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EXAMINING THE SELECTIVITY AND RESPONSE TIME OF A BILIRUBIN ION SELECTIVE ELECTRODE

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ABSTRACT

Bilirubin is primarily produced as a by-product of hemoglobin degradation in the liver. About 60% of full-term and 80% of preterm neonates are affected by hyperbilirubinemia, making the bilirubin concentration in serum crucial for monitoring liver health and identifying hyperbilirubinemia. Potentiometric sensor is used for the determination of free bilirubin which has a linear response range of 0.1 µM to 1000 µM bilirubin. This range includes the 5.0-500 µM range of clinically significant bilirubin levels in serum. Despite the presence of additional blood anions in the serum such as lactate, pyruvate, deoxycholate, phosphate, and chloride, the polymeric ion-selective membrane of the sensor exclusively detects "unbound" or free ionic bilirubin. The sensor responds quickly to the change in bilirubin concentration which further improves the usability of this sensor.

Keywords: Bilirubin, Ion selective electrode, Selectivity, Hyperbilirubinemia, Potentiometry

INTRODUCTION

In vertebrates, Heme that is present in human biological fluids conjugated with glucuronic acid, complexed with albumin, or unbound is broken down to create bilirubin, a tetrapyrrole. It can be found in digestive fluid as a byproduct of digestion. Bilirubin (BR) concentrations in blood and urine are essential indicators of liver health. Free BR can accumulate in tissues and its solubility is pH-dependent. Conjugated bilirubin (CB), also known as unbound bilirubin, is coupled in the liver with glucuronic acid. Unconjugated bilirubin (UCB), which is water-insoluble and binds to albumin in serum, is different from it. Unbound BR can accumulate in tissues and its solubility is pH-dependent **[1]**.

In situations of hyperbilirubinemia, which is a condition that affects roughly sixty percent of full-term babies and eighty percent of pre-term newborns, the serum concentration of unbound bilirubin is crucially important to determine. Despite the fact that the reference level of free BR in the serum of healthy people ranges from 5 to 34 μM, **[2]** the quantity of free BR in neonates with hyperbilirubinemia is approximately 500 μM. It is critical to keep track of the serum level of free BR in hyperbilirubinemia.

The accumulation of free bilirubin in brain tissue is capable of causing irreversible injury. This injury has the potential to be fatal due to various neurologic impairments, convulsions, aberrant reflexes, and abnormal eye movements. Kernicterus or bilirubin encephalopathy refers to the dysfunction of the brain that is caused by bilirubin. Newborns with normal total bilirubin levels but excessive free bilirubin levels suffer from acute bilirubin encephalopathy. This syndrome develops when BR passes across the blood-brain barrier and damages the brain and neurological system permanently. Therefore, doing a free bilirubin assay at the point of service will make it simpler to carry out the suggested treatment procedures, which will lower the chance of passing away or suffering long-term brain damage **[3]**. Changes in pH have a big impact on this approach. The direct spectroscopic method removes interference from proteins containing heme but is unable to measure free BR **[4]**. Transcutaneous bilirubinometry measures skin reflectance. In the majority of hospitals, this technique is utilized, and the results are susceptible to being affected by the patient's skin pigmentation. These procedures have several significant drawbacks, including the fact that they are labour-intensive, that they call for the use of specialized instruments, that they are both pricey and unreliable, that they require many stages for sample preparation and

separation, and that they call for the use of reagents that are both expensive and prone to instability. In addition, none of these techniques can determine the free BR; rather, they can only measure the overall BR, which includes both the complicated and the free components.

The majority of deaths caused by hyperbilirubinemia take place in low-resource settings, where the condition is identified predominantly based on jaundice's visual symptoms (yellowing of the eye whites or the epidermis). Detection with the use of the eyes is neither quantitative nor dependable. Analytical methods that can be used to quantify BR include fluorimetry, voltammetry, amperometry (observation of BR's redox reaction on an electrode surface), enzymatic sensors (using bilirubin oxidase), chemiluminescence, Piezoelectricity, high-performance liquid chromatography, and capillary electrophoresis.

Because of the following, these approaches are not appropriate for the quick, dependable, and economical evaluation of free BR at the point of service or in environments with limited resources.

