IJBPAS, October, Special Issue, 2023, 12(10): 523-534
ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)
'A Bridge Between Laboratory and Reader'
www.ijbpas.com

POTENTIOMETRIC SENSOR FOR ESTIMATION OF FREE BILIRUBIN IN BLOOD SERUM USING ION SELECTIVE MEMBRANE

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Received 7th July 2023; Revised 9th Aug. 2023; Accepted 30th Sept. 2023; Available online 15th Oct. 2023

https://doi.org/10.31032/IJBPAS/2023/12.10.1053

ABSTRACT

Bilirubin is primarily produced in the liver when hemoglobin is broken down. Understanding the bilirubin concentration in serum is essential for assessing liver health and diagnosing hyperbilirubinemia (which affects 60% of full-term & 80% of preterm infants). Potentiometry based on (ISEs) has undergone an evolution due to advancements in the sensitivity & selectivity values of ISEs, the incorporation of latest elements, novel recognizing approaches (from conventional potentiometry to dynamic electrochemistry technique), & a greater theoretical comprehension & prediction of ISE potentiometric sensitivities. The sensor's polymeric ion-selective membrane monitors only free ionic bilirubin (also known as "unbound" BR, or BR that is not bound to albumin perhaps additional entities of complexity), regardless of the existence of more anions in serum, such as chloride, phosphate, pyruvate, deoxycholate, & lactate. When bilirubin is detected in a pH of 8.4 sodium phosphate buffer, the linear response of the sensor encompasses

bilirubin levels in serum that is clinically significant. This potentiometric biosensor was successful in detecting free BR in human serum. The created potentiometric sensor is a potential instrument for the fast, precise, & targeted analysis of blood serum-free bilirubin, which may have clinical diagnostic applications

Keywords: Bilirubin, Jaundice, Biosensors, Potentiometry, Free Bilirubin, Ion Selective Electrode, Hyperbilirubinemia

INTRODUCTION

Bilirubin is produced during the breakdown of hemoglobin. Because of their hydrophobicity, the great majority of bilirubin molecules are attached to albumin & carried in the circulation as a water-soluble protein-bilirubin complex, with only a small fraction remaining free **[1]**. Bilirubin (BR) is a bile pigment that can be present in two different forms in human blood: conjugated bilirubin (Bc) & unconjugated bilirubin (Bu). Bilirubin measurement is crucial for the diagnosis, prognosis, & treatment of diseases that affect the blood in neonates along with adults. Serum albumin is noncovalently bound to Bu, & using anionic detergents or organic solvents, this connection can be easily disrupted. Because the molecule is nonpolar, it is insoluble in water. However, bilirubin which has been covalently linked to glucuronate is a polar molecule that dissolves in water & is not associated with any proteins in the plasma. The normal range for serum bilirubin is 0.3–1.9 mg/100 mL. Iron deficiency or coronary heart disease is associated with low bilirubin levels. The presence of bilirubin concentrations exceeding 2.5 mg/100 mL is the cause of hyperbilirubinemia. In conditions of hyperbilirubinemia, bilirubin accumulates in different tissues, causing jaundice, icterus, hepatitis, mental illness, cerebral palsy, & even death can all be traced back to metabolic issues (in newborns only) **[2]**. Free bilirubin (BR) values range from 5-34 μM in healthy persons to over 500 μM in babies with hyperbilirubinemia **[3]**. The conjugated form of bilirubin is between 0.001 and 0.004 g/L, while the unconjugated form is between 0.002 and 0.007 g/L. There is also a small amount of unconjugated bilirubin that is not attached to a protein. Between 0.002 and 0.012 g/L is the normal amount of bilirubin in the blood serum **[4]**.

