the presence of Zn/HCl to $-NH_2$ group.[20] Using Zn and HCl for reduction of nitro-compounds to amines is a prevalent way. Moreover, it is an advantageous process for leaving non-hazardous salts.[47-49] The RFA protonated in the acidic medium and the protonated RFA ($RFAH^+$) ultimately forms an ion pair in $CHCl_3$ in a 1:1 stoichiometric ratio with the monoanionic dye (BCG^- or BPB^-) at pH maintained around 3 in the potential reaction pathways shown in Scheme 1.[50]



Scheme 1. The reaction pathway of forming $RFAH^{+}\cdot BCG^{-}$ and $RFAH^{+}\cdot BPB^{-}$ ion pair complexes.

The structure of the FA, RFA, BCG, BPB, $RFAH^+ \cdot BCG^-$ and $RFAH^+ \cdot BPB^-$ was ascertained by FTIR spectroscopic analysis (Figure 2).

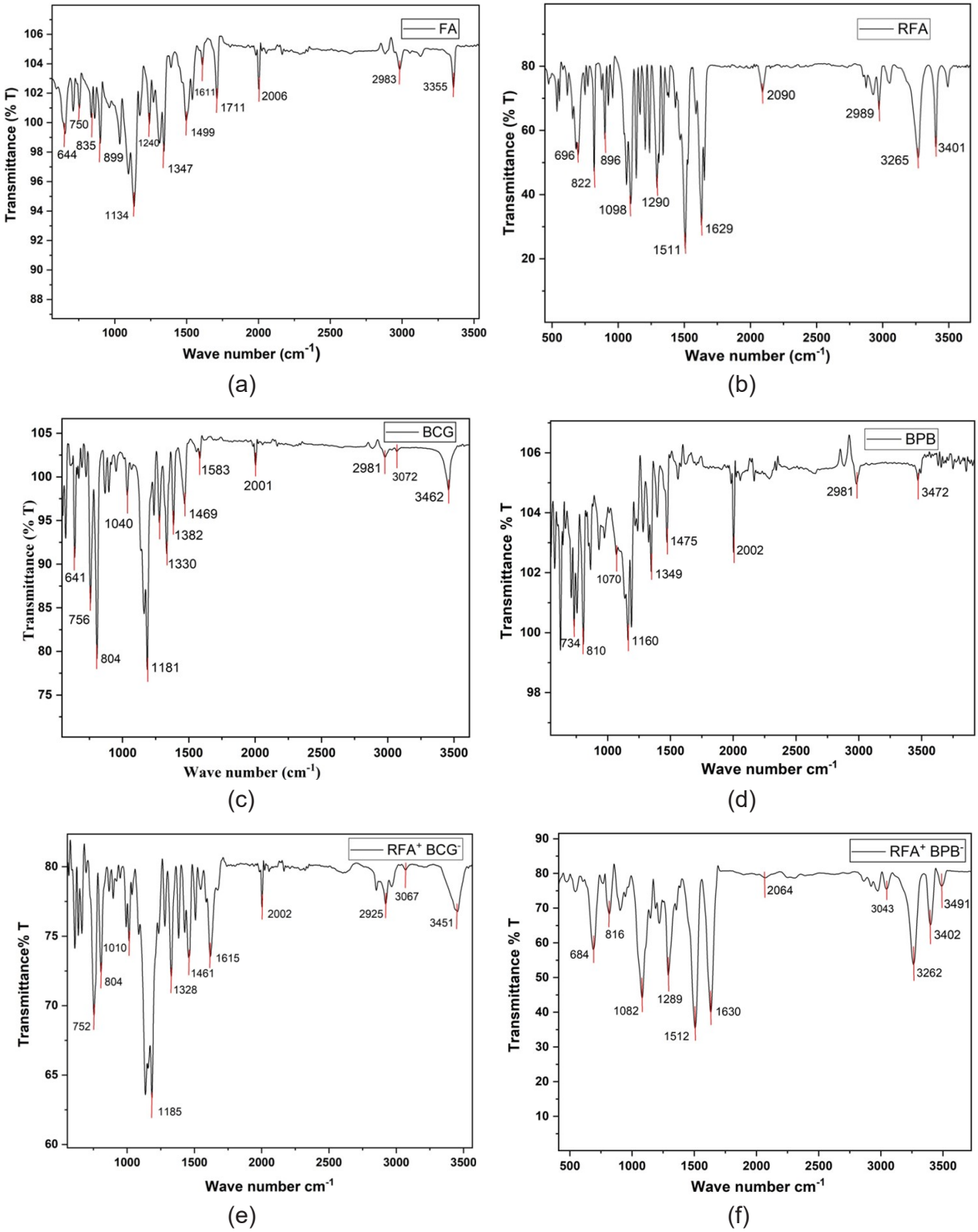


Figure 2. The FTIR spectra of the (a) FA, (b) RFA, (c) BCG, (d) BPB, (e) $RFAH^+ \cdot BCG^-$ and (f) $RFAH^+ \cdot BPB^-$.

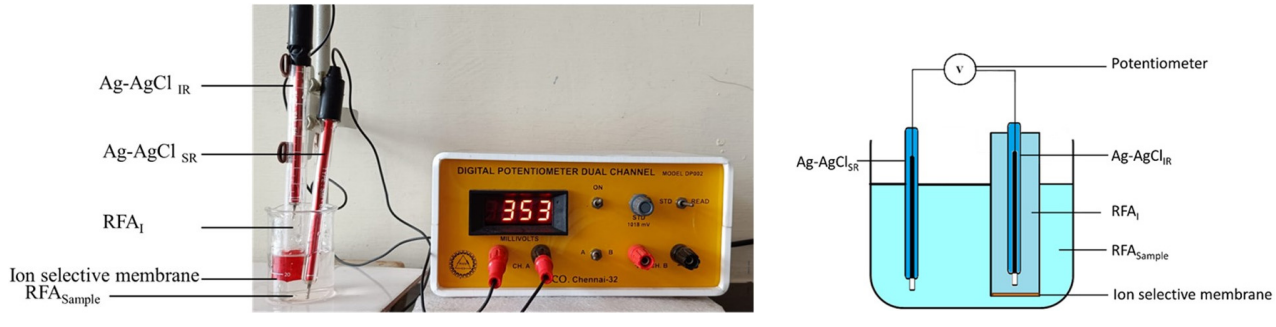
The FTIR spectra of FA display distinct bands at 1499 cm^{-1} and 1347 cm^{-1}, corresponding to the symmetric and asymmetric stretching of the Ar-NO_2 group, respectively, and a band at 899 cm^{-1} attributed to NO_2 scissoring. Carbonyl carbon shows the absorption at 1711 cm^{-1}. Additional absorption bands at 1611 cm^{-1} and 3355 cm^{-1} are linked to the carbonyl and N-H stretching of N-substituted amides followed by the N-H out of plane bending noticed at 644 cm^{-1}. In addition to this, a band at 1240 cm^{-1} indicates the aromatic amide. The C-CF_3 stretching mode has