In-vitro Assessment of the Adaptive Howland Current Source for Bioimpedance Haemolysis Measurements

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***Abstract*—Haemolysis, the rupture of red blood cells, is a significant concern that can occur at any stage during blood collection, transportation or handling. Haemolysed blood used for transfusions can lead to reduced oxygen saturation, inflammation, and renal dysfunction. Established methods for determining the haemolytic condition of blood involve visual inspection of the sample colour or the use of chemical reagents. There is a need for a method that would allow for in-situ detection of haemolysis in real-time without the need for specialised personnel or cumbersome facilities. While high-bandwidth bioimpedance has been proposed as a method for doing so, conventional portable instrumentation is limited in bandwidth (sub-MHz). This work presents whole blood bioimpedance measurements using the previously published Adaptive Howland Current Source (AHCS). Using a custom-designed electrode interface, in a tetrapolar square topology, blood samples were interrogated before and after lysis. Measurements taken with a commercial impedance analyser were found to be informative in determining haemolysis and were compared with measurements using the AHCS system showing satisfactory results up to 3 MHz.**

***Index Terms*—Bioimpedance, haemolysis, AHCS, MEHCS, Howland Current Source, Tetrapolar impedance, blood sample integrity, blood analysis**

I. Introduction

The integrity of blood samples during collection, storage, preservation, transportation, and handling, is crucial for both transfusion and diagnostic testing. Haemolysis is the rupturing of RBC cell membranes, resulting in an outward flux of cytoplasm containing haemoglobin into the blood plasma [1]. It can occur due to factors such as improper collection techniques, excessive agitation, extreme temperatures, prolonged storage, or unsuitable storage solutions. Mechanical damage, incorrect needle gauge, or overexposure to light can also lead to haemolysis, compromising the quality and reliability of the sample [2], [3].

Despite the crucial importance of detecting haemolysis, it is mostly done through simple visual inspection, as haemolysis causes discolouration of the plasma [3]. However, this technique has been reported to be unreliable, unreproducible, inaccurate, and reliant on expert judgment [3]–[5]. Haemolysis can be determined optically using the haemolysis index, however it uses different diluents which introduce spurious turbidity in the blood samples [6], [7]. Another promising method is through the use of microfluidic techniques, which involves calculating the concentration of blood plasma through a membrane filter, however it requires sample preparation [8].

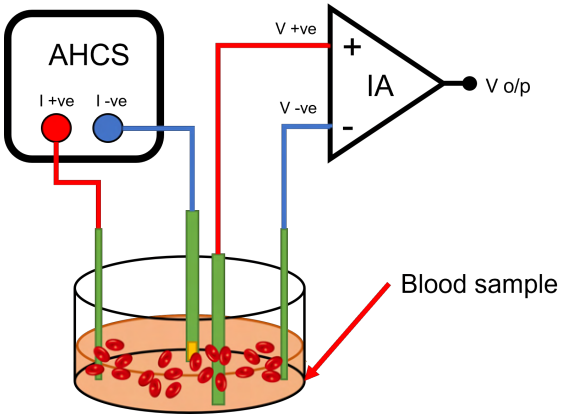

Fig. 1: Bioimpedemitric measurement of haemolysis.

Multi-frequency electrical impedance (or Bioimpedance) measurement has recently emerged as an alternative diagnostic method, which requires no chemical additives, sample preparation, or expert handling. Bioimpedance has been used to quantify blood parameters [9]–[11], and to specifically measure haemolysis [12], [13]. A. K. Tran *et al.* [14] carried out 100 kHz to 100 MHz bioimpedance spectroscopy on conditions mimicking haemolysis, showing that the conductivity of blood decreases with increasing haemolysis. This being due to the cytoplasm containing haemoglobin having a lower conductance than plasma. The work also reported that electrode polarisation is dominant in lower frequencies range with the effect diminishing close to 1 MHz and at higher frequencies, allowing for better measurement of haemolysis.

