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Full Paper

Fabrication and Validation of Novel Fexofenadine Sensor using Alizarin Red S as Ion Exchanger on PVC Matrix for Assay in Pharmaceuticals and Spiked Human Urine Samples

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Abstract- This study depicts the plan, optimization, validation, and utilization of a novel polyvinylchloride (PVC) lattice-assisted membrane sensor to quantify fexofenadine hydrochloride (FFH) by utilizing Alizarin Red S (ARS), β-cyclodextrin (β-CD) and nitrophenyl octyl ether (NPOE) as an ion-exchanger, ionophore, and plasticizer respectively. The PVC network-assisted FFH-ARS sensor answers in <15s with super Nernstian conduct for FFH over 2.5×10^{-6}-1.25×10^{-3} mol L^{-1} in the pH of 2.0 to 5.5 range. The regression coefficient acquired for the alignment plot is 0.9921. The determined Nernstian slope of the line is 56.18±1.25 mV/decade. The detection limit (LOD) is viewed as 3.5×10^{-7} mol L^{-1}. Validation results clarified its appropriateness to assay FFH precisely and definitively. The sensor is a decent one for robust and rugged capability with a mean RSD of 4.39%. The outcomes of the interference study confirmed the non-interference of foreign ions while measuring the potentials. Statistical comparison of the outcomes confirms the good agreement of results of the proposed analytical procedure with the reference one. The percentage of mean recovery of FFH utilizing the proposed FFH-ARS sensor was 98.56 and 95.61% for the tablets and spiked human urine respectively, and this affirmed the selectivity of the solid-state electrode for FFH.

Keywords- Fexofenadine; Sensor; Alizarin Red S; Nernstian behaviour; Ionophore; Statistically agreed

1. INTRODUCTION

Fexofenadine hydrochloride (FFH) is a second-age allergy medication, acquainted with the IUPAC name (±) α, α - dimethyl 4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)- 1-piperidinyl]-butyl]-benzene acetic acid hydrochloride (Figure 1). It has been utilized to get relief from physical symptoms of seasonal hay fever and in chronic urticarial therapy [1]. When histamine binds to receptors, cells get activated and release substances that have allergic side effects, such as sneezing. FFH blocks H_1 receptor, preventing histamine from activating cells that possess non-sedating histamine receptors [2].

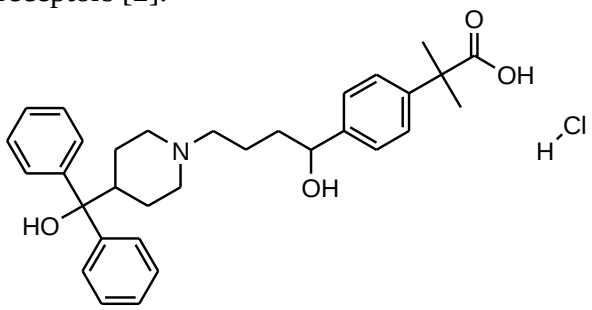


Figure 1. Chemical structure of FFH

The Monographs of both United States and European Pharmacopeia portray the liquid chromatographic procedure for the quantification of FFH [3,4]. FFH in the pure form, pharmaceutical formulations, and spiked body fluids have been quantified by the titrimetric [5-7], UV-visible spectrophotometric [1,7-20], spectrofluorometric [2,21], thin layer chromatographic [19,22], HPTLC [23-26], HPLC [1,20,27-51], UHPLC [52-54], LC-MS/MS [55,56], electrophoretic [57] voltammetric [58] and conductometric [9,59] techniques by different workers, as per the vigorous literature survey conducted on the analytical methods for FFH.

The solid-state potentiometric sensor electrodes were constructed by Abbas et al (2004) using reinecke salt as an ion-exchanger and di-octyl phthalate as a plasticizer for the direct potentiometric measurements in the quantification of FFH in tablets [60]. The reported sensor has the LOD value of 1.3×10^{-6} M and is applicable to determine 2.5×10^{-6} to 1×10^{-2} to M FFH. The slope for the calibration line was 62.3 mV/decade. It involves the potentiometric titration between FFH and phosphomolybdic acid with the use of a developed sensor working as an indicator electrode.

The reported methods like fluorimetry [20, 21], chromatography [22-56], electrophoresis [57], and voltammetry [58] require sophisticated equipment, complex sample preparation step, and prolonged analytical time. The earlier potentiometric method using the sensor made of reinecke salt exerts with higher detection limit and latent period of 25-45s. The cyclic voltammetry procedure has severe limitations such as the need for axillary and high-cost electrodes and the upkeep of stern experimental conditions.

The utilization of inexpensive ion-associating agents such as Alizarin Red S has not been described by any of the previous workers especially to construct potentiometric sensors. When evaluated about ion-selective electrodes, potentiometric methods are superior with short analysis time, better LOD, involving a simple design, cost-effective, wide operating range, high selectivity, minimum sample pre-treatment, excellent accuracy and precision, and ease of measurement process.

