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Electrochemical Sensors for Berberine Hydrochloride Determination in Commercial Products and Bio-Fluids

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The present study describes the development of three electrochemical sensors conducted by the interaction of berberine hydrochloride (BRB) with phosphotungestic acid (plastic membrane), sodium tetraphenyl borate (coated wire), and ammonium reineckate (coated graphite) sensors. Under optimum experimental conditions the effect of membrane content, type of solvent mediator, soaking time, hydrogen ion concentration of the test solutions and the estimation of the analyte in the presence of foreign species were studied. The data confirmed that the developed sensors gave potential responses of 54.10 ± 0.5, 57.00 ± 0.5 and 59.00 ± 0.5 mV decade^{-1} at ambient temperature for (berberine–phosphotungstate, BRB-PT), (berberine–tetraphenylborate, BRB-TPB) and (berberine–reineckate, BRB-AR) sensors, respectively, over drug concentration ranges of 1.0×10^{-7} - 1.0×10^{-3}, 1.0×10^{-8} - 1.0×10^{-2} and 1.0×10^{-7} - 1.0×10^{-2} mol L^{-1} with lower detection limits 5.0×10^{-8}, 5.0×10^{-9} and 5.0×10^{-8} mol L^{-1} for the above suggested sensors, respectively. The suggested sensors provide high selectivity, precise and sensitivity for the determination of BRB within the average pH range 3-9. Validity of the method was performed for the suggested electrochemical probe following ICH guidelines. The suggested sensors were favorably exploited for the estimation of BRB in commercial products and bio-fluids.

Keywords: Berberine hydrochloride; Sensors; Plastic membrane; Coated wire; Coated graphite.

1. INTRODUCTION

Berberine hydrochloride (BRB) is a quaternary ammonium benzyl-isoquinoline alkaloid (Figure 1). It is isolated from different Chinese herbal plants and has diverse pharmacological effects. BRB has antidiabetic, neuro protective properties, anti-lipid peroxidation potential as well as anti-atherosclerosis activity, and also improve polycystic syndrome [1-5]. It is a conventional Chinese medicine component, which possesses a diversity of pharmacological potentials. For thousands of years, it is commonly recommended as an anti-inflammatory, antibacterial, antifungal and gastrointestinal remedy [6, 7]. However, owing to its hydrophobic nature, such as poor stability and bioavailability, its applications were restricted for a long time.

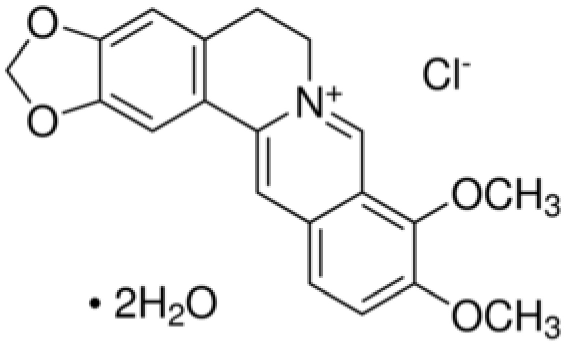

Figure 1. Structural formula of Berberine hydrochloride

Recently, the commercial products of BRB have improved to attain good prospects for medical applications. Furthermore, care of cancer and new strategies have been improved. Thus, new developed systems for drug analysis could be developed for anticancer drug preparation in Chinese medicine [8-14].

Various analytical probes have been addressed for BRB quantification, including high performance liquid chromatography [15-18], separation with thin layer chromatography [19, 20], electrophoresis [21], gas chromatography [22], UV-Vis spectroscopy [23], chemiluminescence [24], fluorescence [25], Potentiometry [26] and Voltammetry [27, 28].

Although, all separation methods give a fast, precise and automatic detection of numerous substances, they still possess many limitations including, the consumption of large quantities of reagents and solvents, expensive devices and require high technical skills [29]. Moreover, spectroscopic methods can also be rapidly applied, and the outcomes are detected as absorbance peaks, but these probes may be exhibited a signal instability [30]. However, the electrochemical techniques, especially potentiometry as a quantitative analytical technique still possess good fortune and high attention, due to its simplicity, stability, excellent throughput and responsiveness.

Electrochemical techniques such as potentiometry have been interested in many scientific applications, pharmaceutical analysis, clinical diagnosis and biomedical applications. Potentiometry is one of the most promising methods that defined as a self-powered method. The detection in these sensors are carried out by gathering the tested analyte under the electrostatic force resulted in the production of potential difference between the surface of designed sensor and a reference one [30].