1.2 BIOSENSORS

Electrochemistry is a common way to make biosensors because it is flexible, quick, cheap, very sensitive, and selective. Because of these benefits, there are now a huge number of different types of electrochemical sensors. One of the best-known & most-used examples is the industrial glucose meter. Most

biosensors that use electricity are voltammetric or amperometric, but more & more potentiometric sensors are being made because they have a lot of benefits. Potential distinctions between an operating electrode & the reference electrode at a defined interface are measured using potentiometry, an electrochemical technique. The potential difference between an operating electrode & reference electrode is determined while a tiny amount of bias current (about 10-15 A) flows. This method is useful because it is easy, small, & doesn't need much power **[5]**. Also, because there is almost no current flow, the method should be less affected by interference effects **[6]** & ohmic drops than voltammetric or amperometric sensors. Lastly, potentiometry is less sensitive to sensor size **[7]**, which means that it can be made smaller without losing sensitivity.

1.3 ION SELECTIVE ELECTRODES

The most common type of potentiometric sensor is an ion-selective electrode (ISE). When an ion moves or changes place at a selective membrane, the membrane potential changes **(Figure 1)**. For a long time, ISEs were initially utilized to determine pH & amounts of electrolytes, however, novel membrane designs made it possible to measure a wider range of analytes **[5, 8]**.

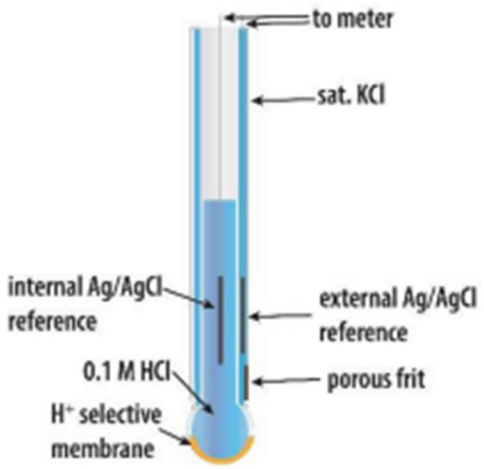

Figure 1: Diagram of a pH-analyzing ISE

Recent developments in this discipline have made it possible to measure neurotransmitters **[9]**, proteins **[10]**, bacteria **[11]**, small molecules **[12]**, & toxins **[13]**.

In this paper, enzyme-free detection of free BR is demonstrated using a potentiometric sensor based on ISE.

2. EXPERIMENTAL

2.1. Materials

Blood serum was collected from PGIMS Rohtak and all the other chemicals such as tridodecylmethylammonium chloride (TDMACl), bis(ethylhexyl) sebacate (DOS), and high molecular-weight (PVC), etc. was gathered via Sunrise Chemicals in Rohtak.

2.2. Fabrication of the BR ISE

To produce ISM, 950 mg PVC, 2020 mg DOS, 20 mg TDMACl, & 10 mL tetrahydrofuran (THF) were combined. We made the mixture one last stir to make sure it was all mixed before putting it into a 6-cm-wide Petri dish. We put a lid on the vessel and left it for an entire night to let the THF vaporize. This formed the ISM, which in turn made the ISE. A piece of ISM measuring ~1.4 cm in diameter & ~1.3 mm in thickness was cut and attached to the tip of a PVC tube that had been sprayed with THF. We injected 3 mL of IFS (inner-filling solution) into the PVC conduit. IFS is a 21 mM sodium phosphate (pH 8.4) buffer containing 17 mM NaCl & 0.5 mM BR. Parafilm was used to seal the tube after a silver wire coated with $AgCl^-$ was placed inside. Cl^- in the ISM must be converted to BR^{2-} for the ISE to function effectively. This was accomplished by soaking the electrode in a 0.5 mM BR (pH 8.4 sodium phosphate buffer) solution for three hours, & then in a 10-µM BR solution for additional 3 hours **[14-26]**.

2.3. Measurements in serum

Combining 1 mL serum with 10 mL sodium phosphate buffer (pH 8.4) containing a suitable quantity of BR (600 µM, 300 µM, & 150 µM) produced the serum solutions **[27]**.