In the ongoing work, a unique potentiometric fexofenadine-selective sensor, that is a solid-state PVC-membrane type, was designed by making use of ARS as an ion-pairing agent or ion-exchanger, NPOE as a plasticizer and β-CD as ionophore. Characteristic parameters (LOD, linearity, standard deviation along with the slope, response time, operative pH, temperature ranges, selectivity, accuracy, precision, robustness, and ruggedness, etc.) of the designed sensor were investigated in detail. The sensor was employed successfully for FFH determination in the pharmaceutical compositions and sample of spiked human urine. The potentiometric and Official USP methods' results were compared.

2. EXPERIMENTAL

2.1. Apparatus

The digitized dual-channel potentiometer (PICO Chennai-32, India) and an Elico (Mumbai, India) pH meter were used to record the potential and pH respectively. Ag/AgCl counter electrode and an aluminum wire as conducting material in an indicator electrode were used for potential measurements.

2.2. Reagents and Solutions

Every one of the chemical substances used in the process was of prime immaculateness and suitable for various analytical approaches. Alizarin red S (ARS), chloroform ($CHCl_3$), dichloromethane (DCM), dichloroethane (DCE), tetrahydrofuran (THF), polyvinylchloride (PVC), dibutyl phthalate (DBP), dioctyl sebacate (DOS), nitrophenyl octyl ether (NPOE) and β-cyclodextrin (β-CD) were purchased from S.D. Fine Chem, Mumbai, India. The pure (99.89%) FFH was practiced as gotten from Sanofi-Aventis Pharma, Mumbai, India, as a gift. Glacial acetic acid was purchased from Merck Ltd, Mumbai, India. Commercial tablets, to be specific, Allegra-120 and Allegra-180 were bought from local stores (Aventis Pharma Ltd., Ankleshwar, India). Distilled water was employed for the entire investigation.

A 0.25% (w/v) solution of ARS was prepared using 250 mg of dye in distilled water; the filtrate was collected through Whatman No.42 filter paper, and used. The reagents such as KOH, NaOH, KH_2PO_4, NaOAc, Na_2CO_3, $NaHCO_3$, CH_3COOH, $AgNO_3$, KNO_3, KCl, H_3PO_4, $NaNO_2$, $CdCl_2$, $CoCl_2$, glucose, sucrose, L-ascorbic acid, urea, uric acid, oxalic acid, glycine, arginine, and talc were purchased from S.D. Fine Chem Ltd, Mumbai, India, and utilized to

prepare solutions of concentration 1mol L^{-1} each by using distilled water. A urine sample was collected from a fit and fine volunteer.

2.3. Standard FFH solution (5.0 mmol L^{-1})

The required quantity of FFH was weighed into a 100mL standard flask, dissolved in glacial acetic acid (5mL), and solution was made up to the mark using distilled water.

2.4. General procedures

2.4.1. Procedure to obtain FFH-ARS ion-association complex

20 mg of FFH was dissolved in 5 mL glacial acetic acid and the volume was raised to 50 mL with distilled water. About 15 mg of ARS was added and dissolved in the above solution, transferred carefully into a 250 mL clean separating funnel. The solution was acidified with 2 mL of H_2SO_4 (1M) to adjust pH between 3.0 and 4.0, and 20 mL of DCM was added to it. The content was shaken vigorously for 60 seconds and layers were allowed for equilibration. The organic layer dried through anhydrous sodium sulphate was collected in a dried beaker. The procedure was repeated twice with 20mL of fresh DCM at a time. The combined extract was kept in a water bath, evaporating the solvent and the resulting solid FFH-ARS ion-association complex was collected, preserved, and used.

2.4.2. Designing of FFH-ARS PVC sensor

The solution containing a composite mixture of 10.0 mg of FFH-ARS ion-associate, 170 mg of PVC, 5 mg of β-CD, and 150 mg of NPOE was prepared in 10 mL THF, transferred to a 5 cm wider Petri Dish and dried at operating temperature for one day. The resulted thin membrane with 2.99% (w/w) in FFH-ARS ion-associate, 50.7% (w/w) in PVC, 1.49% in β-CD, and 44.78% (w/w) in NPOE was used to cover one end of plastic tube utilizing THF and allowed to dry for one day at operating temperature. A 5 mL each of 5.0 mmol L^{-1} FFH and 2.0 mmol L^{-1} KCl solutions were filled into the tube. Ag-AgCl electrode was immersed into the tube and taken into connection to the potentiometer. The electrode was immersed in 5.0 mmol L^{-1} FFH solution for 4 – 5 h prior to its use.

2.4.3. Procedure for bulk drug

a. Construction of calibration curve

Suitable aliquots of 5.0 mmol L^{-1} standard FFH drug solution equivalents to 2.5×10^{-6}-1.25×10^{-3} mol L^{-1} FFH were measured and taken in different 25 mL standard flasks. The volume of solution in each flask was adjusted to 10 mL with distilled water and the pH was brought to the range of 2.0 to 5.5 with 1 M H_2SO_4 or 1 M NaOAc solution. The solution was made up to the mark with distilled water and the content in each flask was mixed well. The

potentials were recorded with a developed membrane sensor using Ag/AgCl reference electrode at 25 ± 2^0 C.