been assigned at 1347 cm^{-1}. The CF_3 stretching mode (Symmetric) appears at 750 cm^{-1}. For isopropyl group attached to carbonyl carbon, C-H stretching occurs at 2983 cm^{-1} and C-H deformation bending, a significant doublet appears at around 1347 cm^{-1}. The band at 1134 cm^{-1} and 2006 cm^{-1} corresponds to C-C-C / C-C skeletal vibration and a overtone region to represent disubstituted benzene. The spectra of RFA demonstrate the reduction of the NO_2 group in FA to an NH_2 group, which is evidenced by bands at 3265 cm^{-1} and 3401 cm^{-1} associated with the N-H stretching of aromatic primary amines. Further bands at 1290 cm^{-1}, 1629 cm^{-1}, and 822 cm^{-1} correspond to the C-N stretching of aromatic amines, N-H bending, and wagging, respectively. For the BCG and BPB, the presence of -OH groups on the benzene rings are indicated by characteristic bands at around 3465 cm^{-1} for the O-H stretching. The C-H stretching and out of plane bending appears at 2981 cm^{-1} and 734-756 cm^{-1}, respectively, the C-H wagging appears in the range of 1000 – 650 cm^{-1}, the C=C stretching at 1583 cm^{-1} and C-C in ring stretching at the range of 1600 – 1400 cm^{-1} indicates the benzene ring. The presence of CH_3 groups on benzene ring in case of BCG are characterised by the appearance of band at 3072 cm^{-1}. The formation of an ionic association complex between RFA and BCG/BBP is confirmed by the broadening of the bands in the range of 3262 – 3401cm^{-1} corresponding to the primary amine group.[41,51–53]