Although impedimetric measurements show promising outcomes in diagnostic applications, most studies have used laboratory-grade high-precision impedance analysers to reach MHz order bioimpedance measurements. While it is highly desirable to develop portable impedimetric systems for in-situ sample interrogation during storage, transfer or handling,

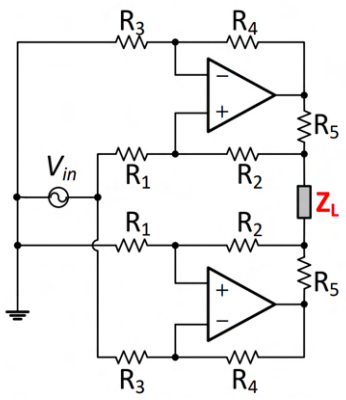

Fig. 2: Mirrored Enhanced Howland Current Source (MEHCS). [18]

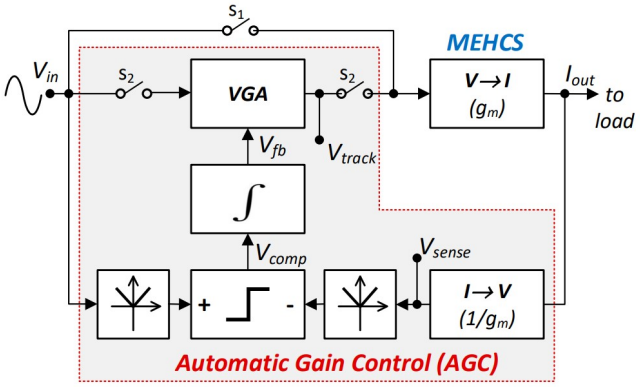

Fig. 3: Functional block diagram for the Adaptive Howland Current Source (AHCS) [18]

such systems are typically hampered by bandwidth limitations. The performance of impedimetric circuitry relies heavily on the current injection stage, which should provide a constant amplitude ($\leq 1\%$ variability) over a wide frequency bandwidth and load variation [15], [16]. In most cases the output current amplitude drops at higher frequencies due to the current injection circuit's output impedance deteriorating. As a result conventional current injection circuitry, typically based on the Howland architecture, can only generate highly accurate currents up to a few hundreds of kHz, with only a handful of designs reporting 1 MHz bandwidth [16], [17]. To address this issue, we have previously described the design of an Adaptive Howland Current Source (AHCS) for high-frequency applications [18]. The AHCS is based on the mirrored-enhanced Howland current source (MEHCS) architecture and uses an Automatic Gain Control (AGC) feedback loop to compensate for the degraded amplitude of the injected signal thus keeping it steady, with less than 1% error for frequencies up to 3 MHz, effectively offering triple the bandwidth than other architectures, including ASIC - based designs. The AHCS overcomes the limitations pointed out above and can be used to interrogate blood at high frequencies for haemolysis detection.

This work proposes the use of the AHCS as a novel method for measuring the impedance of in-vitro blood samples to detect haemolysis. A schematic diagram of the setup is shown in Fig. 1. Two electrodes connected with the Adaptive Howland Current Source (AHCS) work as a current injecting electrode. The remaining two electrodes are connected to an instrumentation amplifier (IA), serving as the voltage measuring electrodes. The interface between the measuring circuit and the biological sample has been achieved using a custom-made PCB electrode designed for a 6-well culture dish with electrodes configured in a square tetrapolar topology. Measurements were taken with MEHCS with and without implementing the AGC feedback loop. The measurements of the system were compared with those of the Agilent A4924A impedance Analyser. Haemolysis was achieved through rapid cooling of blood samples, and confirmed by microscopy and haematocrit. The system showed good performance in measuring the impedance of the samples from 100 kHz to 3 MHz.

II. System Description

A. Adaptive Howland Current Source (AHCS)

MEHCS is widely used for biomedical impedimetric applications for its high output impedance and true differential output, providing a relatively wide bandwidth [17]. The load (Z_L in Fig. 2) is connected between two opposite polarity single-ended Howland Current Sources, symmetrically sourcing and sinking current. Due to limitations including resistor mismatch, stray capacitance and Op-Amp limitations, MEHCS' performance deteriorates at higher frequencies (>100kHz).

