Supplementary Information

A low-cost miniature immunosensor for haemoglobin as a device for the future detection of gastrointestinal bleeding

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S1 Comparison with literature

EIS technique requires a signal generator that can generate low amplitude AC signals, and a frequency response analyzer that works synchronously with the signal generator to measure the response of the sensor. Although current commercial devices are unsuitable for use as ingestible sensors since there are bulky, expensive, and power hungry, numerous studies have proposed small size devices which can be converted to an ingestible EIS sensor. Several researchers [1–4] have suggested using an AD5933 (7.8x6.2x2 mm, £17, Analog Devices, USA) chip which includes a 12-bit, 1 MSPS current-output DDS (direct digital synthesizer) as a signal generator, and a 12-bit ADC (analog-to-digital converter) with on-board discrete Fourier transform (DFT) engine for signal acquisition. Frequency resolution of the chip is 27 bits and capable of generating frequencies less than 0.1 Hz. However, it is only capable of measuring impedances from 100 Ω to 10 MΩ. Also, its 1 MSPS sampling rate is not enough to generate high-quality sinusoidal waves and its ADC resolution is 12-bits which makes it harder to measure low level signals. Brown et al. (2022) has suggested using an ADuCM355 (7.8x6.2x2 mm^2, £19, Analog Devices, USA) which is a system-on-chip device including an ARM Cortex-M3 microprocessor with other peripherals to implement EIS and voltammetric protocols. It includes a waveform generator, and an on-board ADC and DFT engine to read and analyze the data. It can generate frequencies less than 1 Hz to 200 kHz. Impedance measurement range is from 0.4 Ω to 10 MΩ. Some researchers suggested using a built-in DAC (digital-to-analog converter) and ADC of microcontrollers[6]. But built-in DACs and ADCs in general purpose microcontrollers have sampling rates generally less than 2 MSPS and resolution generally less than 12 bits. Gervasoni et al.[7] suggested using a high-speed external DAC and ADC but this method is expensive and requires high-speed processors such as FPGA (field programmable gate array) which are power-hungry devices. All these works use a digital lock-in amplifier or DFT which demodulate the signal after it is converted to a digital value by ADC. However, higher sampling rate and resolution results in cost and power trade-offs in these methods. Comparison of the devices is given at Table S1.

Table S1. Comparison with literature

Reference	Cost	Size (mm^2)	Frequency Range	AC Output Range (mV_{RMS})
Our Device	£31	33x20 (19x7.9 in capsule)	DC -100 kHz	1-30 or 10-300 (depends on selected DDS I/V converter resistor)
Zhang et al.[1]	N.A	N.A	10 Hz-10 kHz	200 mV (fixed)
Chuang et al. [2]	N.A.	N.A	1 kHz- 100 kHz	N.A
Salahandish et al. [3]	$80	83x60	1 Hz-5 kHz	10
Liu et al.[4]	$100	N.A.	1-100kHz	1.99, 0.97, 0.383, 0.198 (V_{p-p})
Brown et al.[5]	$60	26x68	16 mHz- 200 kHz	N.A.
Pruna et al.[6]	$300	120x70	0.1 Hz-10 kHz	500
Jenkins et al.[8]	$105	74x89	DC-100 kHz	10 -100 (4 pre-programmed)
Sensit Smart[9]	$1250	43x25	16 mHz- 200 kHz	1-250

S2 Cost of materials for the EIS device

Table S2. Cost of the materials

Name	Description	Quantity	Unit price (£)	Total (£)
Passive elements	Resistors, capacitors, and inductors	44	0.01	0.44
AD9834	DDS	1	12.11	12.11
EFM8LB12	MCU	1	1.23	1.23
MAX5394	Digital Potentiometer	2	1.61	3.22
MCP3561	24-bit ADC	1	2.62	2.62
PI5A3157	Analog switch	2	0.3	0.6
DS1020-05	Electrode Connector	1	0.2	0.2
FT200XD	USB/I2C converter	1	1.73	1.73
USB connector	USB Mini-b connector	1	1.31	1.31
OPA4354	Quad Op-amp	2	2.6	5.2
LP2985	4.5 V LDO	1	0.85	0.85
MCP1700	3.3 V LDO	1	0.41	0.41
PCB	4-layer circuit board	1	1	1

Total cost: £30.92

S3 Dummy-cell measurements

Precision resistors which have 0.1% tolerance and 10 ppm/°C temperature coefficient in 0805 SMD package were used as dummy-cell.

