Development of a Sulfonephthalein Dye-Based Potentiometric Sensor for Cost-Effective Assay of Clobazam in Pharmaceuticals and Spiked Human Urine Samples

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Abstract
Clobazam (CLB) is a benzodiazepine and a central nervous system depressant. This first ever reported paper introduced a novel potentiometric sensor designed for the simple cost-effective assay of CLB in pharmaceuticals and spiked human urine (SHU). The sensor leveraged an ion association complex between CLB and bromophenol blue (BPB), which is a sulfonephthalein dye, integrated into a poly vinyl chloride matrix, with dibutyl phthalate as plasticizer, and prepared in tetrahydrofuran. IR spectral data were obtained to confirm the formation of CLB-BPB ion-association complex. For CLB, potentiometric measurements of the sensor yielded a linear calibration curve in the range of 9.3×10^{-5} to 4.8×10^{-4} M, with a regression coefficient of 0.9712. The slope of 30.35 indicated Nernstian behaviour of the sensor, corroborating two N atoms in CLB. The sensor demonstrated excellent performance, with a mean accuracy of about 98%, recovery from tablet formulations of 96.83%, and recovery over 96% from SHU samples. The interference study revealed the inactive role of common cations and anions, while measuring CLB potential. Additionally, the sensor showed excellent accuracy, precision, robustness and ruggedness, which makes it highly suitable for routine assays in pharmaceutical and physiotherapeutic laboratories. This method provides a reliable tool for monitoring CLB levels, addressing the need for effective therapeutic drug monitoring.

Keywords: BPB; CLB; ion-association complex; pharmaceutical and potentiometric sensor; spiked human urine sample.

Introduction•

Clobazam - CLB/7-chloro-1-methyl-5-phenyl-1,5-benzodiazepine-2,4-dione- (Fig. 1) is a benzodiazepine antiepileptic drug shown to be effective in many types of refractory epilepsy, which has been used since 1984. It preferentially binds to α_2

•The abbreviations list is in page 93.

subunit of Gamma-aminobutyric acid receptors over α_1, rendering it less sedating and more suitable for chronic administration than other benzodiazepines [1-3].
Although routine therapeutic drug monitoring (TDM) of CLB is not common, emerging evidence suggests that it could be valuable in cases with suspected pharmacokinetic alterations, such as drug–drug interactions [4], liver disease [5] and extreme age [6]. CLB is primarily metabolized in the liver with minimal renal elimination and it has a longer half-life than its parent drug, which contributes to its pharmacological effects [7]. Consequently, monitoring both CLB and its active metabolite, N-desmethylclobazam (NDMCLB), is essential for effective TDM.

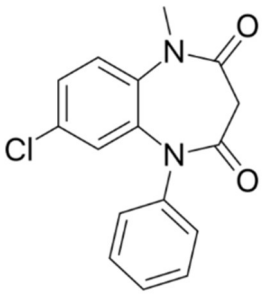


Figure 1: CLB molecular structure.

Various analytical techniques, including GLC [8-10], LC-MS/MS [11-13] and HPLC [14-21], have been employed to measure CLB and NDMCLB in biological samples. However, methods for quantifying CLB in pharmaceuticals are limited, and the reported ones have utilized HPLC [22-24]. European Pharmacopoeia describes the monograph for assaying CLB in pharmaceuticals by spectrophotometry [25], which involves measuring the drug in alcohol, at 232 nm. However, the above methods often come with several limitations. GLC and LC-MS/MS typically require significant expertise, specialized equipment and extended analysis times, which can lead to increased costs and complexity. Additionally, widely used HPLC methods are often time-consuming and resource-intensive, with extended sample preparation and analysis times [22-24].
This study aimed to address this gap by developing a potentiometric method using a novel ion-association complex membrane sensor for CLB detection in pharmaceutical formulations and spiked human urine (SHU). The proposed method relies on recording the potential of CLB sample solution, and calculating the concentration using the calibration curve. Thus, this paper is novel regarding other already reported studies on CLB detection in either pharmaceuticals or SHU samples.

Experimental

Apparatus

PICO Chennai-32 potentiometer is a precision instrument used for measuring EMF and determining electrical potential. The spectrophotometer provided by Agilent Technologies Ltd. was used to record absorbance and transmission of samples. An Elico pH meter (Mumbai, India) was used in all pH measurement and recordings.

Materials and methods

Analytical grade chemicals and reagents and distilled water were used throughout the work. Glycerol was generously supplied by Rankem, while tetrahydrofuran (THF), dibutyl phthalate (DBP), dioctyl phthalate (DOP), dibutyl sebacate (DBS) and nitrophenyloctyl ether (NPOE) were provided by SD Fine-Chem Limited. Poly vinyl chloride (PVC) was sourced from MP Biomedicals LLC. Bromophenol blue (BPB) powder was supplied by Merck. Methanol was obtained from Fisher Scientific, sulphuric acid (98 %) from Avra Ltd., and hydrochloric acid (95 %) from Merck. Whatman filter papers (125 mm, no. 1001 125), Ag-AgCl and SC electrodes, and Al wire were acquired from local commercial distributors. Pure CLB was obtained from Abbot Laboratories, and it was used without further purification. Two brands of CLB-containing tablets, namely, Clozam (20 mg CLB) and Czam (10 mg CLB), both from Abbot Laboratories, were procured from local pharmacy shops.