The calibration plot of potentials versus log[FFH] was constructed and used to find the unknown FFH concentration. The concentration of FFH in samples was computed using a derived regression equation.

b. Procedure for tablet analysis

The previously weighed twenty tablets (Allegra-180 and Allegra-120) were powdered separately and the quantity precisely identical to 134.5 mg of FFH was taken in two separate 50 mL volumetric flasks and added 35 mL of 5% acetic acid (v/v) to them. The components were mixed well for about 20 min and the volume was raised to 50mL with 5% acetic acid. The solution was filtered with the use of filter paper (Whatman No.42) and a required aliquot was examined as per the procedure mentioned above.

c. Procedure for analysis of spiked human urine

A sample of urine was collected from a healthy female volunteer and filtered. Two milliliters of it were spiked with 3 mL of FFH (5 mmol L^{-1}) in a beaker and mixed well. The pH of the content was adjusted between 2 and 5.5 by adding 1 M H_2SO_4 or 1 M NaOAc. The volume was raised to 25 mL using distilled water and then the potential was measured. The unknown concentration of FFH was computed using either the derived regression equation or calibration curve.

d. Interference study

5 mL of pure FFH solution of strength 5.0 mmol L^{-1}, 10 mL of water, and 1 mL of solution of interferents (1 mol L^{-1}) were taken in a beaker and blended for 5 minutes. The pH was adjusted between 2.0 to 5.5; volume was raised to 25 mL with distilled water, and homogenized. Potentials of the solution were measured using the proposed sensor with respect to the Ag-AgCl reference electrode and the concentration of FFH was computed.

e. Determination of selectivity coefficient ($K_{FFH.I}$) of sensor

Varying aliquots (1 to 10 mL) of 5 mmol L^{-1} FFH were taken in 50 mL beakers and added 1.0 mL of interferent of strength 1.0 mol L^{-1} to it. The pH of the solutions attuned to the range of 2.0 to 5.5, adjusted the volume to 25 mL with water followed by thorough mixing. Similar set of solutions were prepared for other interferents also. The potentials (E_{Cell}) were measured utilizing FFH-ARS sensor.

The E_{Cell} were plotted against the log[FFH] and located the point of intersection. The $K_{FFH.I}$ is calculated for each interferent using the following formula [60]:

$$K_{FFH.I} = \frac{[FFH]_E}{[I]_E{}^{Z_{FFH}/Z_I}} = \frac{[FFH]_I}{[I]_{add}{}^{Z_{FFH}/Z_I}}$$

where, $[FFH]_E$ is the strength of FFH to generate indistinguishable $E_{Cell,}$ $[I]_E$ is the strength of interferents to generate indistinguishable values of $E_{Cell,}$ z_{FFH} is the cationic and anionic charges on FFH, and z_I is the charge on added interferent, $[FFH]_I$ *is* the concentration of FFH in the internal solution and $[I]_{add}$ is the concentration of interferent present or added to FFH solution.

3. RESULTS AND DISCUSSION

The FFH-ARS ion-association complex has been exploited as a recognizable material in this work. Scheme -1, Shows the probable reaction in the acidic medium between FFH and ARS in the stoichiometric ratio 1:1 to lead yellow ion association complex, extractable with DCM [6]. Absorption spectrum was plotted for the ion-association complex formed regarding the reagent blank (figure 2) and observed a maximum absorption at 440 nm. The membrane was designed by using FFH-ARS ion-association complex, β-CD, and NPOE in PVC assisted matrix and used as a sensor to quantify FFH potentiometrically. The systematic representation of the electrochemical cell constructed using the designed membrane sensor for FFH determination is as shown below:

$$Ag\text{-}AgCl_{IR} \,\|\, FFH_I,\ (5\ mM),\ KCl\ (2\ mM) \,|\, Membrane \,|\, [FFH]_{Sample} \,\|\, AgCl\text{-}Ag_{SR}$$

where 'Ag-$AgCl_{IR}$' and 'Ag-$AgCl_{SR}$' are reference Ag-AgCl electrodes immersed into internal reference FFH (FFH_I) and sample solution $[FFH]_{Sample}$, respectively. 'Membrane' is a sensor contrived with an ion- associating agent (ARS), ionophore (β-CD) and a plasticizer (NPOE) in the PVC matrix.

The E_{Cell} and $[FFH]_{Sample}$, are related through the followingNernst equation [61]:

$$E_{Cell} = K + 0.05916\, log[\,FFH]_{Sample}$$

Where K indicates the potential of reference electrode, Liquid junction potential, the asymmetry potential, the activity coefficient of FFH, and $[FFH]_I$.