3. RESULT AND DISCUSSION

3.1. Mechanism of sensing

The use of ISEs in conjunction with potentiometric sensing makes it possible to detect ionic substances. Potentiometry can be used to detect BR since it is twice charged at pH levels close to functional levels. Over ninety-nine percent of BR has a -2 net charge in pH ranges of 7 - 8.4 (pKa1 = 4.4 & pKa2 = 4.10). The two parts of this configuration are the reference electrode (which supplies a steady electrical potential for sample) & ISE. The electromotive force (emf) is measured using a potentiometer by determining the variation in electrical potential among these two electrodes. The ISM is the part of ISE that is responsible for its selectivity; a polymer (PVC, polyvinyl chloride) is used to give the membrane mechanical stability. To break down the membrane's constituents & give the ISM ion mobility, a plasticizer called bis(ethylhexyl) sebacate, or DOS, is used in ISM. Tridodecylmethylammonium, or TDMACl, is a lipophilic ion-exchanging cation that maintains a constant concentration of BR^{2-} in ISM. The IFS, a water-based

solution with a steady proportion of BR^{2-} & Cl^-, & the sample solution have sandwiched the ISM. To measure emf, an $AgCl^-$coated Ag wire connected to potentiometer is inserted into IFS. The sensing approach involves the production of charge separation (BR^{2-} & Na^+), It causes an electrical voltage to be produced at the sample's and ISM's interface. BR^{2-} activity in the sample determines the value of this electrical voltage. According to the Nernst equation, the emf is total amount of all interfacial potentials, which are all almost uniform excluding at the contact between the ISM & the specimen. **[28]**.

$$emf = E^0 + \frac{RT}{zF}\ln a = E^0 + \frac{59.2 \quad mV}{z}\log a$$

where z is the BR's charge (-2 at pH 8.4), E° is standard potential in millivolts (mV), R is gas constant, T is temperature, F is Faraday's constant, & 'a' is BR's activity. For every 10 times decrease in movement of BR at ambient temperature, theoretically, emf is increased with 29.6 mV (also called Nernstian response).

3.2. Response of BR ISE

A Practical Segment demonstrates exactly the potentiometric bilirubin sensor (BR ISE) is constructed. **Figure 2** illustrates the emf of this ISE at 10^3 µM, 10^2 µM, & 10 µM BR concentrations in a sodium phosphate buffer using a pH of 8.4. Since the phosphate buffer provides a steady ionic capacity for evaluation substance, we can suppose the BR has a steady efficient movement & plot emf versus its concentration (instead than the movement) of the BR. Since BR is particularly accessible in alkaline water-based formulations & over 99% of BR is charged at 8.4 pH having a total charge of -2, this pH range was selected for the buffer. The ISE had a quick speed of reaction (1-2s) & the emf elevated right away after we diluted the sample to lower the concentration of BR in it (the speed at which we could do dilution was a constraint on how quickly we could quantify the response time). **Figure 3** depicts the linear reaction of the BR ISE, which represents 5 stages of magnitude (0.10 µM - 10^3 µM BR) of BR concentrations. The slope of the correlation between the magnitude of the emf (in mV) & the logarithm of the BR concentration (in µM) was -27.4 mV/decade, which is close to the incline predicted by theory, which was -29.6 mV/decade. This linear spectrum is appropriate to encompass the range of clinically significant free BR concentrations in both healthy individuals & patients with hyperbilirubinemia (30-500 µM) **[29]**.