Procedure for preparation bulk drug solution

About 5 mM solution were prepared by dissolving the required quantity of pure CLB in methanol and diluting it to 50 mL mark, in a volumetric flask, using the same solvent. After thorough mixing, it was used for method development.

Procedure for preparation of CLB-BPB ion selective membrane electrode

25 mg pure CLB were precisely weighed and put into a 25 mL beaker, to which 0544 g BPB dye and 25 mL chloroform were added. The mixture was stirred until the compounds dissolved. The solution was then evaporated to dryness on a water bath, resulting in a residue, which was weighed and combined with 150 mg DBP and 200 mg PVC. Subsequently, 10 mL THF were added to dissolve the mixture, which was then poured into a 10 cm Petri Dish, and left overnight, to form a film. To make the ion-selective electrode, the above film/membrane was cut-immobilized at one end of a glass tube, using THF. At the other end, an Al wire was securely attached with insulation tape. The electrode was filled with 3-5 mL 0.005 M standard CLB (CLB_I) and 2 mL 1 M KCl solutions. It was then dried overnight, and soaked into the CLB solution, for 5 h. This electrode was employed as a sensor, for measuring the potential of CLB sample solutions (CLB_{Sample}), as shown (Fig. 2).

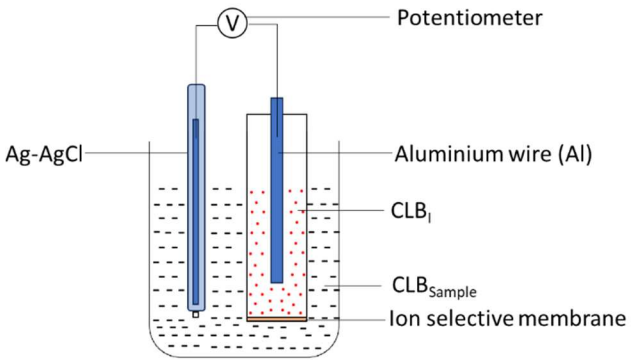


Figure 2: Schematic diagram of potentiometric cell with proposed ISE.

Systematic representation of the potentiometric electrochemical cell with designed-working and Ag-AgCl reference electrodes is given below:

$$\text{Ag-AgCl} \,\|\, \text{CLB}_{\text{Sample}}\ (\text{aq}) \,|\, \text{Membrane} \,|\, \text{CLB}_{\text{I}}\ (\text{aq, 0.005 M}),\ \text{KCl}\ (\text{aq, 1 M}) \,|\, \text{Al}$$

General procedures

Procedure for bulk drug

Different volumes of 5 mM CLB solutions (0.45, 0.75, 1.0, 1.5, 2.0 and 2.4 mL) were measured and transferred into separate 25 mL volumetric flasks. Subsequently, 2 mL 1 M H_2SO_4 and 3 mL 1 M KCl were added to each flask, and the volume was brought up to the mark with distilled water. The solution's pH was kept from 0.9 to 5.5. The potential for each solution was then measured using a specially designed membrane electrode, with Ag-AgCl reference electrode. Potential's difference values were plotted against logarithm of CLB concentration, and the slope was analysed for consistency with Nernstian behaviour. CLB concentration in the sample was determined using Eq. (1) [26]:

$$E_{Cell} = K + 0.05916/\text{z}\ log[\text{CLB}]_{Sample} \qquad (1)$$

where K represents combined effects of reference electrode's potential, liquid junction potential, asymmetry potential and activity coefficients of both CLB and CLB_I. Symbol z denotes overall charge on CLB, which corresponds to the number of protonated nitrogen atoms in it. For CLB, value of z is + 2.

Procedure for tablets

Thirty tablets were carefully weighed and finely powdered. An amount of powder corresponding to 75 mg CLB was placed into a 50 mL volumetric flask, followed by the addition of 30 mL methanol. The mixture was shaken for 20 min, to ensure CLB dissolved, and then the flask was filled to the mark with methanol. The solution was thoroughly mixed and filtered using Whatman No. 1 filter paper. Appropriate aliquots of the filtrate were then taken for the experiment.

Procedure for analysis of SHU with CLB sensor

A sample of urine was collected from a healthy female volunteer and filtered. 2 mL filtered urine were spiked with 2 mL 5 mM solution CLB in a beaker and thoroughly mixed. The mixture's pH was adjusted to a range from 0.9 to 5.5, as required. The volume was then adjusted to 25 mL with distilled water. Potential was measured using CLB-BPB ion-selective membrane electrode. CLB concentration was determined using derived regression equation or calibration curve. Approval for conducting urine analysis in this research was obtained from Ethical Committee (ECR/387/Inst/KA/2013/RR-19), as per Letter No. JSS/MC/PA/5659/2024-25, from November 6, 2024.