This limitation can be overcome using a feedback loop to adaptively control the amplitude of the driving input voltage as we demonstrated in designing the AHCS (Fig. 3) which we developed, tested and previously published in [18]. The circuit utilizes a MEHCS with an AGC mechanism to stabilise the amplitude of the output current using a variable gain amplifier (VGA). When the switch S_2 (Fig. 3) is closed, the VGA takes in the input signal (V_{in}) and transfers it to the MEHCS. The feedback input of the VGA (V_{fb}) is determined through the difference in the amplitude level of the output signal of MEHCS and the input signal (V_{in}). When the output signal gets attenuated at high frequencies, the V_{fb} increases the gain of the VGA to increase the input of the MEHCS, thus stabilising the output amplitude.

The AHCS performs better in terms of bandwidth than other architectures reported in the literature for current injection sources. The output of the AHCS can be as low as 100 μA, compared to other systems reporting an output of 500 μA [19]. The amplitude error of the output current is less that 1%. For a 2 mA p-p output current, the total harmonic distortion of AHCS is reported to be less that 0.2%. The system also showed stable performance for different R-C loads varying over the bandwidth, making it a suitable candidate for bioimpedance measurement.

Fig. 4: PCB electrode setup for the 6-well culture dish.

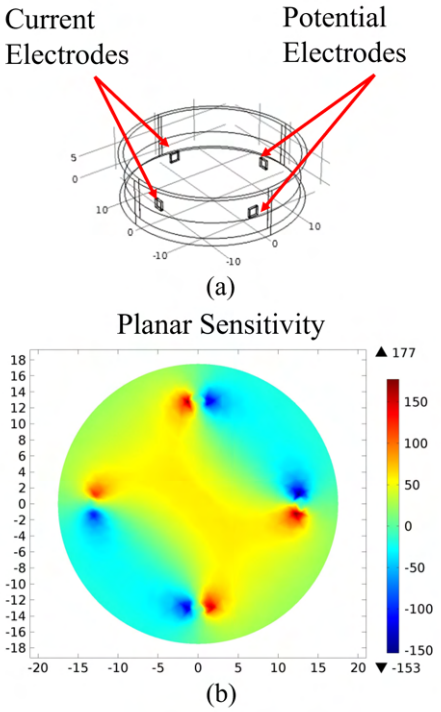

Fig. 5: COMSOL Multiphysics® simulation (a) Electrode configuration (b) Planar sensitivity.

B. Electrode Setup

Custom electrodes were designed to accommodate a 6-well culture dish, based on our prior work [20], using a PCB setup. The main board and electrodes are made of FR4 PCB material board (Fig. 4), with gold-plated electrodes for biocompatibility. The electrodes (inset of Fig. 4) are soldered to the main board, which has the connections leading to the connector points at the end of the board, to which the external circuitry is connected via jumper wires.

A FEM simulation of the electrode setup was done in COMSOL Multiphysics® (Fig. 5). The tetrapolar square setup of electrodes (Fig. 5(b)) ensures higher sensitivity in the central region of the dish. The planar sensitivity distribution at the bottom of the dish (Fig. 5(c)) shows a high sensitivity region of 100 cm^2. This implies that the impedance of the specimen placed in the central 100 cm^2 region of the dish will contribute more to the bulk measured impedance.

C. Blood Sample

1.5 mL of whole human blood was used for each sample, and was EDTA treated to prevent coagulation. Haematocrit (HCT) was measured using a haematology analyser (Dymind DH35) (Table I). Transmission DIC microscopy images were taken using Amscope Transmission microscope (Fig. 6(a)). Blood was lysed by freezing at -80^{0}C for 30 min, thawed and warmed to 22^{0}C. HCT was measured and microscopy images were taken to assess degree of haemolysis (Fig. 6(b)).