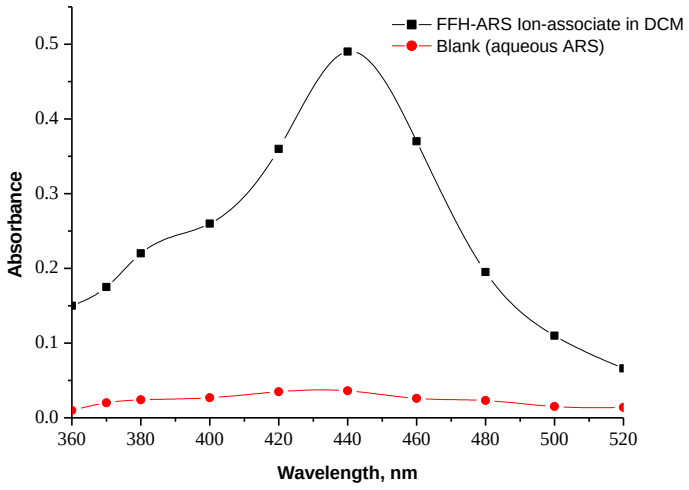
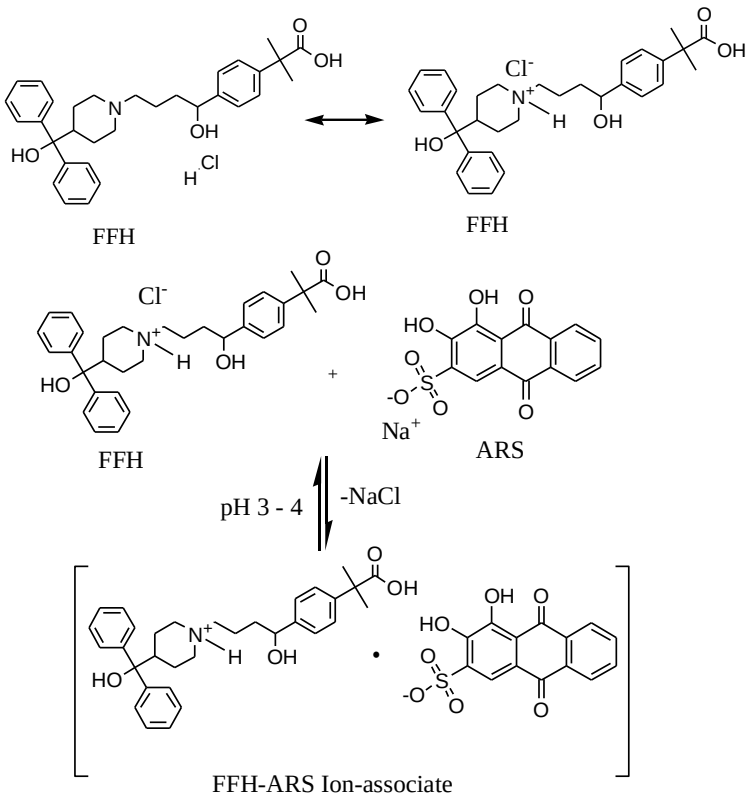


Figure 2. Absorption spectra of FFH-ARS ion-association complex and ARS blank



Scheme 1. Reaction pathway for the formation of FFH-ARS complex

3.1. Optimization of parameters

3.1.1. Membrane composition

Optimum amounts of the matrix, plasticizer, ion exchanger, and ionophore for the purposed membrane have been considered after monitoring the results of a series of preliminary experiments for each parameter. The effective functioning of the membranes prepared in sensing FFH potentiometrically was checked. The membrane sensor prepared with 10.0 mg of FFH-ARS ion-associate, 170 mg of PVC, 5 mg of β-CD, and 150 mg of NPOE was found as a selective one for consistency in measurements. Moreover, the slant got for the plot with this membrane was because of the Nernstian response. The volume of THF viewed helpful for the dissolution of materials was 10mL. Much variation has not been noticed in using more than 10 mL of THF. The solvent was evaporated in 24 h after pouring into Petri Dish.

3.1.2. Choice of plasticizer

The different amounts of various plasticizers such as NPOE, dioctyl phthalate (DOP), dibutyl phthalate (DBP) and Dibutyl sebacate (DBS) were employed as plasticizers to construct appropriate membranes. The membrane sensor responded well with the consistent potential as well as with the Nernstian behavior when the quantity of NPOE was 150 mg. The amount NPOE greater than 150 mg prolonged the evaporation. Thus, 150 mg of NPOE was confirmed to be a more suitable plasticizer for rapid sensing of FFH using the FFH-ARS sensor. The

remaining plasticizers did not yield satisfactory outcomes regarding calibration, Nernstian behavior, response time, and reproducible E_{Cell} values. The results obtained for the choice and optimization of plasticizers are included in Table 1.