The ion-pair complexes obtained were employed in the fabrication of the membranes. These membranes can selectively interact with $RFAH^+$. The equilibrium distribution of $RFAH^+$ at the sample-membrane junction is impacted by the in-situ ion exchange that occurs when the corresponding ISEs are submerged in RFA solutions. The potentiometric response of ISEs with polymeric membranes is largely dependent on this interaction at the interface. Changes in the concentration of RFA in a solution are therefore translated into an electrical potential by the ISE. The voltage has a theoretical relationship with the ionic activity's logarithm.[54,55]

Photography of the electrochemical cell and Schematic diagram of ISEs are included in Figure 3 and its schematic representation for determining FA using the developed sensors is illustrated as below:

$$Ag\text{-}AgCl_{SR} \,\|\, RFA_{sample} \,|\, Membrane \,|\, RFA_{I}\,(5\ mM),\ KCl\ (1\ M) \,\|\, AgCl\text{-}Ag_{IR}$$

Where, the electrodes submerged in the sample solution (RFA_{sample}) and internal standard solution (RFA_I), respectively, are Ag-$AgCl_{SR}$ and Ag-$AgCl_{IR}$. The Nernst equation provides the relationship between E_{Cell} and $[RFA]_{Sample}$.[42]



(a) (b)

Figure 3. (a) Photo of the constructed electrochemical cell using the proposed ISE; (b) Schematic diagram of potentiometric cell with the proposed ISE.

Optimization of parameters

Membrane composition

To construct a suitable membrane for FA assay, a series of trials were conducted using various amounts of ion associate complex, plasticizer, matrix substance, and solvent. The resulting membranes, each with different compositions of these components, were used to create ion-selective electrodes (ISEs). Their performance in sensing RFA was evaluated potentiometrically. For ISE-A, membranes containing 20 mg of the RFA-BCG ion associate complex, 75 mg of DBP as a plasticizer, and 250 mg of PVC as the matrix substance in 10 mL of THF proved to be effective. Similarly, membranes containing 200 mg of PVC, 50 mg of DBP, and 20 mg of the RFA-BPB ion association complex in 10 mL of THF demonstrated RFA estimation reliability for ISE-B. Appropriate Nernstian behaviour was not shown in attempts to set up calibration lines with varying material quantities that differed from these concentrations.

Choice of Plasticizer

To find the best composition for the membranes, tests were conducted using varying amounts of various plasticizers, including DBP, DOP, NPOE, and DBS. With 75 and 50 mg of DBP for ISE-A and ISE-B, respectively, the membrane sensors consistently showed promising Nernstian behavior. Regarding the repeatability of E_{Cell} values, response time, Nernstian behavior, and calibration, other plasticizers did not yield good results. The results of plasticiser optimisation are summarised in Table I.

Table I. Overview of the plasticizer optimization outcomes

Plasticizer	Amounts (mg)	ISE-A		ISE-B	
		Slope* ± SD	CL# at 95%	Slope* ± SD	CL# at 95%
DBP	50.0	51.23±1.12	1.08	62.11±1.14	1.87
	75.0	58.78±1.01	0.75	46.26±0.99	1.45
	100.0	52.32±1.13	1.13	51.00±1.07	1.09
	125.0	54.38±0.99	1.11	54.89±1.12	1.13
	150.0	51.42±0.79	1.14	54.77±1.00	1.22
DBS	50.0	41.45±1.21	1.41	40.13±1.16	1.28
	75.0	42.43±1.07	0.98	41.22±1.32	1.33
	100.0	42.28±0.99	1.03	39.24±0.89	1.10
	125.0	43.23±0.98	0.79	38.51±1.23	1.49
	150.0	45.22±1.36	1.78	38.67±0.79	0.94
DOP	50.0	48.34±1.43	1.33	49.61±0.79	1.16
	75.0	49.19±1.07	1.98	47.16±1.17	1.39
	100.0	48.81±1.36	1.78	46.67±1.16	1.33
	125.0	48.65±0.89	1.19	48.56±1.87	1.97
	150.0	49.37±1.03	1.24	48.97±1.35	1.91
NPOE	50.0	51.26±0.85	1.15	48.32±1.11	1.53
	75.0	50.22±0.54	0.92	47.07±1.21	1.34
	100.0	49.65±0.99	1.37	47.65±0.81	0.98
	125.0	50.00±0.77	0.98	45.33±0.69	0.87
	150.0	51.55±1.23	1.47	46.99±1.03	0.83