They are unable to estimate the total amount of free and complex BR and quantify free BR.

- They call for instruments that are both difficult and costly.
- As a result of the numerous sample preparation and separation procedures they entail, they ought to be carried out by qualified individuals only.
- They require reagents that are both costly and prone to instability.

Ion-selective electrodes (ISEs), which are distinguished by characteristics such as high measurement sensitivity, high selectivity, and simple operation, are a viable alternative to free BR detection **[5, 6]**. Ion-selective electrodes are a type of sensor used to assess the amount of a particular ion in a solution. The ion of interest in this instance is bilirubin. Bilirubin is a crucial biomarker for a variety of medical conditions, particularly liver and blood disorders **[7-12]**. An ion-selective membrane, a thin layer that selectively permits the passage of some ions while inhibiting the passage of others, makes up the electrode **[13-16]**.

In this paper, a potentiometric sensor made of an Ion selective electrode containing 20mg TDMACl and 0.5 mM of BR in an Inner filling solution (IFS) is used. This sensor was analysed for its response time and selectivity in the determination of free BR.

The main objectives include:

- Response Time Assessment
- Selectivity Evaluation

MATERIALS AND METHODS

Bilirubin ion selective electrode (BR ISE) was used and evaluated in this paper. All the chemicals used for the preparation of BR ISE are purchased from Sunrise Chemicals Rohtak. Fabrication of BR ISE is not in the purview of this research paper.

Potentiometric sensor, used in the evaluation, was made using BR ISE having 20 mg TDMACl, which is the main component of ion selective membrane (ISM) and 0.5 mm of BR in IFS. The sensor was analysed for response time and selectivity evaluation in the determination of BR.

- **Response Time Assessment:** The response time is the period of time from the contact of electrode with the sample to getting a detectable signal in the measuring device. In this investigation, the rate at which the electrode responds to changes in bilirubin concentration was determined. Real-time monitoring and clinical applications necessitate a rapid response time.

  Electromotive force (emf) was measured using the potentiometer and in parallel time was recorded. Relation between emf and time was established to study how quickly the sensor responds to achieve emf at a particular BR concentration.

- **Selectivity Evaluation:** In this part, selectivity of the ion selective electrode towards bilirubin was investigated. It was determined if the electrode responds to bilirubin ions only and no other ions that were present in the biological sample. This selectivity is essential for accurate and reliable bilirubin measurements.

  To understand the selectivity of the sensor, selectivity coefficients were calculated for all interfering ions present in the serum. Then, emf measurements were done with solution containing only interfering ions and with different concentrations of bilirubin. Emf was plotted with respect to time to understand the selectivity.

RESULTS AND DISCUSSION

Evaluation of Response Time of BR ISE

Response time is duration from the moment the electromotive force (emf) over time slope reaches a limiting value based on the experimental conditions and accuracy requirements to the point when an ion-selective electrode and a reference electrode come into contact with a sample solution (or the time when the concentration of the ion in a solution changes). Electromotive force (emf) is assessed using a potentiometer to determine the bilirubin ion-selective electrode's reaction time, and time is also monitored in parallel.

Table 1: Relation of emf with time in BR ISE with 20mg TDMACl

Time (s)	emf (mV)
0	81.00
1	108.00
2	108.01
3	108.01
4	108.00
5	108.00
6	108.00
7	108.00
8	108.01