Table 1. Results of choice and optimization of plasticizer for FFH-ARS membrane

Plasticizer	**Amounts, mg**	**Slope* ±SD**	**Confidence limit (CL) at 95%**
DBP	50.0	46.12±2.11	2.61
	75.0	48.02±0.95	1.18
	100.0	51.23±1.11	1.37
	125.0	52.23±1.48	1.83
	150.0	52.22±0.96	1.19
DBS	50.0	49.18±1.88	2.33
	75.0	50.31±1.12	1.39
	100.0	51.99±1.33	1.65
	125.0	53.98±2.15	2.66
	150.0	54.18±2.21	2.74
DOP	50.0	48.18±2.25	2.79
	75.0	49.22±2.11	2.61
	100.0	52.87±0.98	1.21
	125.0	52.55±2.11	2.61
	150.0	53.11±2.22	2.75
NPOE	50.0	44.11±1.47	1.82
	75.0	51.02±1.56	1.93
	100.0	53.56±2.00	2.48
	125.0	55.00±1.11	1.38
	150.0	56.18±1.25	1.55

*Mean value of five determinations

3.1.3. The concentration of FFH in the internal reference solution

A calibration plot of E_{cell} against log[FFH] using FFH-ARS sensor with varying concentrations of internal FFH and KCl solutions was prepared and evaluated for Nernstian behavior. The result obtained with 5 mL of 5.0 mmol L^{-1} FFH and 5 mL of 2.0 mmol L^{-1} KCl solutions was highly satisfied with the expected Nernstian response of the sensor. A good correlation was seen between potentials and log[FFH]. The calibration curve obtained between potentials and log[FFH] is presented in Figure 3.

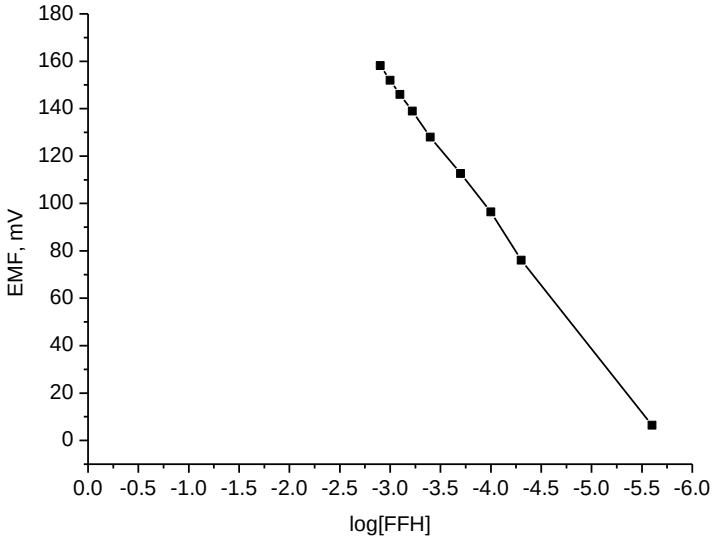


Figure 3. Calibration plot of E_{Cell} against *log[FFH]* for FFH concentration ranged from 2.5×10^{-6} to 1.25×10^{-3} mol L^{-1} under optimum conditions with FFH-ARS Sensor

3.1.4. Electrode conditioning time

The sensor was conditioned by soaking it in the standard solution of FFH for different intervals of time. The observation of this study has evolved the need for immersing the sensor in FFH solution at least for 4.5 to 5 h. In this manner, the time span expected to prepare the membrane's dynamic surface for successful use at 25° C was found as 4.5 h. The impact of the standing time of the sensor in FFH on the potential is given in Figure 4. This assessment also endorsed that, the dried sensors might be kept in any closed opaque vessel and can be used further after conditioning by soaking in standard FFH for 4.5 h.

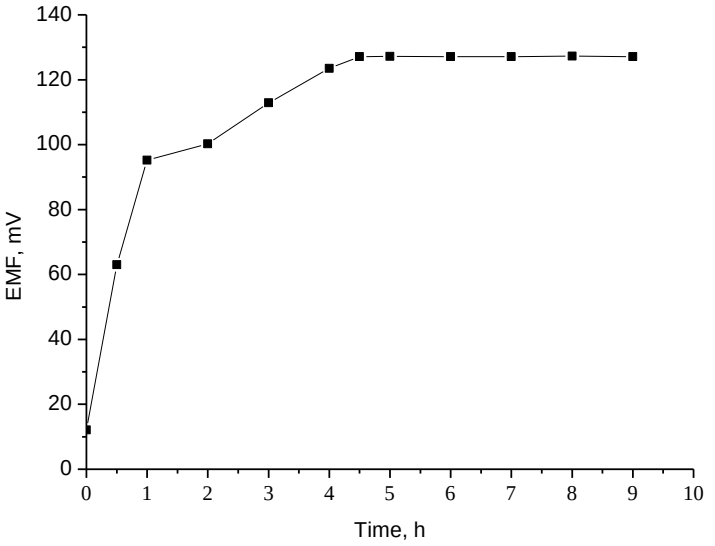


Figure 4. Effect of conditioning time on the potential of 4×10^{-4} M FFH solution using FFH-ARS against Ag-AgCl reference electrode

3.1.5. Effect of pH

The effective pH for measuring steady-state and reproducible potentials (E_{Cell}) using the designed sensor with the Ag-AgCl reference electrode was evaluated. The E_{Cell} was recorded for FFH solution of fixed concentration in the pH range of 0.5 to 8. The pH of solutions was adjusted by adding appropriate volumes of either 1 M NaOAc or dilute NH_3 solutions before the measurement of potentials. The consistency in potential was established over the pH range of 2.0 to 5.5. At pH other than covered in this range, the sensors' behaviour was not Nernstian. Otherwise, potentials were observed as quite unusual.