#Confidence limit / *Mean value of five determinations

Internal reference solution

Various volumes and concentrations of RFA and KCl solutions were tested as internal standards to create calibration plots of E_{cell} versus -log [RFA] using ISE-A and B. The best results for FA quantification were obtained using an internal standard solution composed of 0.005 M RFA and 1 M KCl in amounts of 4 mL and 1 mL, respectively. This combination yielded Nernstian slopes of 58.8 ± 0.96 mV/decade for sensor A and 62.11 ± 0.83 mV/decade for sensor B. Figure 4 displays the calibration curves that show how potentials and -log [RFA] relate to one another.

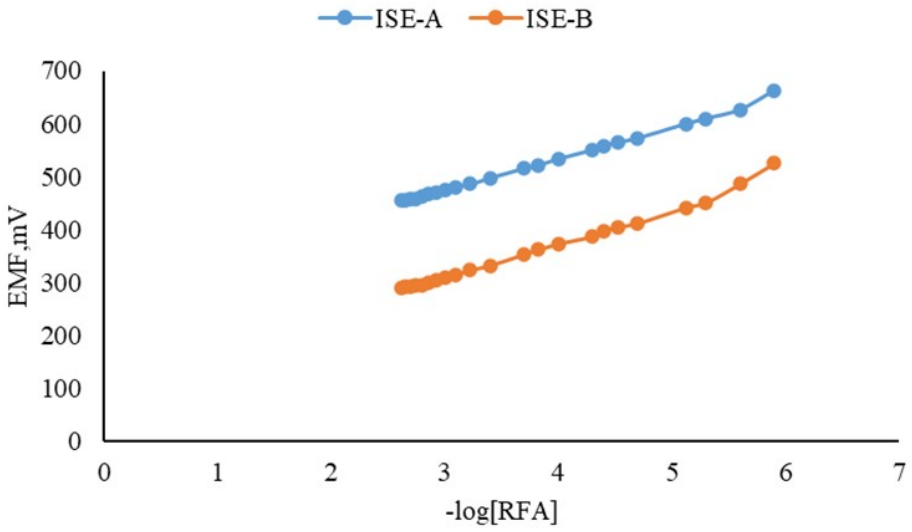


Figure 4. Calibration curves of EMF v/s -log [RFA] using ISE-A and ISE-B.

Electrode conditioning time

The ISEs' surfaces were activated by soaking them in a standard solution of 5×10^{-3} M RFA at 25 ± 2°C for various durations. The experiments showed that a soaking time of approximately 3.5 hours is needed to achieve a stable and consistent potential. The impact of this conditioning time on sensor performance is depicted in Figure 5. Additionally, it is suggested that the sensors be kept in a closed vessel for storage and reused for FA quantification after soaking in the standard RFA solution for about 3.5 hours.