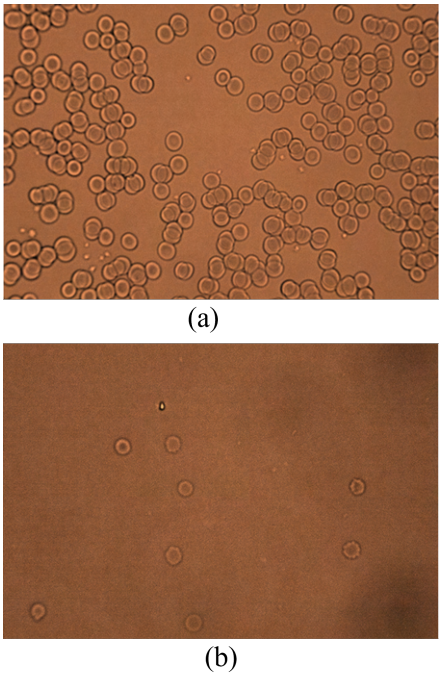

Fig. 6: Microscopic image of (a) whole blood (b) lysed blood.

III. Experimental Procedure

The AHCS was powered with a $\pm$10V DC power supply. The input to the AHCS was taken from an AC signal generator. The amplitude of the injected current was maintained at $1mA$ by changing the amplitude of the input signal and monitoring the output waveform of the circuit across a high precision (0.01% tolerance) sensing resistor of 1kΩ.

The electrodes were cleaned and disinfected and the setup was placed in the dish so that the electrodes reached the bottom surface. 1.6 mm of the electrodes were dipped into the sample. The sample covered the base of the culture dish and was maintained at 22^{0}C.

The setup was connected to the Agilent A4924A impedance analyser, with the injection current set to 1mA. The frequency was swept from 10kHz to 15 MHz with a bandwidth setting of 5 (high precision). Subsequently, the setup was connected to the AHCS mentioned in section II. The potential across the potential electrode was measured using an oscilloscope. The

TABLE I: Hematocrit count of whole blood and lysed blood

Sample	Whole blood (%)	Lysed Blood (%)	Difference (%)
S-1	45.7	9.3	36.4
S-2	44.2	1.1	43.1

TABLE II: Spearman's rank Correlation Coefficient between the measurements from analyser and AHCS

Sample 1		Sample 2	
Whole blood	**Lysed blood**	**Whole blood**	**Lysed blood**
0.9966	0.9870	0.9954	0.9924

frequency of the input signal ranged from 100 kHz to 4 MHz in decade intervals. Data from the oscilloscope was filtered in MATLAB®. The values were divided by the injection current (1mA) to determine the impedance of the sample. This was repeated to determine the impedance of the blood sample following haemolysis.

IV. Analysis

A. Impedance spectrum of blood

The impedance of whole blood and lysed blood has been determined using a standard Agilent A4924A impedance analyser from 100 kHz to 4 MHz. The magnitude of impedance across the spectrum for whole blood and lysed blood for both samples S-1 and S-2 is shown in Fig. 7. There is a difference between the impedance magnitude of whole blood and lysed blood across the spectrum, where lysed blood has a higher impedance than whole blood. Impedance values of both whole and lysed blood converge to the same values at frequencies around 5 MHz. This difference in measured impedance portrays that haemolysis can be detected through impedimetric measurements. The variability in the impedance value at a particular frequency between the two samples can be attributed to the difference in haemolysis levels (Table I). A discontinuity in the data can be seen around 1.095 MHz which is caused by the bridge imbalance of the analyser.

B. Comparison of measurement between analyzer and circuit

The impedance of whole and lysed blood has been measured using the Adaptive Howland Current Source (AHCS) from 100 kHz to 4 MHz. The AHCS can be configured with and without the Automatic Gain Control (AGC) feedback mechanism. The values from the AHCS has been adjusted for DC offsets. Fig. 7 compares the impedance of whole and lysed blood measured with the analyser and the AHCS including and excluding AGC feedback. The graph shows that the circuit performance is improved with the AGC feedback and follows a similar trend as the analyser at higher frequencies. Whereas, excluding the AGC feedback degraded the impedance from 200 kHz onwards. The high correlation between the measurements of the analyser and the AHCS with AGC feedback is quantified through Spearman's rank Correlation Coefficients (Table. II). This shows the superior performance of the AHCS with AGC feedback for multi-frequency measurement of haemolysis.