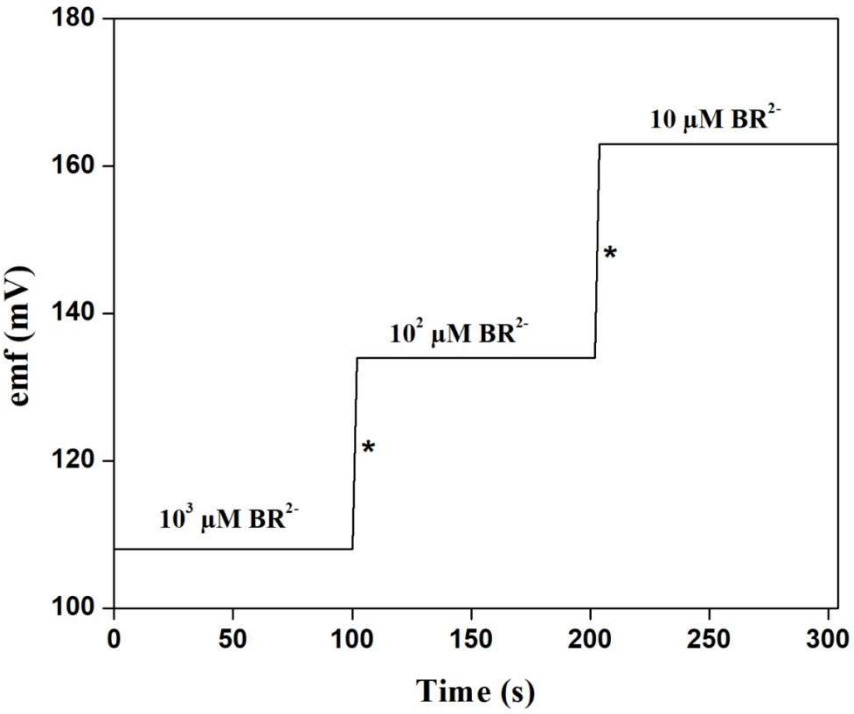

Figure 2: Graph showing emf values at different bilirubin concentrations with time. [The asterisk (*) shows a ten times change in BR concentration in the sample.]

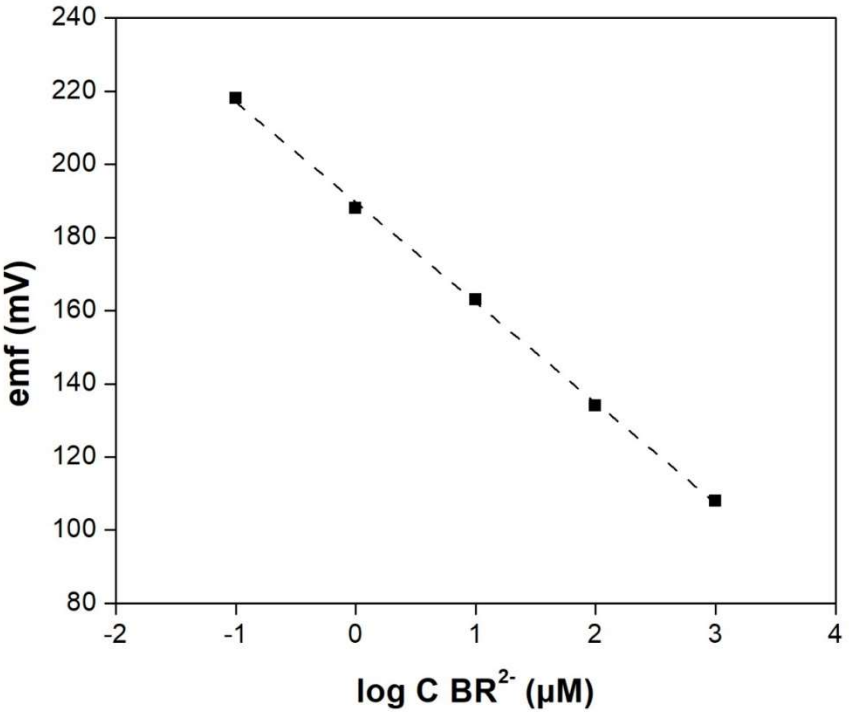

Figure 3: Graph illustrating the linear relationship between emf & the logarithm of Free BR concentration

3.3 Assessments of Serum

We created three identical serums (& consequently identical albumin) containing test solutions but varied BR concentrations (600 µM, 300 µM, and 150 µM) to evaluate BR-ISE's performance in identifying free BR in human serum. These BR values were selected as they encompass the spectrum of BR concentrations detected in the serum of newborns with hyperbilirubinemia. The spectrum of serum albumin concentrations is between 35 - 55 mg/mL (~500 ~ 800 µM). The test solutions were created by diluting serum (1:10) via phosphate buffer (pH 8.4). Therefore, every solution comprised a greater quantity of free BR & the same amount of albumin-bound BR (50~80 µM).

