

Influence of 4,4,4-Trifluoro-1-phenyl-1,3-butanedione on Selectivity of All-Solid-State Lithium Ion Electrode Prepared by Using 6,6-Dibenzyl-1,4,8-11-tetraoxacyclotetradecane as Ionophore and Its Application in Human Serum Analysis

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In this work, we aimed to increase the selectivity of lithium selective electrodes containing lithium ionophore VI in the membrane matrix by using 4,4,4-Trifluoro-1-phenyl-1,3-butanedione (HBTA). Potentiometric response characteristics of the prepared lithium selective electrodes were investigated. Increasing the ratio of HBTA molecule in the membrane cocktail from 0.5% to 1.5% provided a better response with respect to the lower detection limit (LDL), linear range, and selectivity. The LDL and linear range of the electrode with 1.5% HBTA were $7.6 \times 10^{-6} \text{ mol L}^{-1}$ and $1.0 \times 10^{-1} - 3.4 \times 10^{-5} \text{ mol L}^{-1}$ respectively, with a slope 54.3 mV/decade ($R^2 = 0.9998$). The potentiometric responses of all electrodes were pH-independent in the range of pH 3-12. The response time of the electrodes was less than 10 s and the lifetime was more than eight weeks. The electrode with 1.5% HBTA showed a better selectivity against sodium (1:575) and potassium (1:270) and was successfully applied for the determination of lithium levels in diluted human serum samples. The obtained potentiometric data were compared with the ICP-MS results at 98% confidence level.

Keywords: PVC membrane, all-solid-state contact electrode, bipolar disorder, lithium, potentiometry

1. INTRODUCTION

Lithium, mainly in the form of citrate and carbonate salts, is widely used as a mood stabilizer for the treatment of patients with bipolar disorder (BD) [1,2]. Although lithium is the gold standard in managing and preventing acute manic or mixed episodes and is prescribed as a first-line therapeutic agent by all international guidelines, a decrease in the use of lithium drugs have occurred over the last

several years [2,3]. Some of the reasons for this decline are the availability of alternative mood stabilizers, the need of regular monitoring at the initial phase of the medication to ensure blood lithium level in the effective range and possible side effects including nausea, tremor, polyuria, weight gain, and cognitive dullness [4]. BD can be classified in 5 different ways depending on the patterns of the episodic mania and depression [5]. In the commonly known BD which is characterized by recurrent mood phases, patients cycle between a close to normal mood and an unhealthy stage which consists of episodes of mania and depression [6]. The therapeutic range of lithium in plasma is very narrow and very close to the level at which toxicity occurs. In general, recommended plasma lithium level is between 0.6 and 0.8 mmol/L at initial and maintenance phases, 0.6-1.0 mmol/L at manic episode, and 0.4-0.8 mmol/L at depressive episode of BD [1]. Toxicity symptoms can be seen above 1.2 mmol/L and the levels greater than 2.0 mmol/L can be fatal [7]. Lithium level in blood, serum, or plasma should be monitored for early determination of any side effect of lithium and to provide optimal dosing [8].

The lithium level in blood or serum can be measured by atomic emission spectrometry [9], atomic absorption spectrometry [10,11], mass spectrometry [12,13], conductometry [14], impedance spectrometry [15], voltammetry [16,17], potentiometry [18–22], spectrophotometry [23,24], and fluorimetry [25–28].

Quantification of the ionic species by potentiometric ISEs is a widely used analytical technique due to its quick analysis, wide working range, ease of use, and low cost [29]. However, the ISEs have some limitations to overcome in order to expand the usage [15]. One of the main drawbacks of the ISEs is the interference of other ionic species. In the case of lithium, the problem is the interference of sodium which has a relatively high concentration in blood ($135\text{--}145\text{ mmol L}^{-1}$) [30]. Although the main component that provides selectivity in a PVC membrane based ISE is the ionophore, lipophilic salts and neutral carriers also increase the selectivity [29].

All-solid-state ion-selective electrodes (ISE) have some advantages over conventional ISEs as they do not require an inner filling solution, which enables easy miniaturization, applications in small sample volumes, lower detection limits, long lifetime and easy maintenance [31,32].