Study of role of interferences

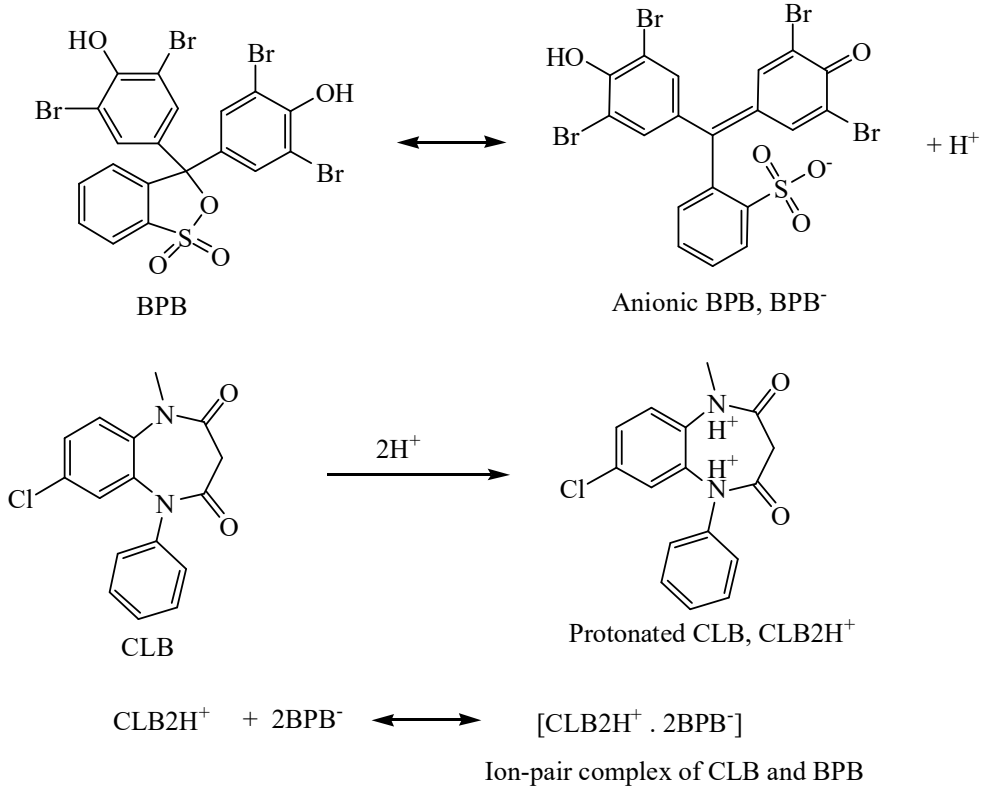
To study the impact of interferences, 5 mL 5.0 mM pure CLB solution was placed into separate beakers. To each beaker, 10 mL water and 1 mL of 1 M interferent

solution were added and stirred for 5 min. The pH of CLB and interferent mixture was adjusted from 0.9 to 5.5, after which the volume was brought up to 25 mL with distilled water, and thoroughly mixed. The solution's potential was measured using the proposed sensor relative to Ag-AgCl reference electrode, and CLB's concentration was determined through regression equation or calibration curve.

Results and discussion

The formation of an ion-association complex between CLB and BPB in chloroform occurs through interactions between basic amine group, in the former, and acidic sulfonephthalein dye, in the latter. Amines, such those in CLB, are nucleophilic, and can readily react with acidic compounds, due to their lone pair of electrons on nitrogen atom. Sulfonephthalein dyes, such as BPB, contain a sulfonic acid group, which is acidic and can deprotonate in a solution, generating a negatively charged sulfonate ion.

When CLB is mixed with BPB in chloroform, amine group in the former can interact with negatively charged sulfonate group in the latter, through electrostatic attraction [26-30]. This interaction leads to the formation of an ion-association complex, where the positive charge on the protonated amine (from CLB) is stabilized by the negatively charged sulfonate group of BPB. With this background, obtained CLB-BPB ion-association complex was employed to construct the membrane sensor for selective determination of the drug. Probable reaction pathway [27-31] between CLB and BPB is depicted in Scheme 1.



Scheme 1: Probable reaction pathway for formation of CLB-BPB ion-pair complex in chloroform.

According to suggested reaction in Scheme 1, CLB and BPB mix in a ratio of 1:2 created an ion association complex, $CLB2H^{+}$-$2BPB^{-}$. An *in situ* ion exchange took place when the corresponding ion-selective electrodes were inserted into CLB solution. This exchange affected equilibrium partitioning of sample ions at the sample/membrane junction. Ion-selective electrodes with polymeric membranes have a potentiometric response that is greatly influenced by this interaction at phase boundary. As a result, variations in CLB concentration in a solution are converted into electric potential by the ion-selective electrode sensor [32, 33].

The formation of am ion-association complex has been confirmed by IR spectra of CLB, BPB and the ion associate between them, as shown in Fig. 3. IR spectral data for BPB reveal key functional groups, with a band at 1175.46 cm^{-1}, corresponding to C-O stretching, and one at 745.5 cm^{-1}, for C-Br stretching, indicating presence of bromine in BPB.