Figure 4 depicts the emf generated by the BR ISE in each of the 3 specimens with total BR contents of 600 µM, 300 µM, and 150 µM. We saw a 10mV increase in the BR ISE's emf as soon as the original BR concentration was reduced from 600 µM to 300 µM. The second step's voltage increased by 14 mV when the overall BR concentration was reduced from 300 µM to 150 µM. According to the calibration curve, each solution contained 80 µM of albumin. Considering a 1:1 association of BR to albumin, actual concentrations of free BR in the 3 specimens were 520 µM, 220 µM, & 70 µM, respectively. As shown in **Figure 5**, the emf shows a linear (negative slope) correlation using the logarithm of the free BR content in serum-containing mixtures **[29]**.

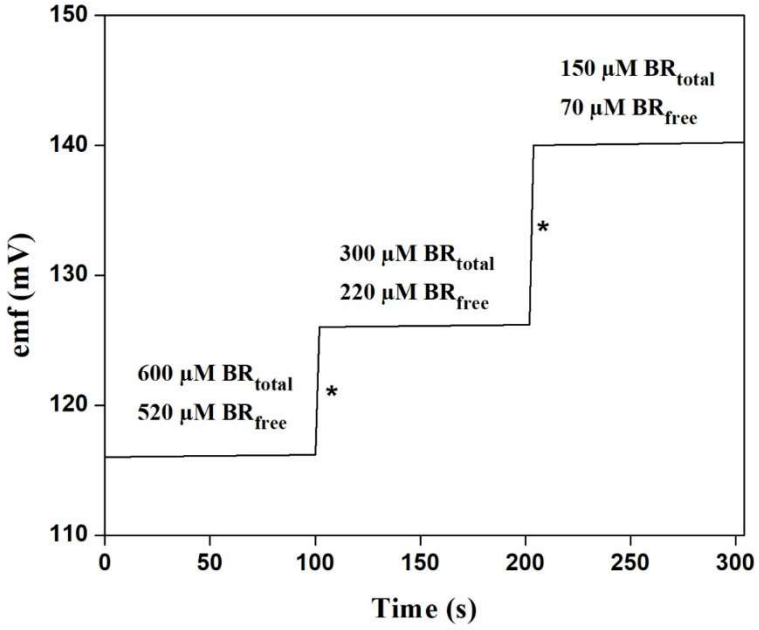

Figure 4: Graph showing emf values of BR ISE in three samples containing 600 µM, 300 µM, & 150 µM of BR. [The asterisk (*) shows where BR samples were changed.]