Table S3. *Mean and standard deviations of dummy-cell measurements*

		Impedance		**Phase**	
		Mean (Ω)	**Std (Ω)**	**Mean (°)**	**Std (°)**
40 Ω	This work	39.97	0.152	-0.198	0.162
	PS-4	40.62	0.078	-0.166	0.457
100 Ω	This work	100.507	0.209	0.276	0.227
	PS-4	100.949	0.099	-0.0484	0.185
1000 Ω	This work	996.421	0.977	-0.126	0.106
	PS-4	998.033	1.494	-0.878	1.012
10500 Ω	This work	10.496k	6.900	0.135	0.166
	PS-4	10.443k	156.547	1.707	1.581
51 kΩ	This work	51.050k	63.464	-0.735	0.597
	PS-4	49.129k	5.852k	11.581	9.897

S4 Transimpedance amplifier

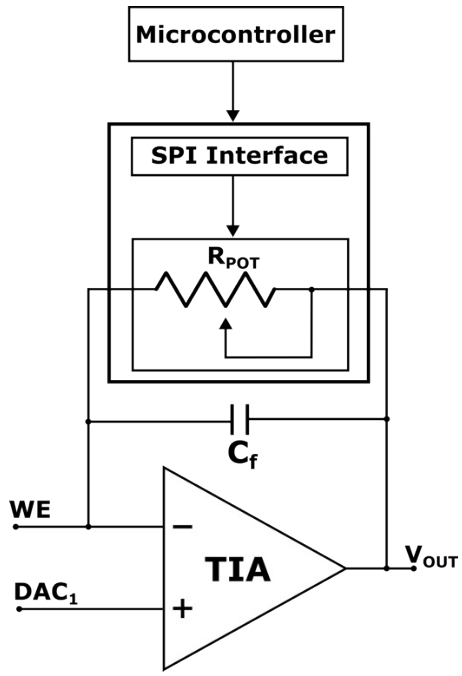

Fig. S1. Transimpedance amplifier and current gain circuitry.

S5 Calibration procedure of the instrument

Six step calibration procedure was applied to calibrate the EIS device. The procedure is shown in Fig. S2.

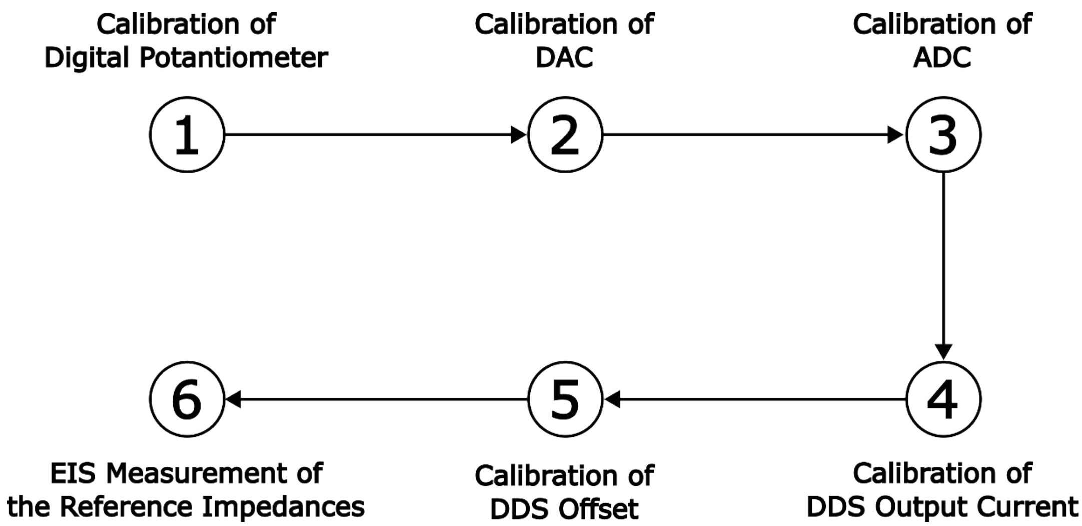

Fig. S2. Calibration procedure of the instrument.