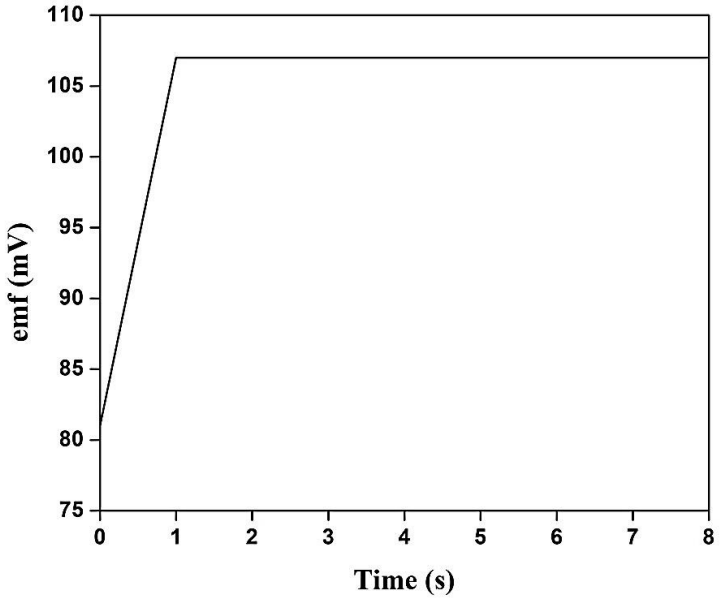

Figure 1: Graph showing the response time of BR ISE with 20 mg TDMACl.

Figure 1 show the response time of BR ISE with 20 mg TDMACl. It was observed that in 1 second, emf reaches the target emf values of BR ISE in a 10^3 µM BR solution. From the above **Table 1 and Graph 1**, it can be concluded that the potentiometric sensor fabricated using ISE (ion selective electrode) has a very fast response time of 1 second and thus it is very quick in identifying free bilirubin.

Selectivity of BR Ion Selective Electrode (ISE)

The ISE's selectivity is determined by the target ion's lipophilicity since the sensor lacks a distinct identifying element (an ionophore). The sensor will be more sensitive to lipophilic ions. The ISE can detect BR above other, less lipophilic substances in blood serum due to its high lipophilicity. This enables it to be detected in a selective manner. To determine the selectivity of the ISEs, we utilized a technique that had been validated in the past (the separate solution approach). In general, the ISE's selectivity of determining BR

against an interfering ion can be determined by equation (1). Logarithm of the selectivity coefficient ($logK_{BR,I}^{Pot}$), gives the numerical measure of the ISE's ability to discriminate against an interfering ion. To put it another way, the ISE's selectivity is reliant on its capacity to distinguish an interfering ion.

$$logK_{BR,I}^{Pot} = \frac{(E_I - E_{BR})z_{BR}F}{RTln10} \quad (1)$$

In this equation, E_I represents the electromotive force (emf) obtained when 1000 µM of the interfering ion (I) is present, E_{BR} represents the emf obtained when 1000 µM of the Bilirubin is present, z_{BR} represents the charge of Bilirubin, the temperature is T, the constant of the gas is R, and the Faraday constant is F.

Using the experimental E_I and E_{BR} values for each solution containing various interfering anions like Salicylate ion, ascorbate ion, lactate, pyruvate, Cl^-, HCO_3^-, HPO_4^{2-}, NO_3^-, and Deoxycholate, the above equation (1) will be applied. By calculating the selectivity coefficient ($logK_{BR,I}^{Pot}$) using the equation, the selectivity of Bilirubin over other interfering anions can be determined.

To comprehend the ISE's selectivity for competing anions in serum, selectivity coefficients were calculated for all of these anions at their maximal physiologically-relevant concentration. This evaluation of selectivity is essential for assessing the specificity and reliability of Bilirubin Ion-Selective Electrodes to accurately measure bilirubin levels, especially when there are ions that can interfere in the measurement.

Physiological serum levels are as follows: HCO_3^-: 17-29mM, Cl^-: 98-106mM, HPO_4^{2-}: 0.4-0.7mM, NO_3^-: 8-68 µM, Ascorbic acid: 36-79 µM, Deoxycholate: 5-10 µM, Lactate: 0.5-1.8mM, Pyruvate: 40-120 µM.

To demonstrate this selectivity, a phosphate-buffered, 8.4 pH solution, containing all of these anions was formulated.

The selectivity coefficient ($logK_{BR,I}^{Pot}$) of BR ISE for BR over possibly interfering ions, I is shown in **Table 2**. It has been calculated from equation (1) by measuring emf for each interfering ion and using emf of BR in 1000 µM solution.