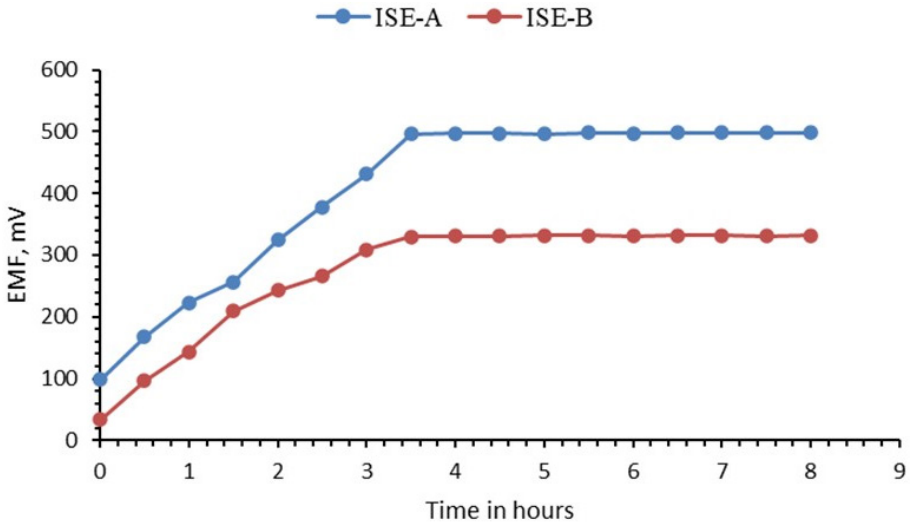


Figure 5. Conditioning time's impact on the 5×10^{-3} M RFA solution's potential employing ISE-A and ISE-B.

Impact of pH

The EMF of 5×10^{-3} M RFA solution was measured using both ISEs to determine how pH affected the EMF value. Na_2CO_3 solution (2 M) was used to maintain a pH between 0.5 and 8. The EMF measurements were stable and consistent within the pH range of 1.0 to 3.8 for both sensors. Beyond this range, as Figure 6 illustrates, the potential values decreased and became erratic. Figure 7 is a bar graph of the slope of the calibration curves at various pH values. An optimal pH of around 3 was selected for all measurements.

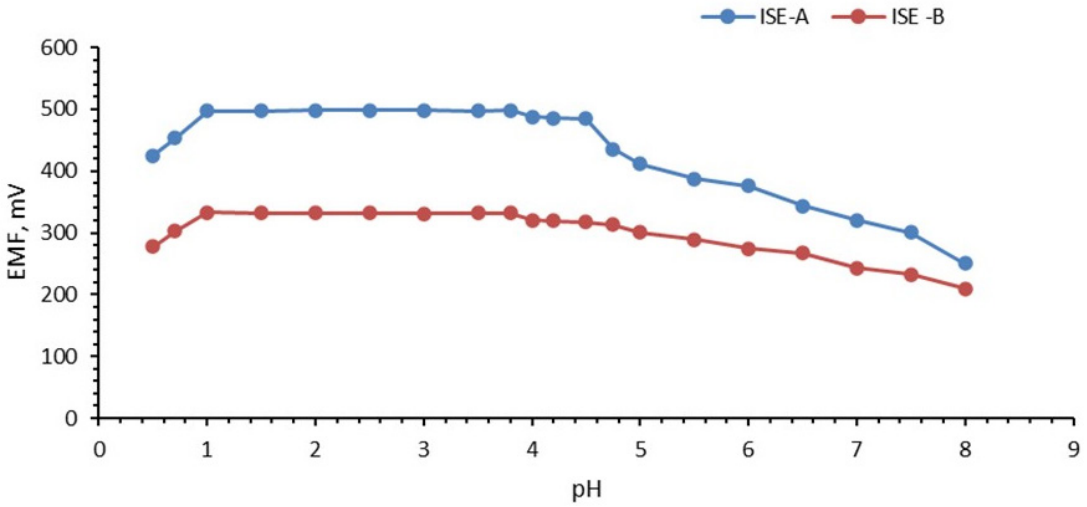


Figure 6. Effect of pH on the 5×10^{-3} M RFA solution's potential using ISE-A and ISE-B.