Potentiometric sensors, plastic or coated polymeric membrane containing electroactive material can quantify various dozens of chemical substances. High molecular weight of polyvinyl chloride (PVC) and different esters of organic acids such as dioctyl sebacate (DOS), dioctylphthalate (DOP) and dibutylphthalate (DBP), etc. are usually used to fabricate these types of sensors. Also, some nitro-ethers has commonly acted as mediating matrix for ion-associate. The ion-associate materials are lipophilic ions or molecules serve to form specific interaction in the membrane with other analyte and pre-determine the selectivity of the related sensor [31].

Nowadays, the extensive attention to these sensors as an impact area of analytical chemistry attracts many researchers to study these sensors as a simple and precise device in chemical analysis [32]. Therefore, herein new different types of BRB sensors were designed using different electroactive materials (BRB-PT, BRB-TPB and BRB-AR) to determine BRB in its authentic samples, commercial formulations and bio-samples. The described potentiometric method was validated to ensure its suitability to analyze the BRB.

2. METHODOLOGY

2.1. Instruments

The potentiometric detection was measured by a digital microprocessor pH-meter HANNA, model 211 (Cluj, Romania). A reference electrode Ag/AgCl and a pH device (Metrohm, model 744) was employed for pH detection.

2.2. Materials and chemicals

All materials used throughout the analytical studies were of high purity and used without further purification. Sodium hydroxide (NaOH, 97.0 %) was supplied by (Winlab, East Midland, UK). Polyvinyl chloride (PVC) powder with high molecular weight, di-butyl phthalate (DBP, 99.0 %), sodium tetraphenylborate (TPB, 98.0 %), phosphotungstic acid (PTA, 99.9 %), ammonium reineckate (AR, 98.0 %), chloroform (99.5 %), acetone (99.5 %), tetrahydrofuran (THF, 99.9 %) and hydrochloric acid (37.0 %) were acquired from (Sigma-Aldrich, Hamburg, Germany). Pure grade of berberine hydrochloride was gifted from Tabuk, Pharmaceutical Co., Tabuk, Saudi Arabia. Berberine® 400 mg/tablet was obtained from local drug pharmacy, Al-Nahdi, Riyadh, Saudi Arabia. Human urine was provided by healthy volunteers and the informed consent was approved for all volunteers before starting this work. Commercial serum was supplied by (Randox Laboratories, Crumlin, Antrim, UK).

2.3. Preparation of BRB solution

A fresh BRB solution (1.0×10^{-2} mol L^{-1}) was accomplished every day by mixing a suitable quantity (0.37 g) of BRB in 100 mL distilled water. To prepare the working solutions, serial dilutions were performed.

2.4. Formation of ion-pairs

The BRB-PT, BRB-TPB and BRB-AR ion-pairs were formed by adding equal volumes of BRB and each precipitating agent PTA, TPB and AR solutions (50 mL, 1.0×10^{-2} mol L^{-1}). The obtained ion-pairs were filtered, washed 3 times with distilled water and dried in ambient temperature for 12 h.

2.5. Membrane composition

The membrane cocktail of each sensor was made separately by mixing 0.19 g of (PVC) and 0.45 mL of plasticizer (DBP) and 0.01 g of every prepared ion-pair (BRB-PT, BRB-T or BRB-AR) in 5 mL (THF). Each membrane cocktail was mixed well in a glass dish (3 cm in diameter) and left aside to evaporate the solvent slowly at ambient temperature to obtain the suitable membrane.

2.6. Sensor designs

Plastic membrane sensor: A round membrane portion was cut and fixed to a plastic tube (6.0 mm diameter) by THF. An internal solution containing 2.5 mL of each BRB and KCL solution (1.0×10^{-3} mol L^{-1}) was poured inside the glass body of the sensor. The reference electrode (Ag/AgCl) was used to complete the potentiometric system. For precondition, the constructed BRB-PT sensor was soaked for 24 h in 1.0×10^{-3} mol L^{-1} BRB solution. The final constructed plastic BRB-PT membrane was obtained and the potential readings were recorded using the following system:

BRB-PT /BRB:KCl /BRB //Ag/AgCl reference electrode.