For CLB, significant bands include: 1685.45 cm^{-1}, associated with C atom in a ring attached to O through a double bond; 1490.68 and 1368.68 cm^{-1}, for C-H bending; 1112.14 cm^{-1}, for C-O stretching; and 748.29cm^{-1}, corresponding to C-Cl stretching, which confirms presence of chlorine atoms in CLB.

In CLB-BPB ion-association complex, IR spectrum shows a carbonyl stretching band at 1686.07 cm^{-1}, similar to that in CLB, suggesting that carbonyl group remained intact in the complex. C-O stretching band shifts to 1185.64 cm^{-1}, slightly different from BPB's 1175.46 cm^{-1}, indicating an altered environment around ester group, likely due to interaction with amine group of CLB. Halogen stretching band appears at 748.29 cm^{-1}, slightly shifted from C-Br stretching in BPB and C-Cl stretching in CLB, which suggests the involvement of halogen atoms in the ion-association complex. Bands at 758.89, 827.34 and 691.2 cm^{-1} correspond to several overlapping absorption stretchings from C-H, C-Cl and C-Br vibrations. These shifts in IR spectra provide strong evidence for the formation of CLB-BPB ion-association complex, with carbonyl, ester and halogen groups playing crucial roles in the interaction between the two molecules.

Carbonyl group's relatively unchanged frequency indicates it was not directly involved in ion-association, but remained part of the overall structure, while shifts in ester and halogen stretching bands further support the formation of the complex, confirming a successful interaction between CLB and BPB.

Furthermore, in the spectrum of CLB-BPB ion-association complex, carbonyl band was more intense, at 1686.07 cm^{-1}, compared to that in pure CLB. This was likely due to increased electron density around carbonyl group, as a result from interactions within the complex, which enhanced vibrational transition, confirming formation of the ion-association complex.

By applying Job's Continuous Variations Method [34], with equimolar concentration of CLB and BPB (2×10^{-4} moles/L), stoichiometry between them was assessed. Findings showed that electrostatic attraction between positive

protonated CLB and BPB anion formed a 1:2 ion-pair. Series of solutions with a combined volume of 5.0 cm^3 were made, each containing CLB and BPB in different molar ratios. Chloroform was used to make the volume to 5 cm^3.