4,4,4-Trifluoro-1-phenyl-1,3-butanedione (HBTA) is an organic extractant used in selective lithium extraction from brines. It has strong selectivity for lithium over other alkaline and earth alkaline metal ions [33]. In this work, however, we examined the effects of varying amounts of HBTA on the selectivity of the all-solid-state PVC-based membrane lithium ISE prepared by using 6,6-Dibenzyl-1,4,8-11-tetraoxacyclotetradecane (Lithium Ionophore VI) as ionophore.

2. EXPERIMENTAL

2.1 Reagents and Solutions

Tetrahydrofuran (THF), high molecular weight poly (vinyl chloride) (PVC), 2-nitrophenyl octyl ether (NPOE), potassium tetrakis(4-chlorophenyl)borate (KTPCIPB), HBTA, and lithium Ionophore VI were purchased from Sigma-Aldrich, and used without further purification in the preparation of the membranes. Graphite oxide (Sigma-Aldrich), epoxy (Macroplast Su 2227, Henkel) and hardener

(Desmodur RFE, Bayer AG) were used in the preparation of the solid contact material. Sterile filtered human serum was bought from Pan Biotech. All standard solutions were prepared using analytical grade nitrate or chloride salts of the relevant cations. Ultrapure water ($18.2 \text{ M}\Omega \text{ cm}^{-1}$) was obtained via Elga ultrapure system (Purelab Flex 4).

2.2 Apparatus

A computer-controlled high-input impedance multi-channel potentiometric measurement system (sensitivity $\pm 0.1 \text{ mV}$) with a custom-made software (Medisen Med. R&D Ltd, Turkey) was used to carry out the potentiometric measurements. All ISE potentials were measured against the silver/silver chloride reference electrode (Ag/AgCl RE) (HI5315, Hanna). The pH of the solutions used throughout the experiment was adjusted by using a combination pH electrode (SenTix 41, WTW) with a benchtop pH meter (inoLab pH 7110, WTW). ICP-MS measurements were recorded by Agilent 7700 device.

2.3 Preparation of the electrodes and potential measurements

The all-solid-state ISEs were prepared with the similar steps described in our previous works [19,29]. Membrane cocktails (Table 1) containing lithium Ionophore VI, PVC, NPOE, KTpCIPB, and HBTA were prepared by dissolving them in 3 ml THF. The all-solid-state contact surface was coated by dipping 5 times into the membrane cocktails. 4 different electrodes prepared in this way were first left to dry in a closed vessel at room temperature overnight, then conditioned in $0.01 \text{ mol L}^{-1} \text{ LiCl}$ solution for at least 12 h before use. Prior to each measurement electrodes were reconditioned for at least 10 min in $0.01 \text{ mol L}^{-1} \text{ LiCl}$ solution. When not in use, electrodes were stored in laboratory at room temperature.

Table 1. Compositions of the different membrane cocktails (w/w %)

Cocktail no.	PVC	NPOE	KTpCIPB	Ionophore	HBTA
Li 1	29	69	0.5	1.5	0
Li 2	29	68.5	0.5	1.5	0.5
Li 3	29	68	0.5	1.5	1
Li 4	29	67.5	0.5	1.5	1.5
Li 5	29	67	0.5	1.5	2

Potential responses of the electrodes at steady state were taken with a cell assembly as follows:

Double junction Ag/AgCl RE | Sample solution | PVC membrane | Conductive material |
Copper wire

3. RESULTS AND DISCUSSION

3.1 Potentiometric behavior of the electrodes

Potentiometric responses were recorded at various standard solutions of lithium (10^{-7} to 10^{-1} mol L^{-1}), which were prepared from the stock solution (10^{-1} mol L^{-1}) by serial ten-fold dilution with purified deionized water. Table 2 shows the potentiometric characteristics of the electrodes that were measured and calculated according to IUPAC recommendations (Figure 1) [34].