Coated wire sensor: This sensor was designed using 10 cm of pure Al wire insulated by polyethylene tube. One end of the wire was circulated and the other end was connected with a pH meter for potential readings. Before coating, the metal surface was carefully cleaned with distilled water, then by acetone and air dried. The cleaned Al surface was immersed several times into the BRB-TPB membrane cocktail to form a thick film. The prepared sensor was soaked for 24 h in the same BRB solution as addressed above. The working potentiometric system was as:

Al wire/membrane (BRB-TPB)/BRB //Ag/AgCl reference electrode.

Coated graphite sensor: This type was obtained after covering a 10 cm graphite rode in length by a polyethylene tube. The surface of one end was immersed in BRB-AR membrane cocktail to form a thick layer and the other end was used for connection with pH meter to record the potential readings of BRB samples. Before the measurements, the obtained sensor was soaked and conditioned as mentioned above.

2.7. Sensor calibration

Approximately, 25 mL aliquots of 1.0×10^{-7} - 1.0×10^{-3}, 1.0×10^{-8} - 1.0×10^{-2} and 1.0×10^{-7} - 1.0×10^{-2} mol L^{-1} standard BRB solutions for the three sensors, respectively were analyzed using the previously mentioned potentiometric systems. The resulted data was graphed vs. – log [BRB].

2.8. Selection of suitable pH

The responses of the suggested sensors can be significantly affected by the pH value. Therefore, the suitable pH value for each sensor was determined by measuring potential reading of the three sensors using 1.0×10^{-4} mol L^{-1} of BRB solution. This study conducted by transferring 50 mL of 1.0×10^{-4} mol L^{-1} of BRB solution to 100-mL beaker and acidified using 0.1 mol L^{-1} HCl to lower the pH. Then the pH was gently elevated by adding 0.1 mol L^{-1} NaOH. The relationship between the pH values and the response of each sensor was plotted and the suitable and safe pH range was determined.

2.9. Effect of foreign species

The influence of various foreign substances on the selectivity of the constructed BRB-PT, BRB-TPB and BRB-AR sensors towards the estimation of BRB in its authentic powder was studied using 1.0×10^{-3} mol L^{-1} of drug and each interferent species. The selectivity coefficient was calculated using the separate solution method [33]. The ability of selection using the three sensors for some sugars and metal ions was tested.

2.10. Time of sensor response

The time of dynamic response of the three constructed BRB sensors was determined from the dynamic potential response corresponding to the drug concentration over the ranges of 1.0×10^{-7} - 1.0×10^{-3}, 1.0×10^{-8} - 1.0×10^{-2} and 1.0×10^{-7} - 1.0×10^{-2} mol L^{-1} BRB-PT plastic, BRB-TPB coated wire and BRB-AR coated graphite sensors, respectively.

2.11. Analytical studies

2.11.1. Quantification of BRB in berberine® tablets

Not less than 20 tablets of Berberine® (400 mg BRB/tablet) were grinded and 0.325 g was dissolved in 100 mL distilled water to obtain 1.0×10^{-2} mol L^{-1} stock solution. Serial dilutions were done to obtain different concentrations of BRB in the ranges of 1.0×10^{-7} - 1.0×10^{-3}, 1.0×10^{-8} - 1.0×10^{-2} and 1.0×10^{-7} - 1.0×10^{-2} mol L^{-1}. The BRB-PT, BRB-TPB and BRB-AR sensors were immersed separately in the drug solutions. The e.m.f. of each sensor system was recorded and the regression equation was used to determine BRB in tablets.

2.11.2. Quantification of BRB in bio-fluids

BRB was quantified in spiked bio-samples. After adjusting the pH of serum sample to pH 6, various drug concentrations were added and the precipitation process was applied to remove any proteins from the resulted samples [34] by stirring 1.0 mL serum with 1.0 mL acetonitrile and 1.0 mL of 0.1 NaOH then 1.0 mL of 5.0 % $ZnSO_4.7\ H_2O$ was added. The obtained mixture was stirred well for 10 min, then filtrated and completed to the mark with distilled water. The dilution process was carried out and then a series of measurements were recorded for BRB concentrations in the ranges of 1.0×10^{-7} -1.0×10^{-3}, 1.0×10^{-8} - 1.0×10^{-2} and 1.0×10^{-7} - 1.0×10^{-2} mol L^{-1}. For urine samples, 10 mL of spiked BRB urine (pH 6) was prepared to final concentrations as described in serum procedure.