Table 2: Lists the selectivity coefficients of BR ISE for anions present in serum

Interfering Ion	$logK_{BR,I}^{Pot}$
BR^{2-}	0
HCO_3^-	-6.05
Cl^-	-6.00
HPO_4^{2-}	-5.30
NO^{3-}	-4.25
Salicylate	-2.80
Ascorbic Acid	-7.70
Deoxycholate	-4.19
Lactate	-6.34
Pyruvate	-6.31

Table 3: Influence of interfering ions solution on BR ISE emf

Concentration	Time (min)	emf
Interfering anions	0	173
	2	174
10 µM BR	2.02	163
	4.02	163
100 µM BR	4.04	134
	6.04	134
100 µM BR + 70 µM albumin	6.06	154
	8.06	154

Table 3 lists the emf values of ISE with 20 mg TDMACl and 0.5 mM BR concentration in IFS in four different solutions (buffering with sodium phosphate and a pH of 8.4). The first solution contains only interfering ions at their maximal physiological concentration, second solution contains 10 µM BR^{2-}, third solution contains 100 µM BR^{2-} and fourth solution contains 100 µM BR^{2-} and 70 µM albumin.

Figure 2 illustrates the electromotive force (emf) measurements of a Bilirubin Ion-Selective Electrode (BR ISE) with 20 mg of TDMACl and 0.5 mM BR concentration in Inner Filling Solution (IFS). The experiment was conducted in four separate solutions, buffered with sodium phosphate at a pH of 8.4. Each solution contained interfering ions at their maximal physiological concentration, as described below:

29 mM HCO_3^-, 106 mM Cl^-, 0.7mM HPO_4^{2-}, 68 µM NO^{3-}, 79 µM ascorbic acid, 10 µM deoxycholate, 1.8 mM lactate, and 120 µM pyruvate.

The asterisks on the graphs shows where the concentration is changed or the solution is changed to a different solution.

A rapid and consistent emf drop with a drift of 0.1 mV/min occurred after adding 10 µM BR^{2-} to the solution that had previously only contained interfering anions. The ISE then had an additional 29 mV reduction in emf magnitude after being submerged in a solution containing 100 µM BR^{2-}. The final mixture had 70 µM albumin and 100 µM BR^{2-}.When the ISE was inserted into this solution, the emf of the BR ISE increased by 20 mV, indicating a lower concentration of free BR than the observed emf of the 100 µM BR^{2-} solution. This finding was made in accordance of the solution's measured emf.

Interpretation: The above graph illustrates how the emf of the bilirubin ISE is affected by the solutions containing interfering anions. The findings suggest that the interfering anions had a relatively minor impact on the emf, as evidenced by the reasonably consistent measurements over the course of the experiment. However, the presence of free bilirubin in the solutions, particularly at higher concentrations (100 µM), causes the emf value to drop noticeably and significantly.