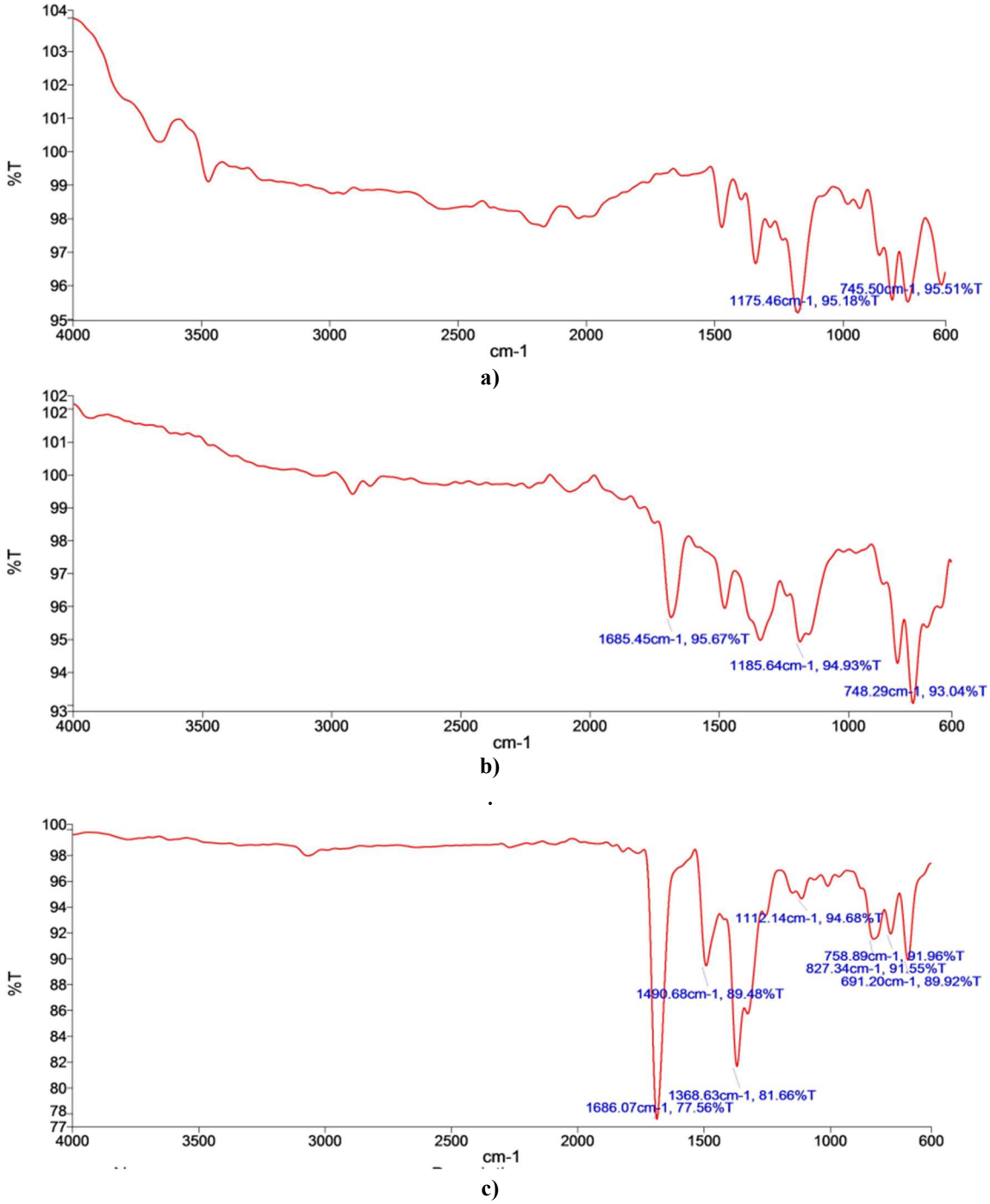


Figure 3: IR spectra- **a)** BPB, **b)** CLB and **c)** CLB-BPB ion associate.

At 410 nm, absorbance of each solution was then recorded. 1:2 CLB:BPB complex was formed, as shown by the maximum on the data graph (Fig. 4), at a mole fraction

for BPB of Xmax = 0.67. Stability constant (K_f) of the ion-association complex was calculated from continuous variation data using Eq. (2) [35]:

$$K_f = \frac{A/A_m}{\left[1 - A/A_m\right]^{n+2} C_M (n)^n} \quad (2)$$

where A and A_m are observed maximum absorbance and absorbance values, when all CLB present is associated, respectively. C_M is molar concentration of CLB at maximum absorbance, and n is stoichiometry at which BPB ion associates with CLB. Log K_f value was found to be 7.77.

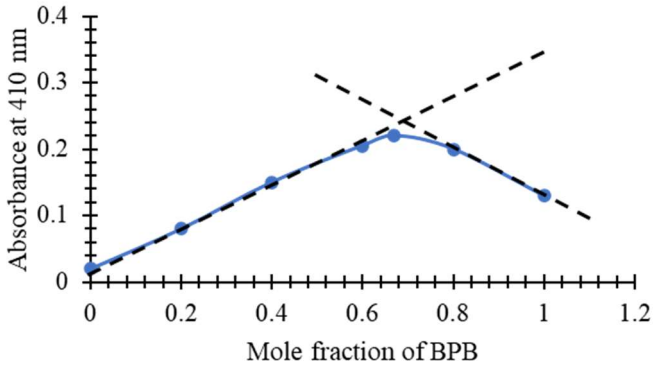


Figure 4: Plot of Job's continuous variations study to evaluate stoichiometry between CLB and BPB in the ion-association complex formation.

Optimization of parameters

Acetone, methanol and chloroform were tried as solvents to dissolve CLB. Results on solubility and stability extent of CLB/methanol solutions showed its good solvent property. Therefore, methanol was used as solvent for the preparation of CLB standard solution.

Membrane composition

Initially, a sequence of trials with different quantities of materials such as BPB, plasticizer and matrix substance were executed to yield optimal membranes. Functionality of the membrane was assessed through potentiometry. The residue of $CLB2H^+$-$2BPB^-$ ion-associate developed with 25 mg CLB, 54.4 mg BPB, 150 mg DBP and 200 mg PVC showed highly reliable results. Additionally, attempts to establish best calibration line with varied material quantities at differing concentrations from those specified above did not exhibit acceptable Nernstian behaviour. Dissolution of materials was found suitable in 10 mL THF, since larger volumes did not significantly alter outcomes.

Choice of plasticizer

DBP, DBS, DOP and NPOE were plasticizers used in the membrane development. For CLB, membranes containing 150 mg DBP consistently displayed potential

responses and Nernstian behaviour. Table 1 provides a summary of performance data for sensors made of various plasticizers in varying proportions.

Table 1: Results of study using membranes made of various plasticizers in varying proportions.

Plasticizer	Weight in mg	CLB sensor	
		Slope* ±SD	Confidence limit 95%
DBP	50.0	22.32±1.02	1.41
	100.0	27.00±1.12	1.55
	150.0	30.35±1.23	1.70
	200.0	38.21±0.79	1.09
	250.0	38.45±0.88	1.22
DBS	50.0	27.23±0.52	0.72
	100.0	31.80±0.89	1.23
	150.0	36.86±1.23	1.70
	200.0	38.18±0.89	1.23
	250.0	40.23±1.03	1.43
DOP	50.0	41.68±1.26	1.75
	100.0	44.29±1.88	2.60
	150.0	46.92±1.45	2.01
	200.0	46.21±0.87	1.20
	250.0	46.00±1.00	1.39
NPOE	50.0	35.26±1.88	2.60
	100.0	40.22±1.57	2.17
	150.0	49.65±1.26	1.75
	200.0	43.00±0.79	1.09
	250.0	42.55±1.00	1.39

*Mean value of five determinations.