3. RESULTS AND DISCUSSION

3.1. Optimization of membrane composition

As previously published [35], there are many parameters can influence the performance of the sensors. Three different membrane constituents were studied. The sensor with 2 % ion pair, 33 % PVC and 65 % plasticizer DBP displays excellent performance properties (slope 54.1 ± 0.5, 57.0 ± 0.5 and 59.0 ± 0.5 mV decade^{-1} at 25 ºC for BRB-PT, BRB-TPB and BRB-AR sensors, respectively, over BRB concentration range from 1.0×10^{-7} - 1.0×10^{-3}, 1.0×10^{-8} -1.0×10^{-2} mol L^{-1} and 1.0×10^{-7} - 1.0×10^{-2} mol L^{-1}, for the above mentioned sensors, respectively (Table 1).

Table 1. Selection of suitable sensor membrane cocktail (w/w %)

Proposed Sensor	w% of PVC	w% of DBP	w% of Ion-Pair	Slope	% RSD	Correlation coefficient	Detection range
Plastic Membrane	33	65	2	54.1 ± 0.5	0.4	0.9999	1.0×10^{-7} - 1.0×10^{-3}
Coated Wire	33	65	2	57.0 ± 05	0.6	0.9999	1.0×10^{-8} - 1.0×10^{-2}
Coated Graphite	33	65	2	59.0 ± 0.5	0.9	0.9998	1.0×10^{-7} - 1.0×10^{-2}

3.2. Performance characteristics of BRB sensors

BRB reacts with PTA, TPB and AR to form stable BRB-PT, BRB-TPB and BRB-AR ion-pairs which are water insoluble but freely soluble in THF. These electroactive materials were tested with DBP as a solvent mediator in the presence of PVC for BRB determination. The responses of the three constructed sensors for the analysis of serial dilutions of BRB solutions were determined (Table 2).

Table 2. Performance responses of BRB-PT plastic membrane, BRB-TPB coated wire and BRB-AR coated graphite sensors

Parameter[a]	BRB-PT sensor	BRB-TPB sensor	BRB-AR sensor
Slope (mV decade^{-1})	54.1 ± 0.5	57.0 ± 0.5	59 ± 0.5
Intercept	556.9	680.6	672.3
Correlation coefficient, r	0.9999	0.9999	0.9998
Linearity (mol L^{-1})	1.0×10^{-7} - 1.0×10^{-3}	1.0×10^{-8} - 1.0×10^{-2}	1.0×10^{-7} - 1.0×10^{-2}
LOD (mol L^{-1})	5.0×10^{-8}	5.0×10^{-9}	5.0×10^{-8}
Time of sensor response/s	45	45	25
Safe pH range	3-9	3-9	3-9
Working sensor life/day	30	50	26
Temperature ˚C	25˚C	25˚C	25˚C
Accuracy (%)	99.7 ± 0.3	99.5 ± 0.7	99.4 ± 0.6
Robustness[b]	99.5 ± 0.5	99.4 ± 0.7	99.5 ± 0.4
Ruggedness[c]	99.6 ± 0.4	99.8 ± 0.3	99.2 ± 0.9

[a]Mean of six measurements [b]Small variations in pH using borate buffer (pH 9.0 ± 1) [c]Comparison of the data with others from different sensors assembled using Metrohm, model 744 pH meter

The constructed BRB sensors gave Nernstian responses using 1.0×10^{-7} - 1.0×10^{-3}, 1.0×10^{-8} - 1.0×10^{-2} mol L^{-1} and 1.0×10^{-7} - 1.0×10^{-2} mol L^{-1} BRB solutions for the above three studied sensors, respectively with cationic slopes of 54.1 ± 0.5, 57.0 ± 0.5 and 59.0 ± 0.5 mV decade^{-1} (Figure 2). The time of response of each constructed BRB sensor was evaluated using the same above BRB solutions. The suggested sensors showed rapid dynamic responses of 45 s for BRB-PT plastic membrane and BRB-TPB coated wire, respectively and 25 s for BRB-AR coated graphite sensor. The lifetimes of the three constructed sensors were 30, 50 and 26 days for the three sensors, respectively, without any sharp changes in their performances.