Table 2. Potentiometric characteristics of the lithium selective electrodes

	Li 1	Li 2	Li 3	Li 4	Li 5
Sensitivity	51.4	53.6	57.4	54.3	52.7
R^2	0.9998	0.9999	0.9995	0.9998	0.9998
LDL	3.3×10^{-5}	2.1×10^{-5}	2.4×10^{-5}	7.6×10^{-6}	2.8×10^{-5}
Linear range	$1.0 \times 10^{-1} - 1.6 \times 10^{-4}$	$1.0 \times 10^{-1} - 1.0 \times 10^{-4}$	$1.0 \times 10^{-1} - 1.4 \times 10^{-4}$	$1.0 \times 10^{-1} - 3.4 \times 10^{-5}$	$1.0 \times 10^{-1} - 1.3 \times 10^{-4}$
pH stability	3-12	3-12	3-12	3-12	3-12
Response time	10-20 s	<10 s	<10 s	<10 s	<10 s

All electrodes exhibited great performance in terms of pH stability and response time. However, the electrode with 1.5% HBTA showed a wider linear response over the range of 1.0×10^{-1} to 3.4×10^{-5} mol L^{-1} ($R^2 = 0.9998$) with a slope of 54.3 mV. Thus, increasing HBTA ratio in the membrane to 1.5% provided a better potentiometric response in terms of LDL, sensitivity, and linear range. One reason for the electrode with 1.5% HBTA outperforms the other electrodes may be the better promotion of complex formation between HBTA and the ionophore with a lithium ion at 1:1 molar ratio.

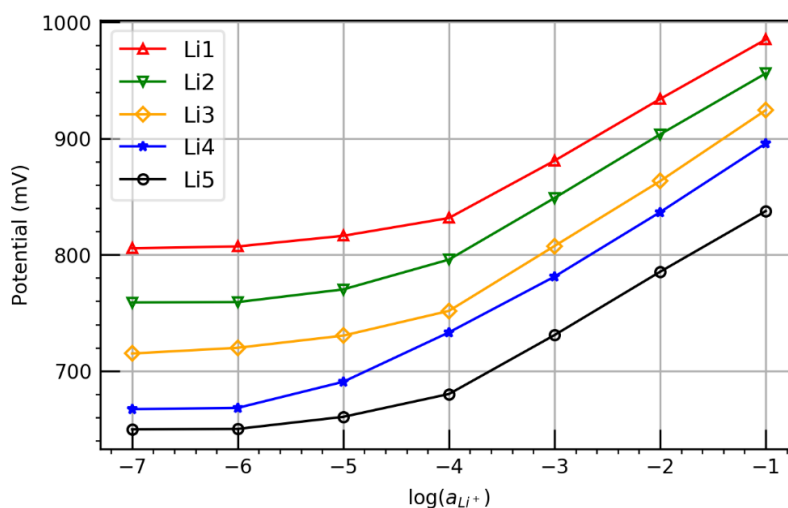


Figure 1. The potentiometric response of the lithium-selective electrodes

The linear response range of the electrode is the range where the linear part of the calibration curve does not deviate more than 2 mV from the curve [35]. The LDL was determined from the intersection of the two extrapolated lines of the calibration curve (Figure 2). The calculated LDL for the electrode with 1.5% HBTA was $7.6 \times 10^{-6} \text{ mol L}^{-1}$.