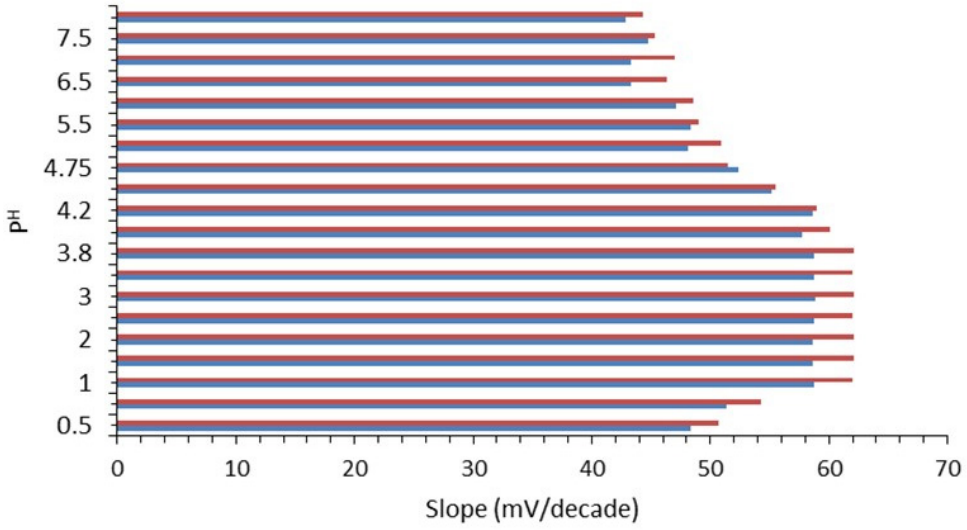


Figure 7. Findings from the assessment of pH's impact on sensor behaviour.

Response time

A typical 5×10^{-3} M RFA solution was used to test the rapid and steady response of the built ISEs. The ISEs exhibited a linear response within 25 seconds of immersion in the analyte solution, as presented in Figure 8. Therefore, it is recommended to take readings after 25 seconds.

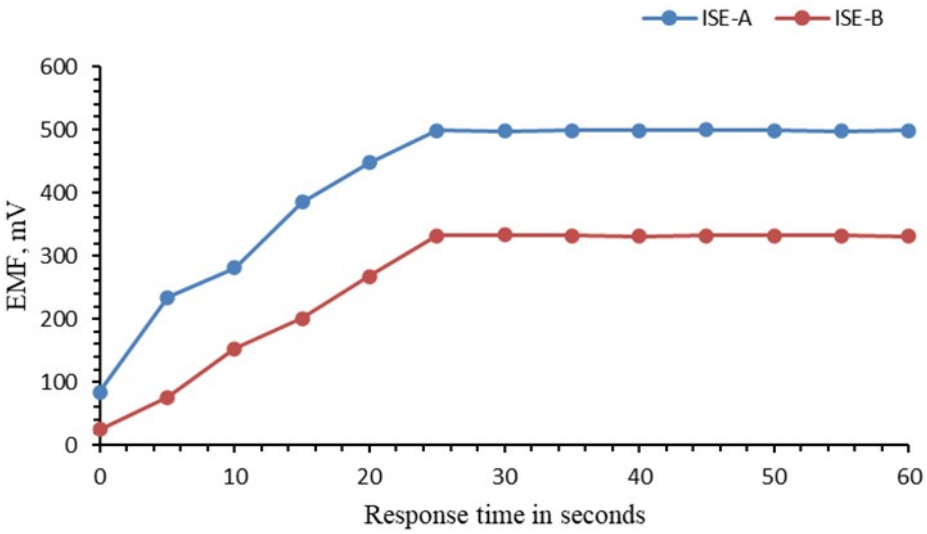


Figure 8. Response time monitored for 5×10^{-3} M RFA solution using ISE-A and ISE-B.

Sensor lifetime

The designed sensors performed exceptionally well, exhibiting a constant mean Nernstian slope of 58.8 ± 0.96 mV/decade for ISE-A and 62.11 ± 0.83 mV/decade for ISE-B in RFA measurements for approximately 52 days. After this period, the Nernstian behavior degraded, indicating a sensor lifetime of 52 days.

Coefficients of selectivity

To assess the selectivity, various interferent solutions of inorganic, organic, anionic, and cationic nature, each with strength of 1 M, were spiked into a pre-examined RFA solution. The calculated selectivity coefficient values arranged in Table II demonstrate that, the additional species did not obstruct the response as every $K_{RFA,I}$ value is less than 1.[42,43]

Table II. The sensors' selectivity coefficients for different interferents

Interferents	$K_{RFA.I}^{*}$	
	ISE-A	ISE-B
H^+	0.253	0.198
Ag^+	0.431	0.384
NH_4^+	0.132	0.098
Na^+	0.072	0.076
K^+	0.119	0.163
Ca^{2+}	0.096	0.101
Co^{2+}	0.348	0.312
Zn^{2+}	0.487	0.431
Cl^-	0.132	0.098
Oxalic acid	0.182	0.168

(continued on next page)

Table II. The sensors' selectivity coefficients for different interferents (continuation)