V. Conclusion

This study employed an impedimetric detection method using an AHCS to measure the impedance of in-vitro blood samples for haemolysis detection. A custom-designed square tetrapolar configuration with PCB electrodes for a 6-well culture dish was used to interface the biological samples with the measuring circuit. The electrode configuration was optimised using a COMSOL Multiphysics® model, which identified the areas of highest sensitivity contributing to the measured impedance. Measurements were conducted with and without the AGC feedback loop, showing a strong correlation of 0.99 between the impedance analyser and AHCS circuit measurements. The results from both the impedance analyser and the AHCS circuit demonstrated that lysed blood exhibits higher magnitude than whole blood, confirming that detecting haemolysis is feasible through these changes in impedance. However, further development is needed to incorporate a signal generator and an output detection system, making it a fully standalone system. Once developed, this standalone system could be an effective in-vitro point-of-care diagnostic tool for detecting blood haemolysis safely and efficiently.

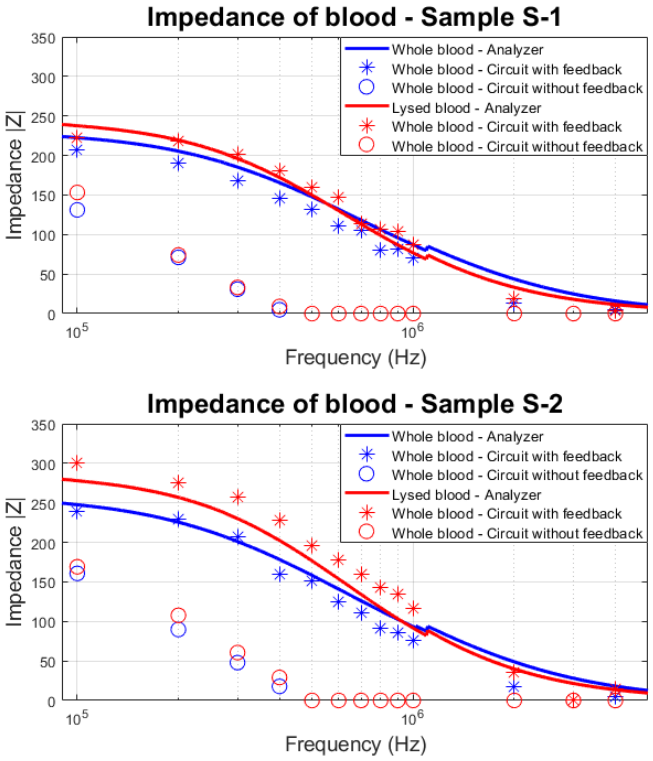

Fig. 7: Impedance spectrum of whole and lysed blood using the impedance analyser and the AHCS with and without feedback.

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References

[1] S. O. Sowemimo-Coker, "Red blood cell hemolysis during processing," *Transfusion medicine reviews*, vol. 16, no. 1, pp. 46–60, 2002.

[2] P. S. Ajmani and P. S. Ajmani, "Storage of blood," *Immunohematology and Blood banking: Principles and Practice*, pp. 37–47, 2020.

[3] N. Choudhury and A. Mathur, "Visual detection of hemolysis in a blood bag before issue," *Asian Journal of Transfusion Science*, vol. 5, no. 1, pp. 61–62, 2011.

[4] K. Janatpour, T. Paglieroni, V. Crocker, D. DuBois, and P. Holland, "Visual assessment of hemolysis in red blood cell units and segments can be deceptive," *Transfusion*, vol. 44, no. 7, pp. 984–989, 2004.

[5] R. Hawkins, "Discrepancy between visual and spectrophotometric assessment of sample haemolysis," *Annals of clinical biochemistry*, vol. 39, no. 5, pp. 521–522, 2002.

[6] G. Lippi, G. Luca Salvagno, N. Blanckaert, D. Giavarina, S. Green, S. Kitchen, V. Palicka, A. J. Vassault, and M. Plebani, "Multicenter evaluation of the hemolysis index in automated clinical chemistry systems," *Clinical chemistry and laboratory medicine*, vol. 47, no. 8, pp. 934–939, 2009.