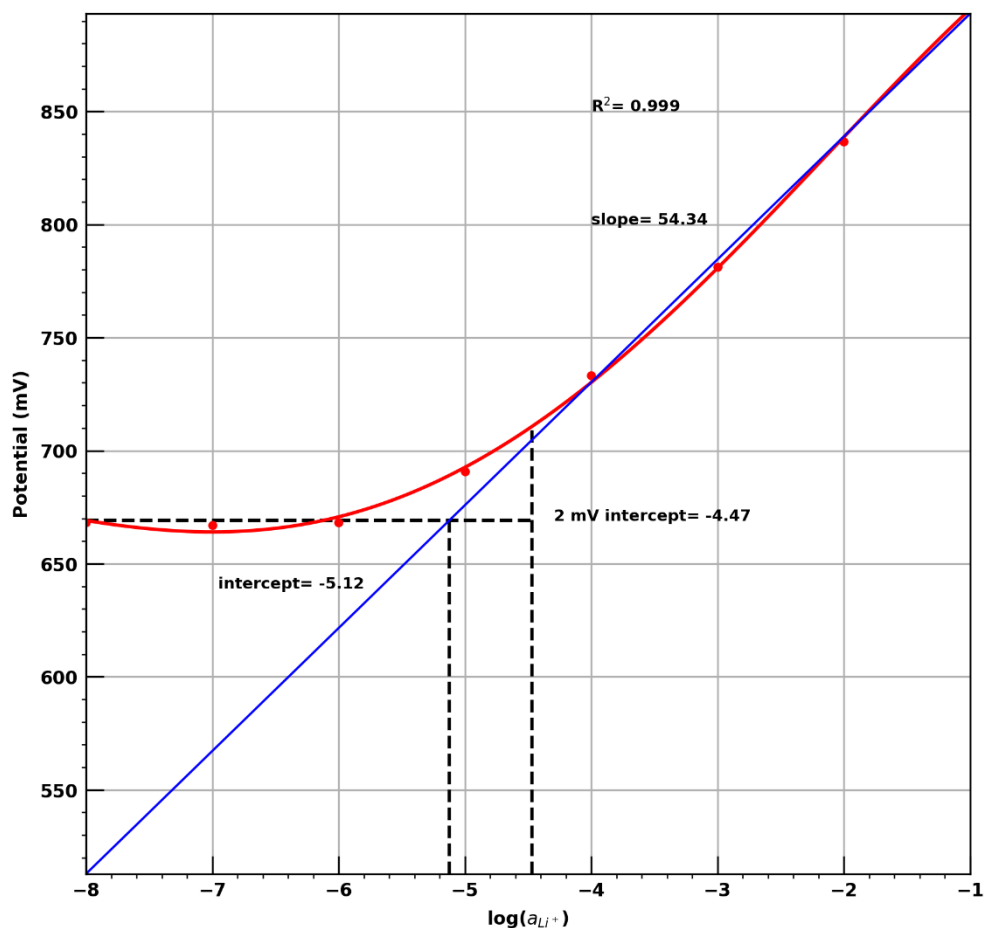


Figure 2. LDL (-5.12) and LOQ (-4.47) of the Li 4 electrode

The effect of the pH on the potentiometric response of the electrodes was investigated over the pH range 2-12 and the response of the electrodes is shown in the Figure 3. Reference pH solutions were prepared by using $1.0 \times 10^{-2} \text{ mol L}^{-1}$ phosphate buffer and included $1.0 \times 10^{-2} \text{ mol L}^{-1}$ lithium ion. The final pH of each solution was adjusted by adding small amounts of diluted KOH or HCl. The potentiometric response of all electrodes was pH-independent in the range of 3-12. The slightly increased potentials at higher pH values could be the result of the diluted KOH while adjusting the pH. The addition of different amounts of HBTA molecule to the lithium-ion selective membrane did not make any significant change at the behavior of the electrode over pH working range.