S6 Electrochemical characterisation

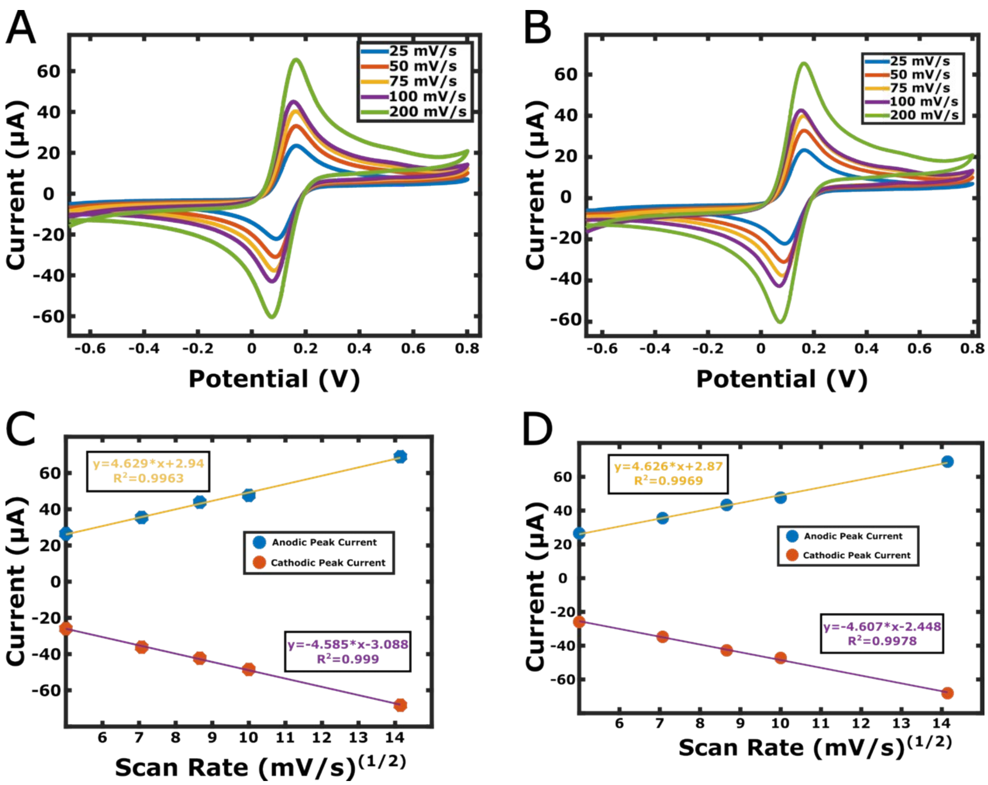

Fig. S3. Electrochemical characterization of the electrodes. A-B) CV measurements at different scan rates with our device and Palmsens-4, respectively. C-D) Relationship between peak current and square root of scan rate for our device and Palmsens-4, respectively.

Table S4. Randles equivalent circuit values.

	R_{sol} (Ohm)	$R_{charge\text{-}transfer}$ (Ohm)	W (Ohm $sec^{-1/2}$)	Q (Ohm^{-1} sec^{n})	n
Our Device	58.37	114.41	175.56	2.17	0.87
PS-4	58.29	108.15	187.53	2.05	0.88

S7 Electrode modification

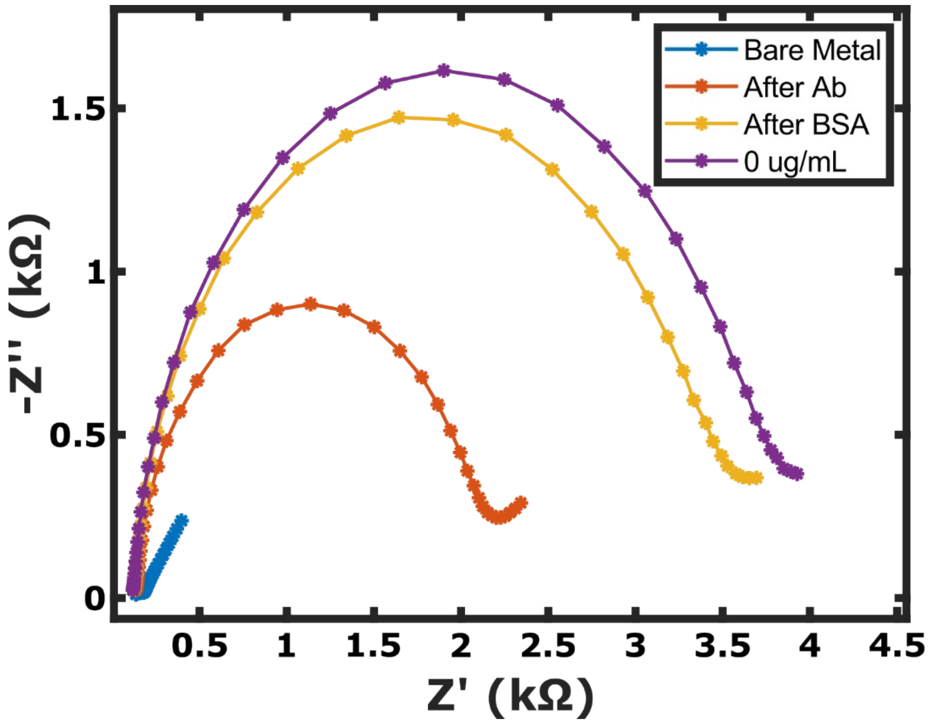

Fig. S4. Stepwise change after coating electrode surface.