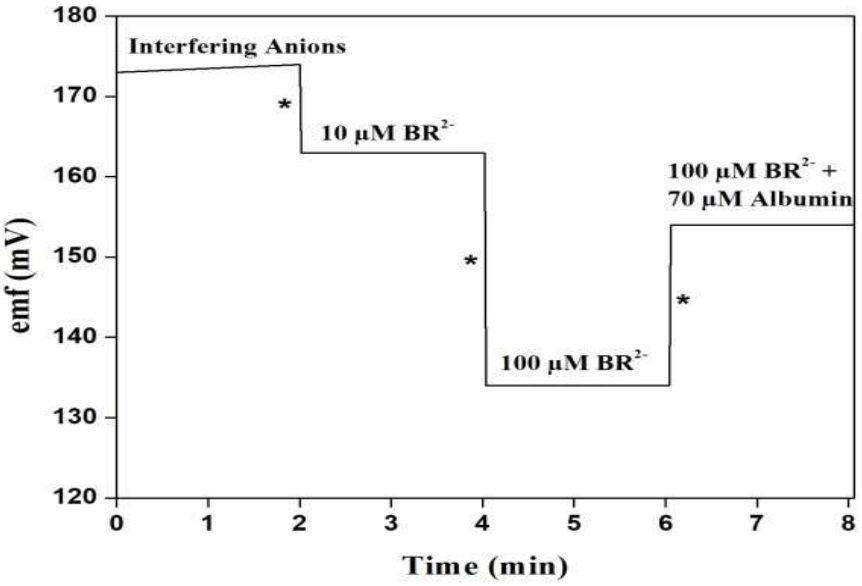

Figure 2: Graph showing the influence of interfering ions solution on BR ISE emf

CONCLUSION

With a very quick response time of just one second, even with all of these complexing substances like albumin and interfering ions, this potentiometric sensor created with 20 mg TDMACl and 0.5 mM IFS is able to detect free BR selectively. Therefore, for label-free detection and selective sensing of free bilirubin in blood serum, this sensor was created. This was done to address the requirement for an accurate method of diagnosing hyperbilirubinemia in newborns in places with limited access to medical services as well as to detect unbound bilirubin in the presence of albumin and other complexing substances. At physiological pH values, bilirubin has a two-fold negative charge, making it detectable via a potentiometric sensor. The following characteristics are included in the potentiometric bilirubin sensor: By integrating with a portable electrochemical detector, At the point of care and in places with few resources, the sensor can be used to detect bilirubin. It can also be used as part of a transmission system for data. The sensor can be used in a way to find albumin without using a name because it only detects free bilirubin and not bilirubin bound to albumin (i.e., the drop in free bilirubin can be used to figure out how much albumin there is). For this reason, albumin might be found without a label. A multiplexed sensor may include the sensor. enabling the simultaneous detection of a large number of minute ionic serum components. The serum electrolytes K+ and Na+ as well as bilirubin are among these compounds. The potentiometric bilirubin monitor can only find bilirubin in an ionic form, which is its only weakness.

As a result, it can only be used in the pH range of 7-9, which is the only region in which it is efficient (where more than 99% of BR has a -2 charge).

REFERENCES

[1] Erlinger, S.; Arias, I.M.; Dhumeaux, D. Inherited disorders of bilirubin transport and conjugation: New insights into molecular mechanisms and consequences. Gastroenterology 2014, 146, 1625–1638.

[2] Balamurugan, T.; Berchmans, S. Non-enzymatic detection of bilirubin based on agraphene-polystyrene sulfonate composite. RSC Adv. 2015, 5, 50470–50477.

[3] Rand, R.N.; Pasqua, A.D. A new diazo method for the determination of bilirubin. Clin. Chem. 1962, 8, 570–578

[4] Chen, J.; Song, G.; Yu He, Y.; Yan, Q. Spectroscopic analysis of the interaction between bilirubin and bovine serum albumin. Microchim. Acta 2007, 159, 79–85.

[5] Zuliani, C.; Diamond, D. Opportunities and challenges of using ion-selective electrodes in environmental monitoring and wearable sensors. Electrochim. Acta 2012, 84, 29–34.

[6] Li, Y.; Chen, Y.; Yu, H.; Tian, L.; Wang, Z. Portable and smart devices for monitoring heavy metal ions integrated with nanomaterials. TrAC Trends Anal. Chem. 2018, 98, 190–200.

[7] Rossotti, FJ.C. and Rossetti, H., The Deiermirrtion of Stability Constants, McGraw - Hill, New York, (1961).

[8] Beck, M.T., Chemistry of Complex Equilibria, Van Nostrand - Reinhold Co., London - New York, (1970).

[9] Ringbom, A, in Analytical Complexation Chemistry, Wiley - Interscience, New York, (1963).

[10] Albert, A. and Sergeant, E.P., Ionization Constants of Acids and bases, Methuen & Co. Ltd., London, New York, (1962).

[11] Vítek, L. (2012). The role of bilirubin in diabetes, metabolic syndrome, and cardiovascular diseases. Frontiers in pharmacology, 3, 55.

[12] Hamoud, A. R., Weaver, L., Stec, D. E., & Hinds, T. D. (2018). Bilirubin in the liver–gut signaling axis. Trends in Endocrinology & Metabolism, 29(3), 140-150.

[13] Narwal, Vinay, *et al*. "Bilirubin detection by different methods with special emphasis on biosensing: A review." Sensing and Bio-Sensing Research 33 (2021): 100436.

[14] Özbek, Oguz, and Caglar Berkel. "Recent advances in potentiometric analysis: Paper–based devices." Sensors International 3 (2022): 100189.

[15] Vítek, L. (2020). Bilirubin as a signaling molecule. Medicinal research reviews, 40(4), 1335- 1351.

[16] Bell, Jeffrey G., *et al*. "Based potentiometric sensing of free bilirubin in blood serum." Biosensors and Bioelectronics 126 (2019): 115-121.