Supporting Information to

Paper-Based Potentiometric Sensing of Free Bilirubin in Blood Serum

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Experimental

Materials: Tridodecylmethylammonium chloride (TDMACl), bis(ethylhexyl) sebacate (DOS), high molecular-weight poly(vinylchloride) (PVC), tetrahydrofuran (THF, inhibitor-free, for HPLC) monohydrogen potassium phosphate, potassium chloride, sodium chloride, sodium hydroxide, sodium nitrate, ascorbic acid, deoxycholic acid, potassium bicarbonate, potassium salicylate, DL-lactic acid, pyruvic acid, and sodium phosphate were purchased from Sigma-Aldrich. Bilirubin was purchased from Toronto Chemical Research. Blood serum (from human male AB plasma, USA origin, sterile-filtered) was purchased from Sigma-Aldrich.

Measurements and Equipment: We measured the response of the sensors using a 16-channel potentiometer (Lawson Labs) at room temperature against a free-flow double-junction AgCl/Ag reference electrode (with a movable glass sleeve junction, 1.0 M lithium acetate bridge electrolyte) purchased from Mettler Toledo. We performed the calibrations of BR through performing successive dilutions of a 20 mL sample. Each 18 mL aliquot removed was replaced with the addition of 18 mL of sodium phosphate buffer (pH = 8.6), and the *emf* was measured for each dilution.

Fabrication of Conventional Ion-Selective Electrodes: The membrane is composed of 990 mg of PVC (poly(vinylchloride), 1980 mg of DOS (Bis(2-ethylhexyl) sebacate, Selectophore grade) and 15 mg of TDMACl (tridodecylmethylammonium chloride). We dissolved these components in 8 mL of THF, stirred the mixture until a homogenous solution was achieved, poured the solution into a petri dish and left it covered overnight; this procedure allowed the THF to evaporate and formed the membrane that provided the ISE. Circular pieces of the membrane with a diameter of ~ 1.1 cm and thickness of ~ 1.2 mm were cut and placed onto PVC tubing, which is wet with THF (causing the membrane to be fused to the PVC tubing). The PVC

tube was filled with 2 mL of an inner-filling solution consisting of 0.5 mM BR in phosphate buffer (pH 8.6) and 15 mM NaCl. The membrane was placed into a solution of 0.5 mM BR for 3 hours before being placed in a 10 μM solution for 3 hours, following the immersion of a Ag/AgCl wire into the inner-filling solution. This procedure allows BR to replace the chloride ion associated with TDMA.

Fabrication of the paper-based ion-selective electrode: We patterned microfluidic zones into chromatography paper using a wax printer and placed the patterned paper in an oven (T = 145 ºC) for 60 s to allow the wax to penetrate the paper fully. Using a laser-cutter (VersaLASER VLS3.50, Universal Laser Systems) we cut stencils for defining regions of ink. We aligned a stencil onto the wax-printed paper, and painted Ag/AgCl ink (C2140310D1, Gwent Group of Companies) onto the stencils, followed by removal of the stencil, and allowed the ink to dry overnight.

The membrane for the paper-based ion-selective electrode consisted of the same ratio of components used in the conventional ISE. We reduced the thickness of the membrane to 0.75 mm for incorporation into the paper-based device by using 660 mg of PVC, 1330 mg of DOS, 10 mg of TDMACl and 8 mL of THF. We poured the membrane solution into a petri dish and left it overnight, thus allowing the THF to evaporate. We cut square segments of the membrane (1.2 x 1.2 cm) and placed them in a solution of 0.5 mM BR for 6 hours to allow the exchange of BR for chloride ions in the membrane.

Measurements with paper-based ISE: To perform a measurement using the paper-based ISE, we taped the bottom layer of the device to a glass slide and added 15 μL of reference solution (1 M NaCl) and 15 μL of sample (different concentrations of BR) to the reference and

sample zones, respectively. Next, we placed the ISM onto the sample zone and covered the ISM with the indicator electrode. We added 15 μL of inner-filling solution (0.5 mM BR and 15 mM NaCl) to the indicator electrode. All solutions were added through pipetting. We placed a second glass slide on top of the device and held it in place by two paper clips. We connected the reference and indicator electrodes to a potentiometer through alligator clips prior to recording a measurement.