Effect of CLB concentration in internal reference solution

In the study, various volumes of CLB with 1 M KCl (from 0.5 to 5 mL) were explored to fix electrodes' internal solution concentration for the analysis. It was observed that using from 3 to 5 mL CLB, with 2 mL 1 M KCl as internal standard solution, consistently yielded best results. Calibration plots of E_{cell} against log[CLB] yielded outstanding results, meeting predicted Nernstian response criteria of the sensor, for those CLB and KCl quantities. Calibration curve constructed using data recorded for varying CLB and KCl concentrations is shown in Fig. 5.

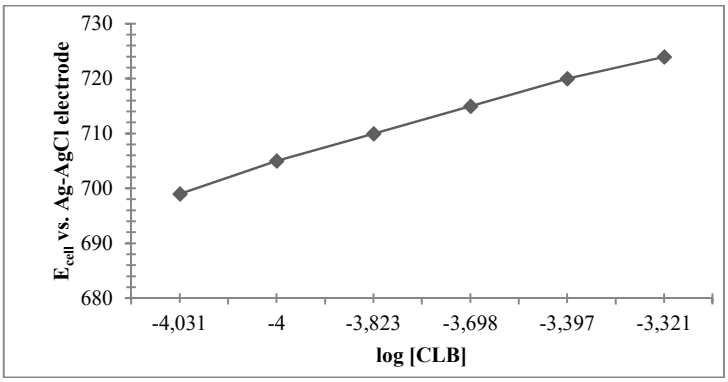


Figure 5: Calibration curve constructed for varying CLB concentration using 5 mL of CLB along with 2 mL of 1M KCl as internal standard solution.

Sensor conditioning time

The sensor was conditioned by immersing it in a standard CLB solution, for different durations. As showcased in Fig. 6, according to the potential values in response to standing time, the dynamic surface required around 5 h of activation, at 25 °C, for optimal performance.

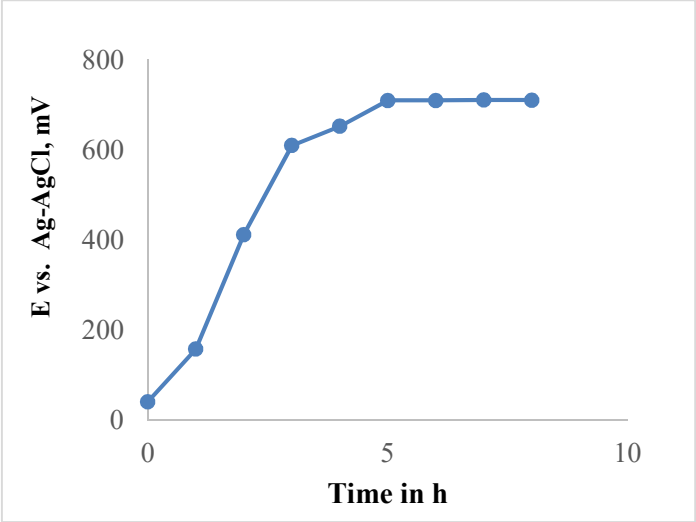


Figure 6: Effect of immersion time on the sensor conditioning in 1.50×10^{-4} M CLB.

Effect of pH

The effect of pH on E_{cell} was examined by measuring the potential of a CLB solution in pH range from 0.25 to 10. Desired pH levels were maintained by adding either 4 M sulphuric acid, diluted ammonia, or 1 M sodium acetate. Stable potential was seen in pH range from 0.9 to 5.5, as shown in Fig. 7.

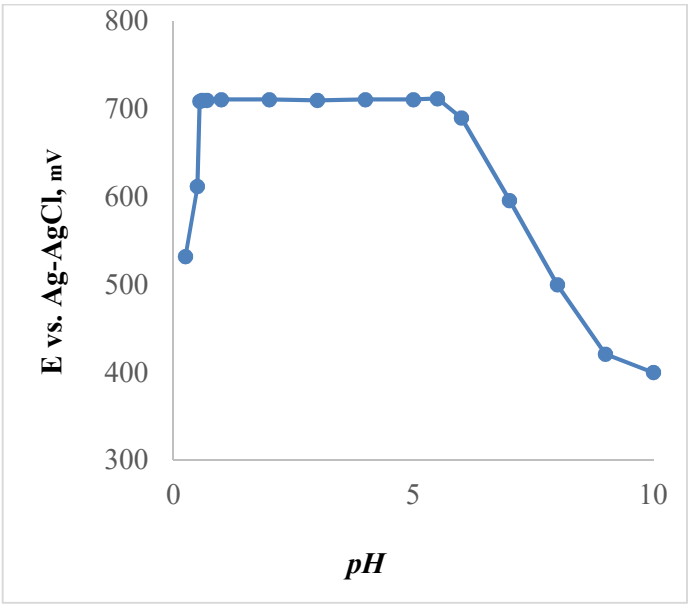


Figure 7: Effect of pH on 1.5×10^{-4} M CLB's potential measured using the proposed sensor.