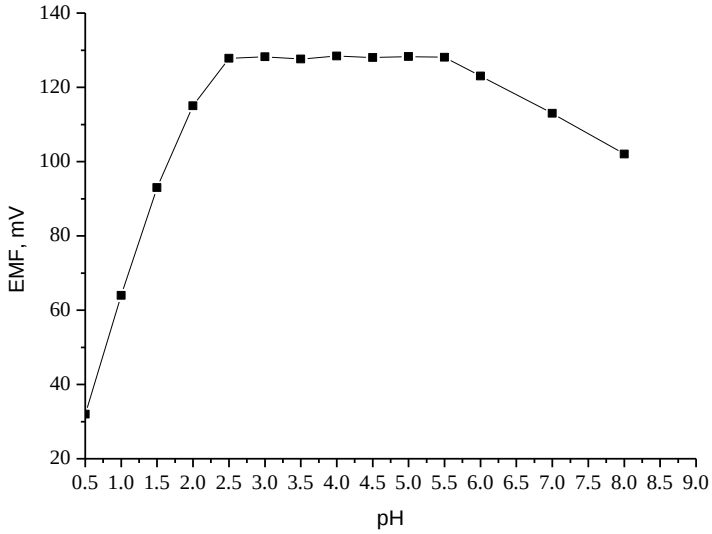


Figure 5. Variation of the potential of 4.4×10^{-4} mol L^{-1} FFH solution measured with FFH-ARS sensor at different pH

Table 2. Results of evaluation of the effect of pH on the behavior of FFH-ARS sensor

pH	Slope of the Calibration plot, mV/decade*	Standard Deviation, SD
0.5	31.2	0.56
1.0	36.88	0.48
1.5	51.22	0.49
2.0	56.17	0.51
2.5	56.18	0.56
3.0	56.19	0.62
3.5	56.13	0.61
4.0	56.15	0.51
4.5	56.14	0.55
5.0	56.19	0.59
5.5	56.12	0.49
6.0	53.21	0.64
7.0	47.12	0.89
8.0	40.12	0.94

*Mean value of three determinations

Hence, the pH range of 2.0 to 5.5 was fixed as ideal for the estimation of the potential of FFH solutions utilizing FFH-ARS sensor. This effect of pH on E_{Cell} of FFH solutions is shown in Figure 5 and the resulting Slopes of calibration lines at different pH are summarised in Table 2.

3.1.6. Response time

The response time of the developed and conditioned FFH-ARS sensor was evaluated. It was found from the experimental observations that the sensor responds to FFH solutions in 30 s. Therefore, it is recommended that after immersing the conditioned sensor into sample solutions of FFH the potentials may be recorded after 30 s.

3.1.7. Lifetime of the sensor

The designed sensor was used frequently on a routine basis for measuring FFH solutions and it was excellent in functioning with a mean Nernstian slope of 56.18 ±1.25 mV/decade up to 62 days. The deviation was observed with the experimental Nernstian slope after 62 days. Therefore, the conclusion drawn and proposed is that the validity of this FFH-ARS sensor is up to 62 days.

3.1.8. Evaluation of selectivity coefficients

The membrane potential (E_m) is generated by the chemical communication between active sites on the membrane and the analyte. The membrane's selectivity has to be assured for a single species because of the factor of the chemical process on the signal. For all intents and purposes, E_m is proportionate to the ionic strength that can interact with the membrane's dynamic destinations. The summed-up Nernst condition that incorporates the interferent's (I) commitment is met as underneath:

Practically, E_m is proportionate to the ionic concentration that can interact with the membrane's active sites. The generalized Nernst equation that includes the interferent's (*I)* contribution is convened as below:

$$E_{cell} = K + \frac{0.05916}{Z_{FFH}} log\ (\,[FFH] + K_{FFH,I}\,[I]^{\frac{Z_{FFH}}{Z_I}}) \qquad (1)$$

where the constant K incorporates reference electrodes, liquid junction, and membrane potentials, Z_{FFH} and Z_I indicate the charges on FFH and I, respectively. $K_{FFH,I}$ is the selectivity coefficient as it may be defined as:

$$\boldsymbol{K_{FFH,I}} = \frac{[\boldsymbol{FFH}]_{\boldsymbol{E}}}{[\boldsymbol{I}]_{\boldsymbol{E}}^{\boldsymbol{Z_{FFH}}/\boldsymbol{Z_I}}} \qquad (2)$$

where, $[FFH]_E$ and $[I]_E$ represents the concentration of FFH and I to yield similar cell potentials (E). The membrane responds to both FFH and I when $K_{FFH,I}$ is unity. The $K_{FFH,I}$ value must always be < 1.0 for the membrane selected [62].