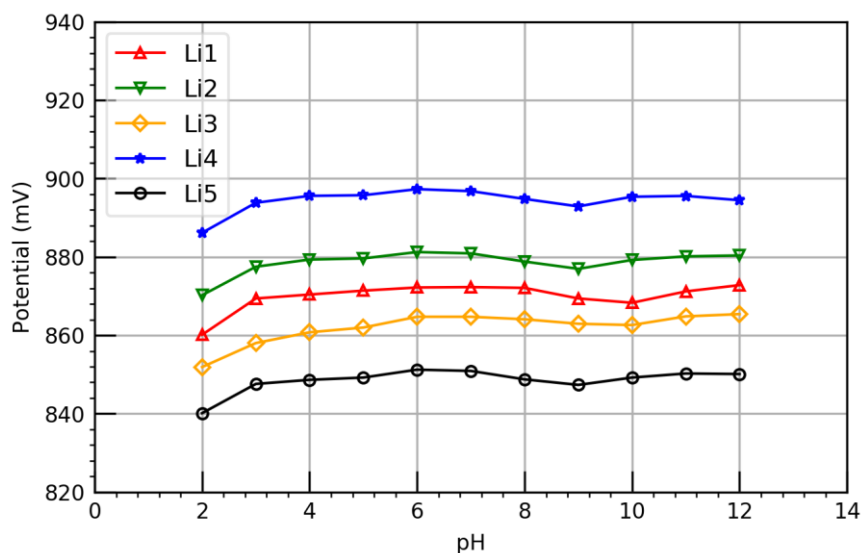


Figure 3. pH response of the lithium-selective electrodes at $1.0 \times 10^{-2} \text{ mol L}^{-1}$ lithium ion concentration

One of the most important characteristics of an ion selective electrode is the selectively responding to the ion of interest in the presence of other ions. The selectivity of the electrodes for a number of alkali and earth alkali ions was measured. The selectivity coefficients were calculated according to separate solution method (SSM) ($a_A = a_B$) and fixed interference method (FIM) proposed by IUPAC [36]. In separate solution method the potentiometric response of the electrode is measured with two separate solutions. One of the solutions contains the main ion A at the activity a_A , the other one contains the interfering ion B at the activity a_B . The selectivity coefficient is calculated from the equation 1:

$$\log K_{A,B}^{pot} = \frac{(E_B - E_A)}{S} + \left(1 - \frac{z_A}{z_B}\right) \log a_A \quad (1)$$

where E_A and E_B are the measured potential values, and z_A and z_B are the charge of the ions A and B. S is the sensitivity of the A-selective electrode [37].

The potentiometric response of the electrode was also measured according to fixed interference method at various concentrations of primary ion (10^{-6} to $10^{-1} \text{ mol L}^{-1}$) while the concentration of an interfering ion was kept fixed ($10^{-2} \text{ mol L}^{-1}$). Then the selectivity coefficient $K_{A,B}^{pot}$ was calculated from the equation 2:

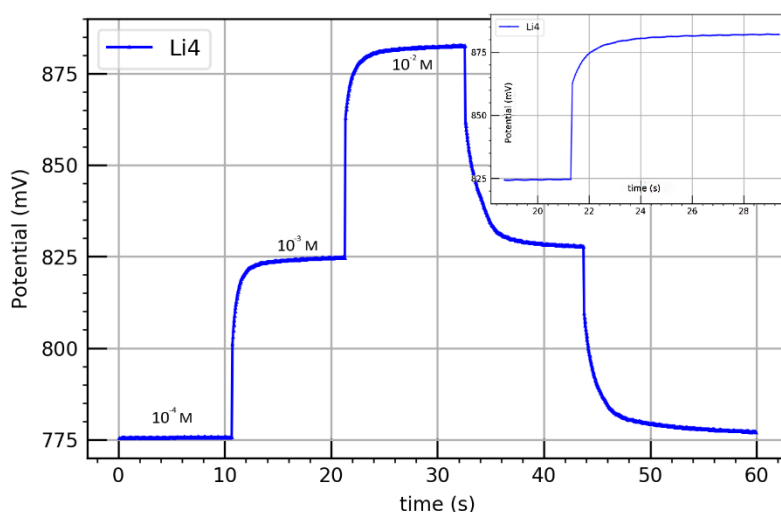
$$K_{A,B}^{pot} = \frac{a_A}{(a_B)^{z_A/z_B}} \quad (2)$$

where a_A was calculated from the intersection of the extrapolated lines of the potentiometric response of the electrodes to the $-\log$ of the activity of the primary ion [36]. The selectivity coefficients for a number of alkali and earth alkali ions calculated according to separate solution method (SSM) ($a_A = a_B$) and fixed interference method (FIM) are respectively given in Table 3.