Table S5. Change in Randles equivalent circuit parameters after each modification step.

Electrode	R_{sol} (Ω)	CPE		R_{CT}	W
		Q (μT)	n (Φ)	(kΩ)	(kσ)
Bare Metal	132.9	0.15	1.1	0.03	1.28
After antibody immobilisation	138.4	0.31	0.94	1900	1.47
After BSA immobilisation	122.9	0.34	0.93	3278	1.59

S8 Antibody immobilisation optimisation

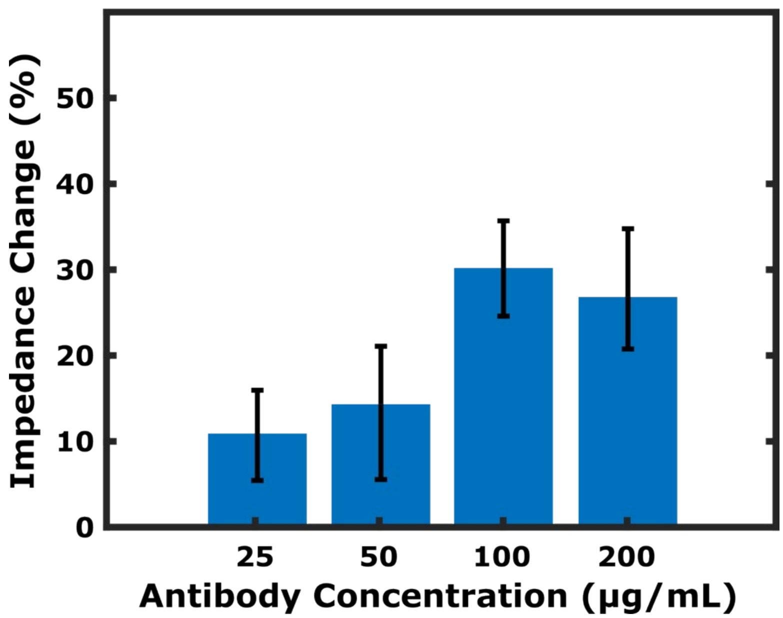

Fig. S5. Response of the sensor with different antibody concentrations.

S9 Response of the sensor with and without antibody

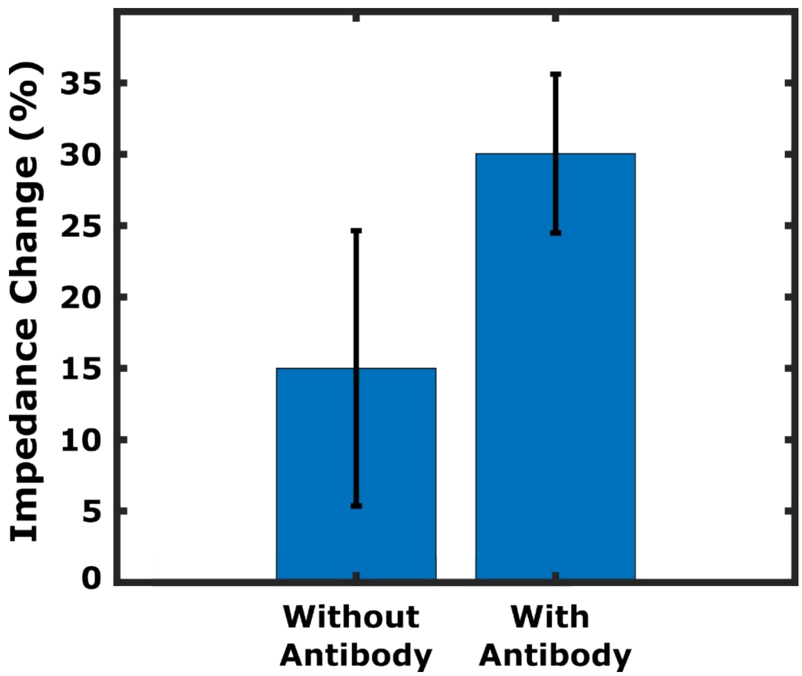

Fig. S6. 10 µg $mL^{-1}$of Hb was measured when surface was covered with and without antibody.

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