Subsequently, as a significant nature of the proposed FFH-ARS sensor, $K_{FFH,I}$ can be assessed utilizing the information of potentials of solutions contained with a known yet fixed amount of I, $[I]_{add}$, and differing measures of FFH. In assessing the value of $K_{FFH,I}$, two well defined linear regions are informative in the plot of E_{cell} against log [FFH]. E_{cell} is a linear function of log [FFH], when the [FFH] is significantly larger than $K_{FFH,I}$ $[I]_{add}$. E_{cell} remains constant when $K_{FFH,I}$ $[I]_{add}$ is significantly larger than [FFH]. The $K_{FFH,I}$ value is calculated using the [FFH] and [I] at the crossway of the two linear regions.

In this way, $K_{FFH,I}$ an indicator value for FFH with various interferent, were determined and are given beneath in Table 3. The sensor was examined with different interferents of the inorganic, organic, anionic and cationic nature. A different solutions containing 1 mol L^{-1} of each interferent was spiked into a pre-examined FFH solution and investigation was done [63, 64]. The determined values of the selectivity coefficient organized in Table 3 demonstrate the non-impedance of the additional species as the determined $K_{A,I}$ is <1. Accordingly, the FFH-ARS sensor conceivably utilized to examine the genuine examples that go with interferents for the FFH specifically.

Table 3. The selectivity coefficients of the sensor for various interferents

Interferent	**Selectivity coefficient, $K_{FFH,I}$***
Ag^+	0.342
NH_4^+	0.123
Na^+	0.425
K^+	0.412
H^+	0.092
Ca^{2+}	0.365
Co^{2+}	0.614
Zn^{2+}	0.444
Glycine	0.090
Urea	0.561
Uric acid	0.851
Glucose	0.213
Oxalate	0.328
Formic acid	0.421
Citric acid	0.333
Tartaric acid	0.413
Benzoic acid	0.210
Salicylic acid	0.120
Phthalic acid	0.213
Boric acid	0.289
Talc	0.122

*Average of 5 determinations

3.2. Validation of sensor

The sensor was examined for linearity, accuracy and precision, robustness and ruggedness as indicated by IUPAC Guidelines [62, 63] and ICH Rules [64]. The evolved-out validation outcomes are mentioned in the sections below hereafter.

3.2.1. Linearity and regression data

The measured potentials and FFH concentrations were linear as described in the plotted Figure 3. The Nernstian conduct is shown by the incline of 56.18 ±1.25 mV/decade for the calibration plot. The curve fitting equation obtained by considering regression data is as follows:

$Y = 56.18 X + 320.06$. The regression coefficient to exhibit highly agreeable linearity between estimated potentials and log[FFH] was likewise determined. The LOD was calculated in accordance with IUPAC Guidelines [62, 63], i.e., from the convergence point of the extrapolated linear portions of the calibration line with x-axis. Other performance characteristic values for the FFH-ARS membrane sensor are given below in Table 4.

Table 4. Sensor's performance features and regression data

Parameter	**Values**
Linear range, mol L^{-1}	2.5×10^{-6} to 1.25×10^{-3}
Limit of detection (LOD), mol L^{-1}	3.5×10^{-7}
Slope (m), mV/decade	56.18
Intercept (b), mV	320.06
Correlation coefficient (R)	0.9997
R^2	0.9994
pH (Optimum)	2.0 – 5.5
Lifetime, days	62

3.2.2. Accuracy and Precision

To study the intra-day variations, 0.2, 0.6, and 1.0 mmol L^{-1} FFH solutions and their seven replicas were analyzed and evaluated. The FFH concentration and %RSD for each level of test solutions were calculated and summarised in Table 5. In inter-day deviations, 0.2, 0.6, and 1.0 mmol L^{-1} FFH solutions and their five replicas were examined over three days. The concentration/amount of FFH found and %RSD values of these samples are also included in Table 5.

Calculated relative error (RE) between the FFH taken and found, as an index of accuracy, for each concentration in intra- and inter-day evaluations are placed in Table 5. The accuracy and precision of the proposed scientific technique utilizing the FFH-ARS membrane sensor were shown by the %RSD somewhere in the range of 2.11 and 4.89%, and %RE values ≤5%.

Table 5. Results indicating the precision and accuracy of the FFH-ARS sensor

FFH taken, mmol L^{-1}	Intra-day variations			Inter-day variations		
	FFH found*, mmol L^{-1}	%RSD	%RE	FFH found$^{\$}$, mmol L^{-1}	%RSD	%RE
0.20	0.208	4.00	5.00	0.194	2.21	3.00
0.60	0.580	3.69	3.33	0.607	4.89	3.50
1.00	1.031	3.00	3.10	0.960	2.11	4.00

*Arithmetic means of seven measurements; $^{\$}$ Arithmetic mean of five measurements

3.2.3. Robustness and ruggedness

Robustness of the proposed sensor was ascertained by deliberately changing the ideal working temperature by 2^{O} C in the examination of 0.2, 0.6, and 1.0 mmol L^{-1} FFH solutions. The %RSD found at the temperature 23, 25, and 27^{O} C were in the reach 2.56 – 4.88 (Table 6). This confirmed the robustness of the method.

The proposed sensor was used for analyzing FFH by three analysts. The three different potentiometers were used to monitor the instrumental variations. The %RSD found were also indicated in Table 6. RSD values ranged between 2.59 and 4.65% in the table below proving the rugged behavior of the sensor.