Determination of Selectivity Coefficients: The selectivity coefficients were determined using the separate solution method (SSM) according to the procedure outlined in the IUPAC recommendations.[1] In order to obtain unbiased selectivity coefficients, we conditioned the electrode in a solution of 1 mM KCl (in sodium phosphate buffer, pH = 8.6) overnight. The measured *emf* of 1 mM of the interfering ions (E_I) was used in the equation:

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

Where R is the gas constant, T is the temperature, F is Faraday's constant, and z is the charge of BR.By using the *emf* response to a 1 mM solution of BR (E_{BR}) and the slope ($z_{BR}F/RTln10$) of the linear response of the BR-ISE, the selectivity coefficients ($logK_{BR,I}^{Pot}$) were obtained and are presented in Table 1.

Preparation of solutions

Preparation of Phosphate buffer 20 mM (pH 8.6): We dissolved 2.74 g of sodium dihydrogen phosphate in 900 mL of distilled water and adjusted the pH by adding 1 M NaOH dropwise until the pH reached 8.6. The solution was then adjusted to 1 L.

Preparation of Solution of 1 mM Bilirubin: We dissolved 29.2 mg of BR in 2 mL of 0.1 M NaOH and adjusted the volume to 50 mL with 20 mM phosphate buffer (pH 8.6).

Preparation of Solutions for Selectivity Studies: We dissolved the required amount of chemical to make 20 mL of a 1 mM solution of the potentially interfering species in 20 mM sodium phosphate buffer (pH 8.6).

Background

Techniques to measure bilirubin

Table S1: Techniques employed for the measurement of bilirubin.

Technique	**Mode of Operation**	**Linear Range (μM)**	**Limit of Detection (μM)**	**Reference**
Electrochemical	Amperometry	4 – 100	4	[1]
Electrochemical	Amperometry	0.01 – 500	0.0001	[2]
Electrochemical	Voltammetry	1.2 – 420	0.025	[3]
Electrochemical	Voltammetry	5 – 600	—	[4]
Electrochemical	Impedance	0.01 – 500	0.005	[5]
Optical	Fiber Optic	0.1 – 300	0.1	[6]
Optical	UV-VIS	0.068 – 17.2	0.068	[7]
Optical	UV-VIS	0.1 – 50	0.04	[8]
Optical	Fluorescence	25 – 50	0.15	[9]
Separation	HPLC-TLS	0.00025 – 0.15	0.00009	[10]
Separation	HPLC	0.01 – 2	0.45	[11]
Separation	Capillary Electrophoresis	10 – 200	9	[12]
Separation	Capillary Electrophoresis	5 – 206	2	[13]

The Van Den Bergh reaction — which involves reaction of diazotized sulfanilic acid with BR and produces the UV-active compound azobilirubin — is highly dependent on pH, and requires a UV-VIS spectrophotometer.[14,15] Analytical methods depending on fluorimetry, and techniques requiring separations, often require time-consuming sample preparation steps and have a high

cost.[16-18] Electrochemical methods (enzymatic and non-enzymatic) have also been employed to measure free BR, although enzymatic sensors suffer from the high cost and low stability of enzymes (bilirubin oxidase, BOx, loses 50% of its activity after 17 h at 37 ^{o}C).[19-21] The currently utilized method for detection of BR in hospital settings is transcutaneous bilirubinometry (a skin reflectance technique). This method has the benefit of being a non-invasive approach, but has a tendency to overestimate the actual amount of BR in Asian newborns while underestimating the amount of BR in white newborns.[22] Furthermore, transcutaneous bilirubinometers are costly and often require expensive calibration standards. This high cost makes their use in resource-limited regions infeasible (and it is in these regions where the majority of deaths stemming from hyperbilirubinemia occur).[23]