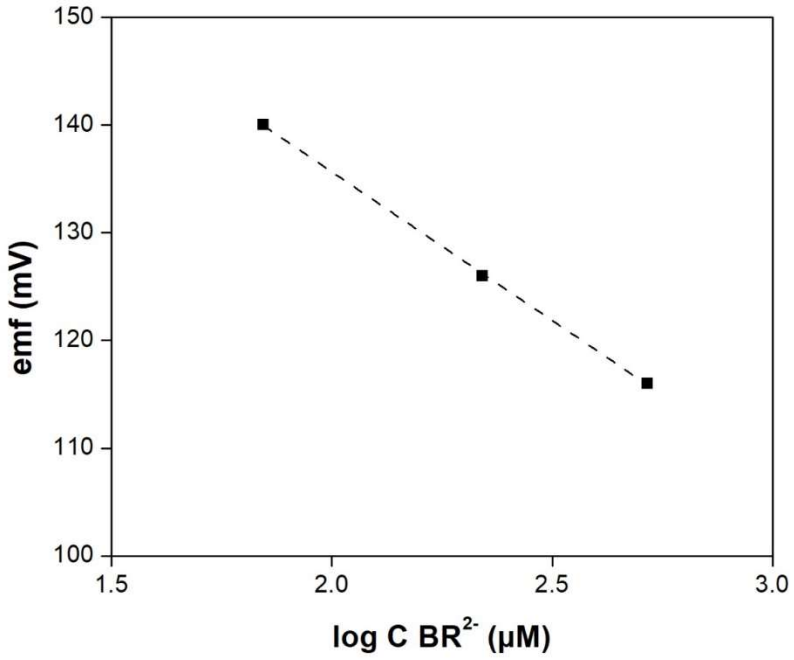

Figure 5: Graph illustrating linear relationship between emf & the logarithm of free BR concentration

To further examine the sensitivity of BR-ISE for identification of free BR, a mixture comprising 400 µM of BR & serum diluted with phosphate pH buffer (pH 8.4, 1:10 dilution) was produced. Using this composition, emf of BR ISE was determined & then used to determine the amount of free BR utilizing the calibration curve. The concentration of free BR in final composition is 321 µM, allowing for the existence of 79 µM albumin (400 µM total BR minus the BR in 80 µM BR-albumin varied yields 320 µM free BR). These findings indicate that BR-ISE is able of detecting free BR in human serum. Furthermore, the BR ISE has the potential to be used for indirect albumin experiments (measuring albumin by bilirubin binding & the subsequent shift in bilirubin concentrations) **[30]**.

4. FUTURE PERSPECTIVE

In the future, potentiometric sensors for estimation of free BR in blood serum using ion-selective membranes hold great potential for transformative advancements in healthcare. Miniaturization and integration of these sensors with microfluidic systems or wearable technologies can enable convenient and real-time monitoring of bilirubin levels, leading to early detection and management of hyperbilirubinemia. Further research focusing on enhancing the sensitivity & selectivity of the sensors can result in enhanced accuracy and reliability of bilirubin measurements. The development of multi-analyte detection

capabilities within these sensors can provide a comprehensive assessment of a patient's health status, facilitating personalized healthcare management. The integration of wireless connectivity and data integration features can enable remote monitoring and seamless transmission of patient data, enabling healthcare professionals to provide timely interventions and recommendations. Moreover, the design and implementation of user-friendly and cost-effective sensor Instruments for point-of-care & home testing programs can empower individuals to monitor their bilirubin levels conveniently and enable early detection of potential health issues. These future perspectives have the potential to revolutionize bilirubin monitoring, enhance patient care, and contribute to improved health outcomes.

5. CONCLUSION

According to the findings of this research, a potentiometric sensor is capable of recognizing free bilirubin in blood serum. The sensor works like ISEs, which are membrane-based sensors that respond only to a certain ion. When it comes to bilirubin, the ISE is responsive to the form of bilirubin that has a negative charge (BR^{2-}). The ISE was set up using a set of liquids with known amounts of bilirubin, and then the sensor was employed for determining bilirubin levels in blood serum. The findings indicated that the sensor could identify bilirubin in the range found in the serum of neonates with hyperbilirubinemia.

The potentiometric monitor is better than other ways of measuring bilirubin in several ways. It is easy to use and doesn't need any sample prep. It works quickly and gives answers in seconds. It is delicate and can find even small amounts of bilirubin. It works only with one type of ion in the blood serum. The potentiometric monitor is a new and potentially useful way to measure bilirubin in blood serum. Additionally, the sensors were employed successfully to measure human serum albumin (HSA).

The potentiometric sensor made in this study could change the way bilirubin is measured in a big way. The sensor is easy to use, quick, sensitive, specific, and is also easy to make, and doesn't cost much. Because of these benefits, the sensor is a useful new tool for diagnosing and treating hyperbilirubinemia.

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