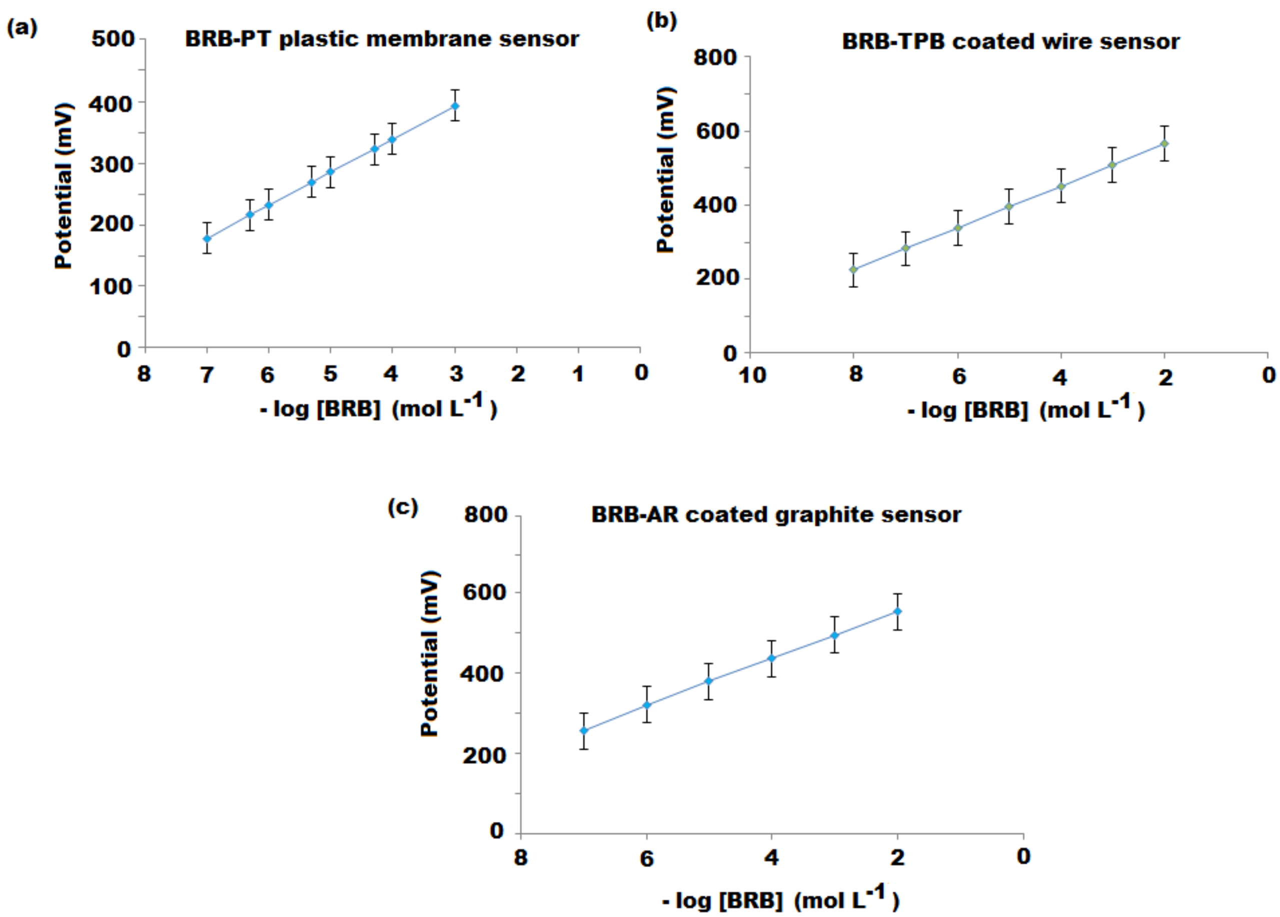

Figure 2. Calibration graphs of (a) BRB-PT plastic membrane, (b) BRB-TPB coated wire and (c) BRB-AR coated graphite sensors.

3.3. Effect of drenching

The performance features of BRB-PT, BRB-TPB and BRB-AR sensors were evaluated as a function of soaking time. This study was conducted by soaking the sensors separately in 1.0×10^{-3} mol L^{-1} of BRB solution and the linear responses were plotted after one day. The suitable time of drenching was found to be 24 h and the potential responses of the three sensors were 54.1 ± 0.5, 57.0 ± 0.5 and 59.0 ± 0.5 mV decade^{-1}, at 25 ºC, respectively. The prolonged drenching greatly affects the lifetime of the three constructed sensors. The effect of continuous drenching of the sensors using 1.0×10^{-3} mol L^{-1} of BRB solution for 5, 10, 15, 20, 25 and 35 days was investigated. It was noticed that the slopes of the constructed sensors were moderately decreased to be 53.8, 55.0 and 53.8 mV decade^{-1} after 10 days for the three suggested sensors, respectively. After continuous drenching to 35 days the slopes were decreased to be 52.3, 45.4 and 50.5 mV decade^{-1}. To regenerate the potential readings of the exhausted sensors, the three sensors were drenched separately in 1.0×10^{-2} mol L^{-1} of BRB solution for 24 h, followed by drenching in 1.0×10^{-2} mol L^{-1} of PTA, TPB and AR solution for 6 h. The plotted calibration graphs showed that the potential responses of the exhausted sensors were increased to be 53.9, 56.10 and 58.80 mV decade^{-1} for BRB-PT plastic membrane, BRB-TPB coated wire electrode and BRB-AR coated graphite sensors, respectively.