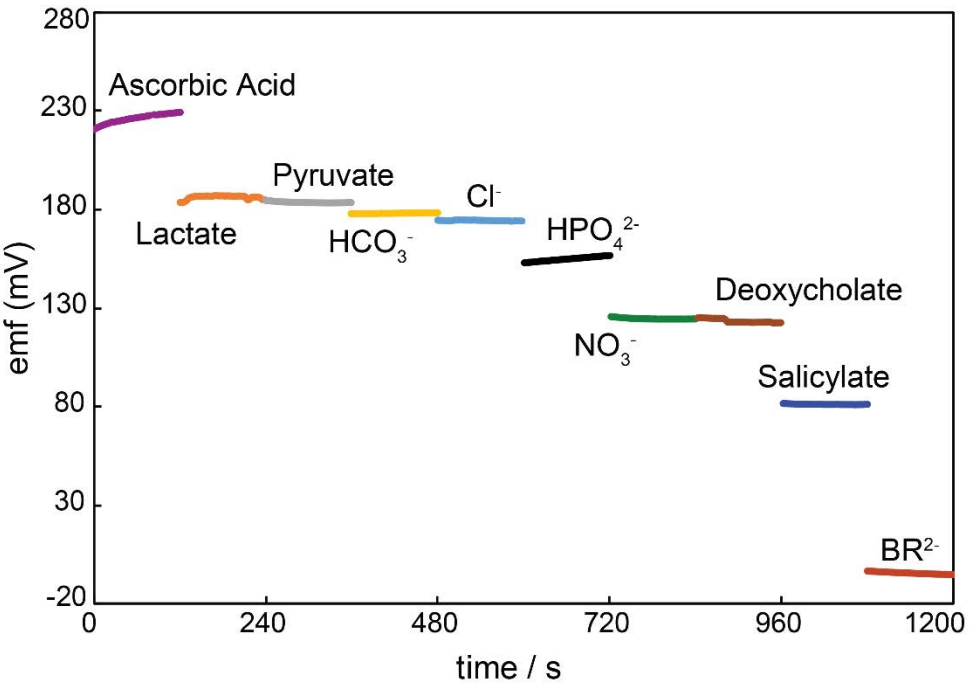

Figure S1. Emf response of the ISE (conditioned in 1 mM KCl) to 1 mM solutions of the specified anions in sodium phosphate buffer (pH 8.6).

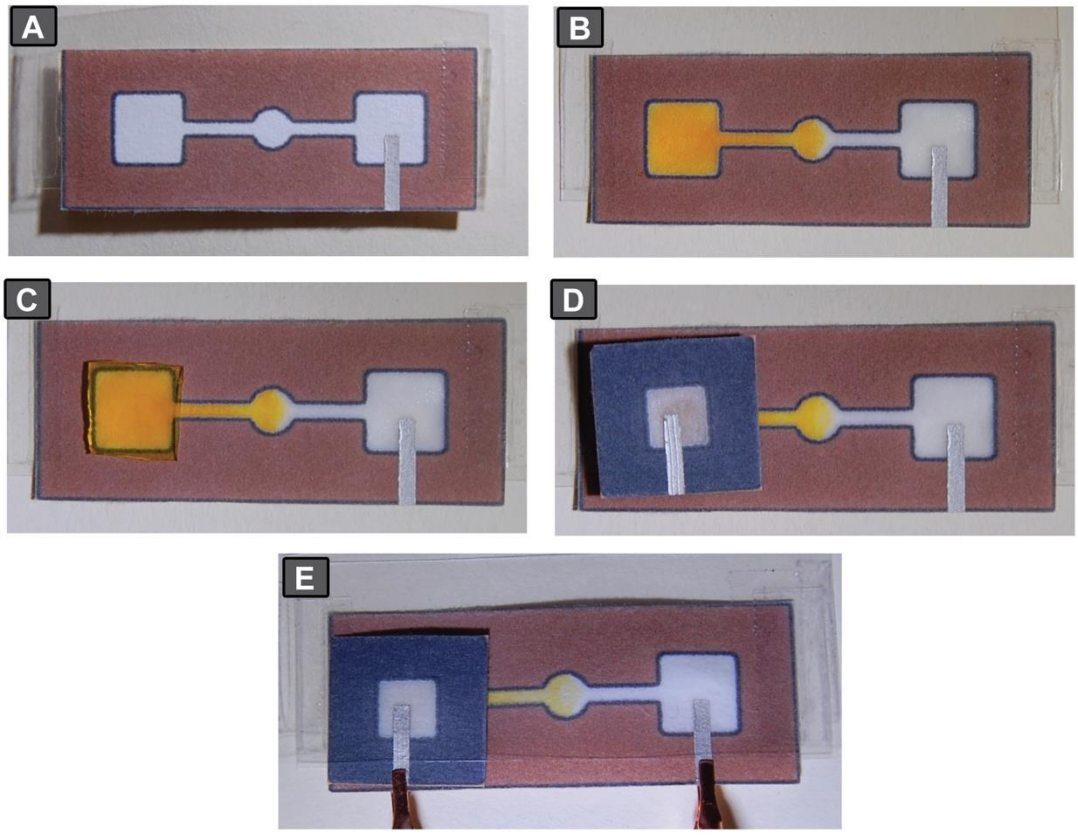

Figure S2. Photographs of the paper-based device. **A.** Bottom layer, **B.** Bottom layer with BR added to sample zone and NaCl added to reference zone. **C**. BR-ion-selective membrane (BR-ISM) placed on sample zone. **D.** Indicator electrode placed on top of the BR-ISM, with added inner filling solution. **E.** Connection to potentiometer made through connecting alligator clips to the Ag/AgCl electrodes.

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