Table 3. Selectivity coefficients ($K_{Li^+,B}^{pot}$) of the electrodes calculated according to SSM and FIM

Electrode No	Method	$\log K_{Li^+,B}^{pot}$						
		Na ⁺	K ⁺	NH ₄ ⁺	Ca ²⁺	Mg ²⁺	Ba ²⁺	Sr ²⁺
Li 1	SSM	-2.38	-1.95	-2.67	-3.89	-4.12	-3.61	-3.96
	FIM	-1.97	-1.72	-2.33	-3.47	-3.81	-3.26	-3.53
Li 2	SSM	-2.76	-2.43	-2.97	-3.82	-4.25	-3.89	-3.65
	FIM	-1.84	-1.96	-2.41	-3.42	-3.74	-3.44	-3.21
Li 3	SSM	-2.48	-2.24	-2.71	-3.14	-3.76	-3.78	-3.83
	FIM	-1.96	-1.88	-2.36	-2.87	-3.34	-3.36	-3.49
Li 4	SSM	-2.71	-2.34	-2.99	-4.00	-4.34	-3.89	-4.06
	FIM	-2.09	-1.82	-2.43	-3.64	-4.00	-3.42	-3.68
Li 5	SSM	-2.44	-2.37	-2.78	-3.25	-3.97	-3.75	-3.71
	FIM	-1.95	-1.83	-2.41	-2.92	-3.58	-3.34	-3.30

The dynamic response time of the electrode was determined by measuring the time required to get a 1 mV difference from the steady potential, after the electrode was immersed in a lithium solution which had 10-fold concentration difference from the previous solution. The average response time of all electrodes was less than 10 s (Figure 4). The electrode was tested once in two days for 8 weeks and no significant change in the slope of the electrode was observed.

**Figure 4.** Dynamic response of the electrode with 1.5% HBTA (Li 4) in the standard solutions of 10^{-4} , 10^{-3} and 10^{-2} mol L⁻¹ lithium ion

To investigate the repeatability of the electrodes, potentiometric response to the 1.0×10^{-2} , 1.0×10^{-3} , and 1.0×10^{-4} mol L⁻¹ lithium ion solutions was measured eight times. The results (Table 4) indicate that the electrodes exhibited repeatable potentiometric response with high accuracy ($SD_{\max} = 1.9$).

Table 4. Mean potentials of the electrodes in various lithium standard solutions after eight measurements (with $\pm$ standard deviation)

Electrode no.	Concentration of the lithium standard solutions (mol L ⁻¹)		
	1.0×10^{-2}	1.0×10^{-3}	1.0×10^{-4}
Li 1	882.3 $\pm$ 1.0	831.1 $\pm$ 1.6	779.8 $\pm$ 1.9
Li 2	864.0 $\pm$ 1.0	808.5 $\pm$ 1.7	760.1 $\pm$ 0.9
Li 3	873.7 $\pm$ 1.2	815.8 $\pm$ 0.9	766.3 $\pm$ 1.7
Li 4	890.6 $\pm$ 1.1	834.1 $\pm$ 1.1	788.7 $\pm$ 0.9
Li 5	785.3 $\pm$ 1.1	730.9 $\pm$ 1.3	680.1 $\pm$ 1.5

The developed electrodes were compared with electrodes which have similar membrane compositions without HBTA reported in the literature (Table 5 and 6). Our electrodes had comparable results with other electrodes. Although the compared electrodes from the literature have almost the same selectivity coefficient, the electrode containing 1.5% HBTA developed here are found more selective when compared. We also found that increasing the HBTA ratio in the membrane to 1.5% enhances the selectivity of the membrane too.

Table 5. Comparison of the prepared electrodes with the electrodes from the literature

No	Membrane Composition	Sensitivity (mV)	Linear range (mol L ⁻¹)	Detection Limit (mol L ⁻¹)	Response time (s)	pH range	Ref.
1	28% PVC 1% ionophore 0.7% KTpCIPB 70.3% NPOE	58.7	-	1.3×10^{-5}	15-30	4-12	[22]
2	28% PVC 1.5% ionophore 0.5% KTpCIPB 70% NPOE	50.3	1.1×10^{-1} – 5.5×10^{-6}	8.0×10^{-6}	10-20	2.5-12	[29]
3	29% PVC 1% ionophore 0.5% NaTPB 69.5% NPOE	53.6	1.1×10^{-1} – 6.0×10^{-5}	2.1×10^{-5}	<10	6-10	[19]