3.4. Effect of hydrogen ion concentration

The effect of pH on the sensor potential of the constructed BRB sensors was conducted by acidifying 1.0×10^{-4} mol L^{-1} BRB solution using 0.1 mol L^{-1} HCl, pH was decreased to be 1.2, and the hydrogen ion concentration was elevated to 12 using 0.1 mol L^{-1} NaOH solution. The measured mV of the constructed sensors was plotted against the pH vales and it was found that the optimum pH range was from 3 to 9 (Figure 3).

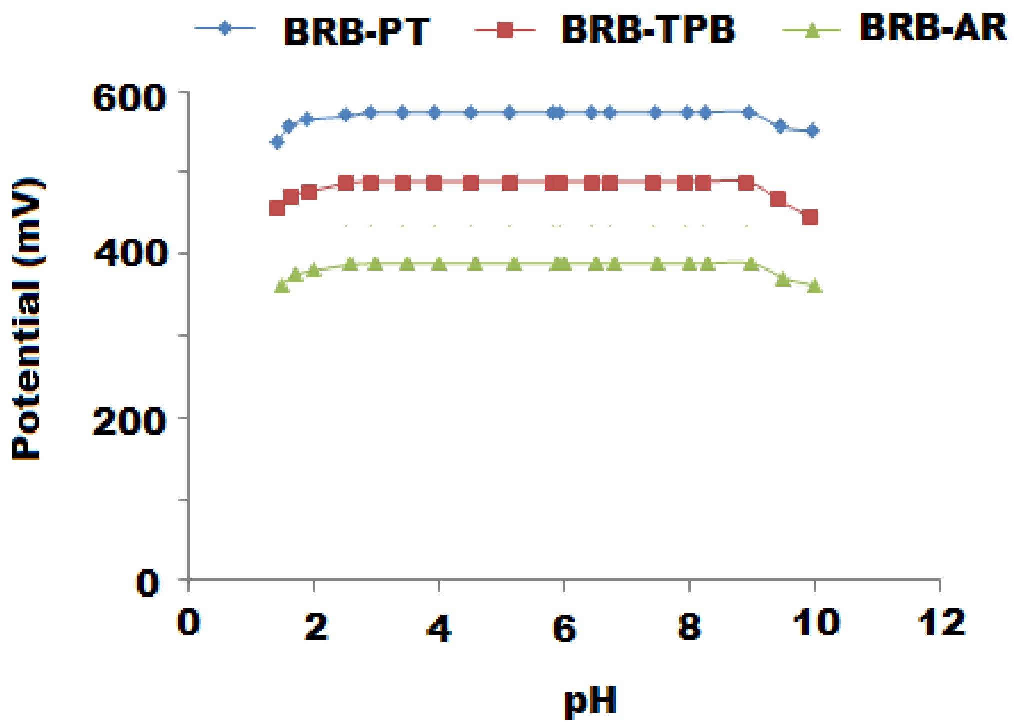

Figure 3. The potential readings of BRB-PT plastic membrane, BRB-TPB coated wire and BRB-AR coated graphite sensors at different pH values

The resulted data showed that below pH 3 the ions of H^+ are increased in the test solution and the potential was slightly increased. However, above pH 9 the OH^- ions are increased causing a decrease in the potential readings [36].

3.5. Influence of foreign substances

The selectivity of the constructed BRB sensors towards the determination of BRB solution was studied by using the separate solution method [33]. The tolerable values were estimated and summarized in Table 3. High selectivity was recorded for the constructed BRB sensors and no significant interference was noticed from certain cations and sugars. This can be due to the difference in permittivity of such ions towards the constructed membrane sensors compared to BRB^+.