Deviations from Nernstian response at pH levels outside this range were likely due to $CLB2H^+$ reduced availability, which limited the ion's active participation. Consequently, the ion-selective electrode sensor effectively translated fluctuations in $CLB2H^+$ concentration in the solution into corresponding changes in electric potential [32, 33]. Resulting slopes of calibration curves for different pH values are summarized in Table 2.

Table 2: Evaluation of pH influence on the performance of the proposed CLB sensor.

pH	Curve slope	pH	Curve slope
0.25	21.66	5.25	30.71
0.50	24.63	5.50	30.70
0.55	30.38	5.75	32.35
1.00	30.78	6.00	36.92
2.00	30.55	7.00	39.52
3.00	30.75	8.00	41.73
4.00	30.81	9.00	42.04
5.00	30.88	10.00	43.12

*Mean value of five determinations.

Response time

CLB sensor, after being immersed in the solution, showed rapid response time of 8 sec., indicating its efficiency in detecting changes. This quick response is critical for real-time monitoring applications, where timely detection is essential. Observed data highlight CLB sensor's ability to deliver prompt and reliable readings, reinforcing its potential utility in various analytical works.

Sensor lifetime

Developed CLB sensor showed excellent performance, consistently maintaining a mean Nernstian slope of 30.35 mV/decade, over a period of 52 days of regular use. However, after this duration, variations in measured potential were seen.

Evaluation of selectivity coefficients

Pre-analysed CLB solution was deliberately combined with various 1 M interfering solutions. Potential was measured using CLB sensor. Studied interferents for selectivity coefficients (K_{IA}) were Ag^+, NH_4^+, Na^+, K^+, H^+, Ca^{2+}, Co^{2+}, Zn^{2+}, glycine, urea, uric acid, glucose, oxalate, formic acid, citric acid, tartaric acid, benzoic acid, salicylic acid, phthalic acid and boric acid. Recorded potentials were used for determining selectivity coefficients [36, 37]. Obtained K_{IA} values, for all interferents lower than 1, indicated that added substances did not cause any interference.

Method validation

The proposed method was validated according to latest IUPAC standards [37-39] and ICH guidelines [40], focusing on evaluating the sensor linearity, accuracy, precision, recovery, sensitivity, robustness and ruggedness for CLB detection.

Linearity of calibration curve, regression data and performance characteristics

Measured EMF demonstrated linear correlation with CLB concentration in solutions within range from 9.3×10^{-5} to 4.8×10^{-4} M (Fig. 4). Nernstian behavior is evident through slopes of 30.35 mV/decade. The slope value further demonstrated relative charge on two N atoms of CLB as 2, as depicted in Scheme 1. Eq. (3) derived from curve-fitting regression data is as follows:

$$Y = 30.35\ X + 695.74 \quad (3)$$

where X is concentration and Y is measured potential.

LOD was determined according to IUPAC guidelines [37, 40], calculated based on the point where extrapolated linear segments of calibration curve intersected x-axis. Additional performance characteristics of CLB membrane sensor are provided in Table 3.

Table 3: Performance characteristics of fabricated CLB membrane sensor.

Parameter	Value
Slope (mV/decade)	30.35
Intercept	695.74
Response time (s)	8
Working pH range	0.9-5.5
Concentration range	9.5×10^{-5} - 4.8×10^{-4}
LOD (M)	9.4×10^{-5}
Stability (days)	> 52

Accuracy and precision

To evaluate intra-day variations, three different concentrations of solutions were tested, each with seven repetitions. For inter-day variations, three distinct CLB concentrations were analyzed, with five repetitions per each. %RSD and %RE shown in Table 4 reflect accuracy and precision of the proposed method.

Table 4: Results of study of the precision and accuracy of CLB proposed sensors.

	Intra-day variations			Inter-day variations		
CLB taken (mmol L^{-1})	**CLB found* (mmol L^{-1})**	**%RSD**	**%RE**	**CLB found** (mmol L^{-1})**	**%RSD**	**%RE**
0.2	0.194	2.23	3.00	0.205	1.44	2.50
0.3	0.291	2.65	3.00	0.292	1.98	2.67
0.4	0.408	2.14	2.00	0.412	1.77	3.00

*Mean value of seven measurements; **mean value of five measurements.

Robustness and ruggedness

To assess robustness, operational temperature was deliberately increased by 2°C, while analyzing CLB solutions at concentrations of 0.2, 0.3 and 0.4 mM. %RSD across temperatures of 23, 25 and 27 °C remained below 3%, demonstrating the robustness of the method. Additionally, three analysts were employed to assess CLB solutions, with three different potentiometers to account for instrumental and

inter-person variability. %RSD, which was less than 2.26% as shown in Table 5, further validated the reliability of proposed CLB sensor.

Table 5: Results of robustness and ruggedness of CLB sensor.