Interferents	$K_{RFA.I}$*	
	ISE-A	ISE-B
Formic acid	0.176	0.173
Boric acid	0.447	0.398
Benzoic acid	0.127	0.165
Citric acid	0.088	0.097
Salicylic acid	0.149	0.124
Tartaric acid	0.131	0.134
Phthalic acid	0.151	0.167
Talc	0.371	0.387
Glycine	0.098	0.101
Oxalate	0.081	0.097

*Mean value of five determinations

Method validation

The sensor was validated according to International Union of Pure and Applied Chemistry (IUPAC)[56] and International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH)[57] guidelines, with results presented in the following sections.

The degree of linearity in the calibration curve and performance metrics

As illustrated in Figure 4, the EMF measurements revealed a linear association with the RFA solution concentrations ranging from 2.5×10^{-6} to 2×10^{-3} M for ISE-A and from 5×10^{-6} to 1.8×10^{-3} M for ISE-B. The linearity, following Nernstian behaviour was demonstrated by slopes of 58.8 ± 0.96 mV/decade for ISE-A and 62.11 ± 0.83 mV/decade for ISE-B. These slopes confirm the 1:1 stoichiometric reaction between RFA and BCG as well as RFA and BPB for forming the ion associate complex. The regression equations derived from the calibration curves are:

$$Y = 58.796\ X + 298.2 \text{ with ISE-A}$$

$$Y = 62.108\ X + 122.6 \text{ with ISE-B}$$

Additional performance characteristics are detailed in Table III.

Table III. Performance characteristics of the developed sensors

Parameters	ISE - A	ISE - B
Linear range, mol L^{-1}	2.5×10^{-6} - 2×10^{-3}	5×10^{-6} - 1.8×10^{-3}
LOD, mol L^{-1}	0.82×10^{-6}	1.64×10^{-6}
LOQ, mol L^{-1}	2.45×10^{-6}	4.86×10^{-6}

(continued on next page)

Table III. Performance characteristics of the developed sensors (continuation)

Parameters	ISE - A	ISE - B
Slope (mV/decade)	58.8 ± 0.96	62.11± 0.83
Intercept, mV	298.2	122.6
R^2	0.9998	0.9993
Optimum pH	1.0 – 3.8	
Life time, days	52	
Response time, s	25	

Assessment of correctness and precision

To ascertain daily and interday fluctuations, two sets of five replicates of RFA solutions at concentrations of 0.2, 0.4, and 0.6 mM were analysed. For the intra-day study, each solution was analysed seven times at three-hour intervals, while for the inter-day study, each solution was analysed five times over three days. The resulting %RSD (Relative standard deviation) and %RE (Relative error) values, listed in Table IV, were <1.2 and <2, respectively, indicating the sensors' correctness and precision.

Table IV. Findings demonstrating the precision and accuracy of the suggested sensors

ISE	RPF taken, mmol L^{-1}	Intra-day*			Inter-day$		
		RPF found, mM	%RSD	%RE	RPF found, mM	%RSD	%RE
ISE-A	0.2	0.198	0.78	1.05	0.198	0.89	1.14
	0.4	0.396	0.95	1.00	0.396	1.09	0.89
	0.6	0.591	0.70	1.49	0.589	0.70	1.81
ISE-B	0.2	0.201	1.12	0.64	0.201	1.06	0.72
	0.4	0.398	0.92	0.40	0.400	0.91	0.08
	0.6	0.601	0.79	0.15	0.603	0.67	0.45

*Seven measurements' average value; $five measurements' average value.

Robustness and ruggedness

Robustness was tested by varying experimental conditions slightly (pH: 3.0 ± 0.2 and temperature: 25 ± 2 °C). The values of %RSD varied from 0.95 to 1.22 for ISE-A and from 0.79 to 1.46 for ISE-B, verifying the method's resilience (Table V). Ruggedness was evaluated through inter-analyst and inter-potentiometric measurements on different days, with RSD values <2, confirming the sensors' ruggedness.