Table 3. The tolerance values of BRB-PT plastic membrane, BRB-TPB coated wire and BRB-AR coated graphite sensors

Interferent	BRB-PT sensor	BRB-TPB sensor	BRB-AR sensor
	K^{pot}_{BRB+}		
Na^+	8.1×10^{-3}	3.5×10^{-3}	2.8×10^{-3}
K^+	4.8×10^{-3}	3.6×10^{-3}	1.9×10^{-3}
NH_4^+	8.4×10^{-3}	3.1×10^{-3}	3.8×10^{-3}
Ag^+	6.3×10^{-3}	2.9×10^{-4}	1.5×10^{-3}
Ca^{2+}	4.6×10^{-4}	2.0×10^{-4}	9.6×10^{-4}
Zn^{2+}	6.0×10^{-4}	1.6×10^{-4}	4.4×10^{-4}
Cu^{2+}	8.8×10^{-4}	2.6×10^{-4}	8.2×10^{-4}
Ni^{2+}	3.8×10^{-4}	1.2×10^{-4}	3.1×10^{-4}
Fe^{3+}	2.4×10^{-5}	1.6×10^{-4}	3.9×10^{-4}
Lactose	7.1×10^{-5}	2.2×10^{-4}	6.1×10^{-5}
Sucrose	7.4×10^{-5}	1.4×10^{-4}	6.5×10^{-5}
Starch	4.1×10^{-5}	2.9×10^{-5}	2.8×10^{-5}
Talc	3.5×10^{-4}	2.0×10^{-4}	3.5×10^{-4}

3.6. Quantification of BRB in bulk powder

Direct potentiometric determination of BRB using BRB-PT plastic membrane, BRB-TPB coated wire and BRB-AR coated graphite sensors was carried out and evaluated from the calibration graphs. The results expressed as mean recoveries of 99.6 ± 0.4, 99.7 ± 0.6 and 99.3 ± 0.9 for the above three mentioned sensors, respectively. The results obtained revealed excellent quantification of BRB in its bulk powder with high percentage recoveries and lower % RSD than 2%. The outcomes were assessed statistically using t-student's test and F- test [37] with respect to others from a previously addressed potentiometric technique [26] (Table 4)**.**

Table 4. The outcomes of BRB quantification in pure drug and a commercial formulation in comparison with a reference method using BRB-PT plastic membrane, BRB-TPB coated wire and BRB-AR coated graphite sensors

Statistical analysis	BRB-PTA sensor	BRB-TPB sensor	BRB-AR sensor	Reference method [26]
Pure sample				
Mean±SD	99.6±0.4	99.7±0.6	99.3±0.9	99.8±0.8
n	6	7	6	6
Variance	0.16	0.36	0.81	0.64
%SE*	0.16	0.22	0.37	0.33
%RSD	0.40	0.60	0.90	0.80
t-test	0.545 (2.288)**	0.252 (2.201)**	1.008 (2.288)**	
F-test	4.00 (5.05)**	1.78 (4.39)**	1.26 (5.05)**	
Berberine® tablets				
Mean±SD	99.2±0.3	99.8±0.9	99.4±0.4	99.6±0.5
n	6	7	6	6
Variance	0.09	0.81	0.16	0.25
%SE*	0.12	0.34	0.16	0.20
%RSD	0.30	0.90	0.30	0.50
t-test	1.715 (2.288)**	0.507 (2.201)**	0.780 (2.288)**	
F-test	2.78 (5.05)**	3.24 (4.39)**	1.56 (5.05)**	

%SE*(%Error) = $\%SD/\sqrt{n}$

** The theoretical values of t- and F- tests at p=0.05 [37].

3.7. Method validation

The suggested potentiometric method to determine BRB using three different sensors was evaluated and validated with respect to the criteria of ICH guidelines [38].

Under optimum measurement conditions, the linearity was plotted between the potential readings of the proposed BRB sensors and serial dilutions of BRB. The constructed BRB sensors showed Nernstian responses over the concentration ranges of 1.0×10^{-7} - 1.0×10^{-3}, 1.0×10^{-8} -1.0×10^{-2} mol L^{-1} and 1.0×10^{-7} - 1.0×10^{-2} mol L^{-1} BRB for the above three studied sensors, respectively with cationic slopes of 54.1 ± 0.5, 57.0 ± 0.5 and 59.0 ± 0.5 mV decade^{-1} (Table 2).

The lower limit of detection (LOD) was calculated for the proposed method using the three different BRB sensors when the potential readings of each sensor dropped by 17.9 mV. The obtained values were 5.0×10^{-8}, 5.0×10^{-9} and 5.0×10^{-8} mol L^{-1} for the above sensors, respectively (Table 2).