Strength of CLB (mM)	%RSD values for varied parameters		
	Robustness (Varying T by 2 ^{o}C)	Ruggedness	
		Inter-analysts	Inter-potentiometry
0.20	2.26	1.72	2.10
0.30	2.03	1.95	2.18
0.40	2.25	1.93	2.12

Application to tablets analysis

Validated membrane sensor was used to analyse five replicates of tablet extracts containing CLB at concentrations of 0.2, 0.3 and 0.4 mM. This analysis provided CLB quantity in each tablet, along with their %recovery and %RSD. Results were consistent with those obtained using reference ultraviolet spectrophotometric method for CLB available as a monograph in EP (Table 6) [25].

Table 6: Results of analysis of CLB tablets using proposed sensor and statistical comparison with results of EP method [25].

Analysed tablets	CLB tablet (mg)	Found*	
		%Label claim ± SD	
		EP method	Proposed method using CLB
Clozam-20	20	98.27±1.44	99.23±1.76 t = 0.95 F = 1.49
Czam-10	10	96.28±0.86	97.85±1.05 t = 2.59 F = 1.49

*Mean value of five measurements.

Experimental t- and F-test values tabulated below 95 % confidence level, indicated the procedure's accuracy and precision. Furthermore, average percentage recovery of CLB, close to 100 % with standard deviation under 2 %, reinforced the similarity between proposed and reference methods.

Recovery study

A 0.05, 0.1 and 0.15 mM solution of pure CLB was added to the three different tablet extract solutions at 0.10 mM concentration each, with respect to CLB, respectively. The pH of the resultant solutions was adjusted to ideal range. The potential of these solutions was recorded following standard procedure. The newly discovered sensor demonstrated its precision with an average recovery of 96.83%, for CLB, and % RSD value below 3%. Recovery results of CLB from the tablet extract mixture and pure drug solution are shown in Table 7.

Table 7: Results of accuracy assessment in recovery study by standard-addition procedure.

CLB from tablet extract	Pure added CLB (mM)	Total CLB found (Mm)	*Recovered %CLB	%RSD
0.10	0.05	0.1048 ±0.002	96.00	1.56
0.10	0.10	0.1981 ±0.005	98.10	2.43
0.10	0.15	0.2446 ±0.007	96.40	2.88

*Mean of three determinations

Application to SHU sample

Potentials recorded for CLB samples with SHU showed no interference from endogenous substances present in urine. Mean percent recovery of CLB from the analysis was 96%, with RSD of 2.89%. This demonstrates the applicability and suitability of CLB sensor for accurately determining CLB concentration in urine, making it a valuable tool for physiotherapeutic administration laboratories.

Conclusion

A first ever reported novel potentiometric sensor for assay of CLB was herein developed, validated and used to determine it in pharmaceuticals. The electrode was fabricated using BPB dye, dissolved in chloroform, and cast into a membrane using a mixture of DBP and PVC in THF. This ion-selective membrane electrode demonstrated a significant response to CLB concentration, exhibiting excellent Nernstian behaviour, with a slope of 30.35 mV/decade. Performance evaluation of the sensor revealed a rapid response time of 8 sec., and maintained consistent reliability over 52 days of continuous use. Calibration curves and recovery studies highlighted high accuracy, with an average recovery rate of 96.83% and %RSD below 3%. Validation with CLB tablet analyses showed results consistent with those from a reference UV spectrophotometric method, confirming the sensor's precision. Additionally, the sensor exhibited minimal interference from common substances, demonstrating robustness and ruggedness across varying conditions. Overall, CLB-BPB ion-selective membrane electrode represents a precise, accurate and efficient method for quantifying CLB in pharmaceutical formulations. Results from application to SHU sample revealed the emergence of the proposed method as a relevant tool to determine CLB, for both analytical and practical applications. Compared to reported chromatographic methods, this technique is particularly advantageous due to its ease of operation, lower cost per analysis and simpler instrumentation.

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Authors' contributions

N. Rajendraprasad, C. Siddaraju and H.C. Prameela: contributed to carry out the research; prepared manuscript draft. **N. Rajendraprasad**: supervised work; suggested experimental modification as and when required; evaluated outcomes; finalized paper for submission to publishing process.

Abbreviations

Ag-AgCl: silver-silver chloride
BPB: bromophenol blue
CLB: clobazam
DBP: dibutyl phthalate
DBS: dibutyl sebacate
DOP: dioctyl phthalate
E_{cell}: cell potential
EMF: electromotive force
EP: European Pharmacopeia
GLC: gas-liquid chromatography
HPLC: High-performance liquid chromatography
ICH: International Conference on Harmonization
IR: infrared
IUPAC: International Union of Pure and Applied Chemistry
KCl: potassium chloride
LC-MS/MS: Liquid chromatography-tandem mass spectrometry
LOD: limit of detection
NDMCLB: N-Desmethylclobazam
NPOE: nitrophenyloctyl ether
PVC: polyvinyl chloride
RE: relative error
RSD: relative standard deviation
SC: saturated calomel
SHU: spiked human urine
TDM: therapeutic drug monitoring
THF: tetrahydrofuran

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