4	29% PVC 1.5% ionophore 0.5% KTpCIPB 69% NPOE	51.4	$1.0 \times 10^{-1} - 1.6 \times 10^{-4}$	3.3×10^{-5}	10-20	3-12	This study
5	29% PVC 1.5% ionophore 0.5% KTpCIPB 67.5% NPOE 1.5% HBTA	54.3	$1.0 \times 10^{-1} - 3.4 \times 10^{-5}$	7.6×10^{-6}	<10	3-12	This study

NaTPB: Sodium tetraphenylborate. All used ionophore was lithium ionophore VI

Table 6. Selectivity coefficients of the electrodes listed in Table 5

No	Method	Interfering Ion				
		Na ⁺	K ⁺	NH ₄ ⁺	Ca ²⁺	Mg ²⁺
1	SSM	-2.67	-2.38	-2.97	-3.90	-
	FIM	-2.55	-2.56	-3.08	-4.01	-
2	MPM	-1.96	-1.49	-2.24	-4.44	-
3	MPM	-2.43	-2.53	-3.42	-3.62	-4.22
	SSM	-2.38	-1.95	-2.67	-3.89	-4.12
4	FIM	-1.97	-1.72	-2.33	-3.47	-3.81
5	SSM	-2.71	-2.34	-2.99	-4.00	-4.34
	FIM	-2.09	-1.82	-2.43	-3.64	-4.00

3.2 Analytical Application

The electrode containing 1.5% HBTA (Li 4) was selected for further analytical applications due to its superior selectivity and response. Application of our electrode to determine serum lithium level was carried out in a similar procedure with our previous works [19,29]. Five milliliter of each serum samples was diluted with five milliliter 1.2, 1.6, 2.0 and 2.4 mmol L⁻¹ Li⁺ solutions in 10 mmol L⁻¹ Tris-HCl buffer (pH 7.3). Calibration of the electrode was performed at various concentrations of lithium ion in 5 mmol L⁻¹ Tris-HCl buffer (pH 7.3) containing 70 mmol L⁻¹ NaCl.

The results obtained via both methods were compared by calculating percentage error (RE%), critical two-tailed student *t*-test, and *F*-test for comparing the standard deviations at 98% confidence level.

Table 5. Lithium levels in serum measured by potentiometry and ICP-MS (mean value $\pm$ SD) and statistical values (for $N = 3$ at 98% confidence level)

Sample No	Potentiometric results ^a (mmol L ⁻¹)	ICP-MS results ^a (mmol L ⁻¹)	$ t_4 $ value ^b	$F_{2,2}$ value ^c	RE% ^d
1	0.60 $\pm$ 0.02	0.55 $\pm$ 0.03	4.31	2.25	9.3
2	0.80 $\pm$ 0.01	0.90 $\pm$ 0.04	6.36	16.00	11.4
3	0.99 $\pm$ 0.01	1.05 $\pm$ 0.05	3.60	25.00	6.3
4	1.17 $\pm$ 0.01	1.17 $\pm$ 0.06	0.22	36.00	0.4

^a Average of three measurements^b Critical two-tailed $|t_4| = 6.96$ ^c Critical two-tailed $F_{2,2} = 99.00$ ^d Potentiometry versus ICP-MS

4. CONCLUSIONS

In this study, all-solid-state lithium ISEs with new membrane compositions were developed and their potentiometric analysis was performed. The addition of HBTA molecule improved the selectivity against the main interfering ions such as sodium and potassium. The 1.5% HBTA containing electrode exhibited the best performance and was successfully applied for the measurement of lithium ion level in human serum samples. The results obtained for diluted human serum sample were comparable with the ICP-MS results. The statistical analysis demonstrated that our electrode (Li 4) can be used reliably for the determination of human serum lithium levels at 98% confidence level. For future studies, membranes with better selectivity should be developed to obtain better results at lower concentrations, and more trials should be done in dynamic and real conditions for use in clinical practice.

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