The accuracy of the suggested potentiometric technique was investigated by determination of BRB in its authentic samples (n = 9). The obtained results were expressed as mean percentage recoveries 99.7 ± 0.3 %, 99.5 ± 0.7 % and 99.4 ± 0.6 % for BRB-PT plastic membrane, BRB-TPB coated wire and BRB-AR coated graphite sensors, respectively (Table 2).

The precision of the designed potentiometric method was evaluated using inter-day and intra-day assays and the results were expressed as percentage relative standard deviation (% RSD). The obtained values for intra-day assay were 0.3 %, 0.9 % and 0.4 % and for inter-day assay were 0.2 %, 0.4 % and 0.2 % for the above three BRB sensors, respectively. All recorded values were less than 2 % revealing excellent precision.

The robustness of the suggested method was tested by evaluating the capacity of the suggested method to remain uninfluenced by small variations in its analytical parameters and supply a sign of its liableness throughout traditional usage. Whereas the ruggedness of the suggested method was studied by measuring similar samples using different laboratories, analysts and instruments (pH-meter, Jenway-3510). The obtained results for robustness were 99.5 ± 0.2 %, 99.4 ± 0.7 % and 99.5 ± 0.4 % and for ruggedness were 99.6 ± 0.4, 99.8 ± 0.3 and 99.2 ± 0.9 % for BRB-PT plastic membrane, BRB-TPB coated wire and BRB-AR coated graphite sensors, respectively (Table 2).

3.8. Sensor response in tablets and bio-fluids

The constructed BRB sensors were applied in the quantification of BRB in its tablets. The obtained results were found to be 99.2 ± 0.3, 99.8 ± 0.9 and 99.4 ± 0.4 % for BRB-PT, BRB-TPB and BRB-AR, respectively (Table 4). The developed sensors displayed high sensitivity, accuracy and precise for the determination of BRB in its tablets. The %RSD is lower than 1%, revealing excellent precision of the suggested potentiometric approach. Further analytical studies were performed for the quantification of BRB in spiked bio-fluids using the constructed BRB sensors. The outcomes in serum were found to be 98.8 ± 0.9, 99.5 ± 0.3 and 99.0 ± 0.6 % for BRB-PT, BRB-TPB and BRB-AR sensors, respectively. Whereas, the results in urine were 99.5 ± 0.5, 99.4 ± 0.6 and 99.3 ± 0.5 % for the above suggested sensors, respectively (Table 5).

Table 5. Results of BRB quantification in bio-fluids using BRB-PT, BRB-TPB and BRB-AR sensors

Statistical analysis	BRB-PTA sensor	BRB-TPB sensor	BRB-AR sensor
Serum samples			
Mean±SD	98.8 ± 0.9	99.5 ± 0.6	99.0 ± 0.3
n	5	7	6
Variance	0.81	0.36	0.09
%SE	0.41	0.22	0.12
%RSD	0.91	0.60	0.30
Urine samples			
Mean±SD	99.5 ± 0.5	99.4 ± 0.6	99.3 ± 0.5
n	5	7	6
Variance	0.25	0.36	0.25
%SE*	0.22	0.22	0.20
%RSD	0.50	0.60	0.50

%SE*(%Error) = %SD/$\sqrt{n}$

From the above results it was observed that coated wire sensor of BRB-TPB was more sensitive and gave higher results than the other two sensors, this can be attributed to the high conductivity of the Al wire which is 3.77×10^{7} S/m at room temperature which can play a promising key in the development of high sensitive potentiometric method for quantification of BRB in different matrices.

4. CONCLUSION

The suggested analytical method was conducted by developing different types of selective BRB sensors with significant analytical performances for the quantification of BRB in its pure drug, commercial formulations and bio-fluids. The three types of BRB sensors showed linear responses with excellent Nernstian responses of 54.1 ± 0.5, 57.0 ± 0.5 and 59.0 ± 0.5 mV decade^{-1} over wide concentrations ranges with PTA, TPB and AR ion pairs, respectively. The lifetimes were 30, 50 and 26 days, respectively. The LOD were 5.0×10^{-8}, 5.0×10^{-9} and 5.0×10^{-8} mol L^{-1}, respectively. The accuracy (%) were 99.7 ± 0.3, 99.5 ± 0.7 and 99.4 ± 0.6, respectively. All sensors provided useful results for determination of BRB drug in various matrices.

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CONFLICT OF INTEREST
No any competitive of interest for this study.

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