Table V. Results of robustness and ruggedness of ISEs

ISE	RPF taken (mM)	% Values of RSD for different parameters		
		Robustness (by varying T)	Ruggedness	
			Inter-analyst	Inter-potentiometric
ISE-A	0.2	1.16	1.62	1.85
	0.4	1.22	1.26	1.63
	0.6	0.95	1.54	1.03
ISE-B	0.2	1.46	1.53	1.64
	0.4	1.16	1.24	1.57
	0.6	0.79	1.47	1.20

Application of ISEs for FA tablets analysis

Tablet extracts at concentrations of 0.2, 0.4, and 0.6 mM RFA were analysed in five replicates using the validated ISEs. Table VI contains a tabulation of the amount of RFA, recovery percentage, and percentage RSD. The computed *t*- and F- values for four degrees of freedom were less than the standard values at a 95% confidence level, which was in line with the reference method's observations. There is no discernible change between the suggested and reference procedures, as seen by the average percentage recovery of RFA being almost 100% with a standard deviation under 2%.

Table VI. Analyses of tablets containing FA using suggested ISEs; statistical comparison of findings with official method

Tablet analysed	FA/tablet (mg)	Found* (%label claim ± SD)		
		USP method	Using proposed sensors	
			ISE-A	ISE-B
Cytomid	250	99.67±1.88	99.81±1.67 *t* = 0.96 F = 2.15	99.92±1.61 *t* = 1.07 F = 1.99

*Arithmetic means of five determinations
(The approximated *t*-value for four degrees of freedom and at the 95% confidence level is 2.77.)
(The approximated F-value for four degrees of freedom and at the 95% confidence level is 6.39).

Recovery study

Using the suggested ISEs and a conventional addition process, the recovery of FA was evaluated. Tablet extract that had been previously studied (0.2 mM) was added to five replicates of pure RFA solutions (0.2, 0.4, and 0.6 mM). A pH of around three was achieved by adding 2 M Na_2CO_3, and the liquid was homogenized after the volume was raised to 25 mL using distilled water. The ISEs were used to gauge each solution's potential. The total RFA found, %RFA recovered, and %RSD were calculated and recorded in Table VII. The % recovery ranged from 99.30 to 100.28 with %RSD values between 0.46 and 1.23, demonstrating the accuracy of the assay procedures.

Table VII. Results of recovery study by following standard-addition strategy

ISE	RFA from tablet extract (mmol L^{-1})	Pure RFA added (mmol L^{-1})	Total RFA found (mmol L^{-1})	%RFA recovered*	%RSD
ISE-A	0.2	0.2	0.398	99.61	1.03
	0.2	0.4	0.597	99.56	0.69
	0.2	0.6	0.802	100.28	0.46
ISE-B	0.2	0.2	0.398	99.60	1.23
	0.2	0.4	0.599	99.95	0.57
	0.2	0.6	0.794	99.30	0.60

*Arithmetic means of five determinations

Utilization in spiked human urine examination

It was found from the spiked human urine analysis that there was no evidence of endogenous matter from urine interfering with the recorded potentials for RFA samples. The mean per cent recovery of RFA was 99.36% with an RSD of 1.12% for ISE-A, and 99.24% with an RSD of 1.25% for ISE-B. These results support the applicability and suitability of the proposed sensors for determining RFA in urine in therapeutic administration settings.

CONCLUSION

The amount of flutamide (FA), both in pure form and in drug formulations, was effectively determined using two newly developed and validated ion-selective electrodes. The innovation of these methods lies in the use of two sulfonphthalein dyes, bromocresol green and bromophenol blue, as agents for ion-pair complexing in the potentiometric measurement of FA. The ability to form a non-polar solvent extractable ion associate complex with the drug is the main factor in the selection of these two dyes for the preparation of ISEs. The methods demonstrated excellent selectivity with mean recoveries of 99.81% and 99.92% for FA from pharmaceutical samples and low standard deviations of less than 2%. RSD values below 2% confirmed the methods' ruggedness and robustness. Among these two ISEs, the sensitivity of ISE-A is higher compared to ISE-B as it covers a wide linear range of 2.5×10^{-6} – 2×10^{-3} M with a LOD and LOQ values of 0.82×10^{-6} M and 2.45×10^{-6} M, respectively. These methods are suitable for routine analytical use in quality control laboratories, as they do not require sophisticated instruments or highly skilled operators.

Conflicts of interest

The authors claim there is no conflict of interest.

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