[7] I. Munnix, M. Raijmakers, W. Oosterhuis, and H. Kleinveld, "Detection of a monoclonal gammopathy by lipemia-index measurement," *Ned Tijdschr Klin Chem Labgeneesk*, vol. 34, no. 4, pp. 248–249, 2009.

[8] J. H. Son, S. H. Lee, S. Hong, S.-m. Park, J. Lee, A. M. Dickey, and L. P. Lee, "Hemolysis-free blood plasma separation," *Lab on a Chip*, vol. 14, no. 13, pp. 2287–2292, 2014.

[9] T. Fuse, A. Sapkota, O. Maruyama, R. Kosaka, T. Yamane, and M. Takei, "Analysis of the influence of volume and red blood cell concentration of a thrombus on the permittivity of blood," *Journal of Biorheology*, vol. 29, no. 1, pp. 15–18, 2015.

[10] J. Li, D. Kikuchi, A. Sapkota, and M. Takei, "Quantitative evaluation of electrical parameters influenced by blood flow rate with multiple-frequency measurement based on modified hanai mixture formula," *Sensors and Actuators B: Chemical*, vol. 268, pp. 7–14, 2018.

[11] J. Li, A. Sapkota, D. Kikuchi, D. Sakota, O. Maruyama, and M. Takei, "Red blood cells aggregability measurement of coagulating blood in extracorporeal circulation system with multiple-frequency electrical impedance spectroscopy," *Biosensors and Bioelectronics*, vol. 112, pp. 79–85, 2018.

[12] H. Fricke and H. J. Curtis, "The electric impedance of hemolyzed suspensions of mammalian erythrocytes," *The Journal of General Physiology*, vol. 18, no. 6, pp. 821–836, 1935.

[13] K. A. Tran, J. Li, H. C. Khon, and M. Takei, "Investigating dielectric properties of hemolysis by electrical impedance spectroscopy," in *7th International Conference on the Development of Biomedical Engineering in Vietnam (BME7) Translational Health Science and Technology for Developing Countries 7*. Springer, 2020, pp. 239–241.

[14] A. K. Tran, A. Sapkota, J. Wen, J. Li, and M. Takei, "Linear relationship between cytoplasm resistance and hemoglobin in red blood cell hemolysis by electrical impedance spectroscopy & eight-parameter equivalent circuit," *Biosensors and Bioelectronics*, vol. 119, pp. 103–109, 2018.

[15] R. Ghorbani and M. Nahvi, "Analysis of performance of howland ac current source for electrical impedance spectro-tomography," *Sensing and Imaging*, vol. 20, no. 1, p. 28, 2019.

[16] L. Constantinou, I. F. Triantis, R. Bayford, and A. Demosthenous, "High-power cmos current driver with accurate transconductance for electrical impedance tomography," *IEEE Transactions on Biomedical Circuits and Systems*, vol. 8, no. 4, pp. 575–583, 2014.

[17] V. Grando Sirtoli, V. Coelho Vincence, and P. Bertemes-Filho, "Mirrored enhanced howland current source with feedback control," *Review of Scientific Instruments*, vol. 90, no. 2, 2019.

[18] I. I. Nwokoye and I. F. Triantis, "A 3 MHz low-error adaptive howland current source for high-frequency bioimpedance applications," *Sensors (Basel, Switzerland)*, vol. 24, no. 13, 2024.

[19] A. Mahnam, H. Yazdanian, and M. M. Samani, "Comprehensive study of howland circuit with non-ideal components to design high performance current pumps," *Measurement*, vol. 82, pp. 94–104, 2016.

[20] M. Rabbani, E. Rahman, A. Al Aishan, M. B. Powner, and I. F. Triantis, "A low-cost, scalable, and configurable multi-electrode system for electrical bio-interfacing with in-vitro cell cultures," *Applied Sciences*, vol. 14, no. 1, p. 162, 2023.