Table 6. Results of robustness and ruggedness of FFH-ARS sensor (expressed in %RSD)

Concentration of FFH, mmol L^{-1}	%RSD values for varied parameters		
	Robustness (Varying T by 2^{O} C)	Ruggedness	
		Inter-analysts	Inter-potentiometric
0.20	3.26	2.61	3.33
0.60	4.88	2.59	4.65
1.00	2.56	4.00	2.98

3.2.4. Application to FFH tablet analysis

The constructed and validated membrane sensor was employed to analyze the five replicates of tablet extracts each of 0.2, 0.6, and 1.0 mmol L^{-1} FFH. The quantity of FFH present, their % recovery, and %RSD were determined. The official USP technique was considered to compare the mean recovery and %RSD of the designed method [1]. The student-t and F-test were applied to individually assess the accuracy and precision of the introduced technique respectively. The t_{Cal} and F_{Cal} values at the confidence level of 95% were < 2.77 (t_{tab}) and < 6.39 (F_{tab}), respectively for four degrees of freedom. Hence, the results obtained by the developed sensor are accurate and précised. The values were tabulated in Table 7.

Table 7. Results of analysis of FFH tablets utilizing developed sensor and statistical comparison with authority USP technique

Tablets analyzed	mg of FFH/Tablet	Found*	
		%Label claim±SD	
		USP method	The proposed method using FFH-ARS sensor
Allegra-180ᵠ	180.0	96.88±1.35	97.12±1.59 ***t*** = 0.25 ***F*** = 1.39
Allegra-120ᵠ	120.0	98.11±1.54	96.23±1.05 ***t*** = 2.29 ***F*** = 2.15

*Arithmetic means of 5 determinations
(Tabulated t-value at the 95 % confidence level and for four degrees of freedom is 2.77)
(Tabulated F-value at the 95 % confidence level and for four degrees of freedom is 6.39)
ᵠMarketed by: Aventis Pharma Ltd., Ankleshwar, India

3.2.5. Recovery study

The proposed FFH-ARS sensor was employed to perform the recovery experiments by following the standard addition procedure. Three different levels of FFH pure solutions, namely, 0.2, 0.4, and 0.6 mmol L^{-1} were spiked into a pre-analyzed 0.4 mmol L^{-1} tablet extract, and potentials were recorded by using the FFH-ARS sensor. Into five replicates of 0.4 mmol L^{-1} tablet extract of FFH, 0.2, 0.4 and 0.6 mmol L^{-1} pure FFH solutions were spiked. The potential of the prepared solutions was recorded after adjusting the pH to optimum range, raising the volume to 25 mL with water followed by thorough mixing. The amount of FFH for each situation was determined and lastly the recovery values were figured. The %recovery of FFH found were from 97.5 to 101.5 (Table 8) with RSD values < 5% showing the accuracy of the introduced technique using FFH-ARS sensor.

Table 8. Results of recovery study by following standard-addition strategy

FFH from tablet extract, mmol L^{-1}	Pure FFH added, mmol L^{-1}	Total FFH found, mmol L^{-1}	%FFH recovered*	%RSD
0.40	0.20	0.595	97.5	3.22
0.40	0.40	0.806	101.5	4.56
0.40	0.60	1.006	100.1	2.36

*Arithmetic means of three measurements

3.2.6. Application to spiked human urine analysis

The potentials recorded for samples of FFH spiked with human urine have not shown any kind of interference from the endogenous substances of urine. The mean percent recovery of FFH from the analysis was 97.21 with an RSD of 2.13% and this supplemented the applicability and suitability of the proposed FFH-ARS sensor in determining the FFH in urine in therapeutic administration laboratories.

4. CONCLUSION

For the absolute first time, as an environmentally-safe methodology, the design, optimization, validation, and utilization of a new and original membrane sensor assisted by a polyvinylchloride (PVC) matrix is proposed to quantify the FFH selectively using ARS, NPOE and β-CD as an ion-pairing agent or ion-exchanger, plasticizer and ionophore respectively. The designed method with the potentiometric sensor is highly economical and selective between the linear order of 2.5×10^{-6} and 1.25×10^{-3} mol L^{-1} of FFH over the wider pH ranges between 2.0 and 5.5 with excellent Nernstian behavior as designated by the slope 56.18±1.25 mV/decade. LOD of the employed sensor was delineated to be 3.5×10^{-7} mol L^{-1}. This sensor is applicable for FFH quantification in pure, tablet, and spiked human urine samples. The outcomes acquired by the statistical tests affirmed the openness of the procedure for examine of FFH with excellent recoveries and agreed better with the official USP technique. Interference was not shown by the excipients present in tablets and hence the method is of the highest selectivity. Hence, the FFH-ARS sensor can be suggested for the potentiometric measure of FFH in quality control and therapeutic administration laboratories on a routine basis Thus, the FFH-ARS sensor can be recommended for the potentiometric assay of FFH in quality control and therapeutic administration laboratories on a routine basis.

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Declaration of interest

The authors proclaim no irreconcilable circumstance in this detailed work.

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