Title: Impedance spectroscopy measurements as a tool for distinguishing different luminal content during bolus transit studies.

Authors: A Ruiz-Vargas¹, R Mohd Rosli², A Ivorra^{3, 4}, J W Arkwright¹

¹The Medical Device Research Institute, College of Science and Engineering, Flinders University, Adelaide, Australia.

²Department of Gastroenterology and Surgery, Flinders Medical Centre, Adelaide, Australia.

³Department of Information and Communication Technologies, Universitat Pompeu Fabra, Barcelona, Spain.

⁴Serra Húnter Programme, Universitat Pompeu Fabra, Barcelona, Spain.

Correspondence: albert.ruiz@flinders.edu.au

13 Abstract

14 **Background:** Intraluminal electrical impedance is a well-known diagnostic tool used to
15 study bolus movement in the human oesophagus. However, its use in the human colon it
16 is hindered by the fact that the content cannot be controlled and may include liquid, gas,
17 solid or a mixture of these at any one time. This article investigates the use of complex
18 impedance spectroscopy to study different luminal content (liquid and gas).

19 **Methods:** An excised section of guinea pig proximal colon was placed in an organ bath
20 with Krebs solution at 37°C degrees and a custom built bioimpedance catheter was
21 placed in the lumen. Liquid (Krebs) and gas (air) content was pumped through the
22 lumen and the intraluminal impedance was measured at five different frequencies (1
23 kHz, 5.6 kHz, 31.6 kHz, 177.8 kHz and 1 MHz) at 10 samples per second. A numerical
24 model was created to model the passage of bolus with different content and compared to
25 the experimental data.

26 **Key Results:** Differences in mean impedance magnitude and phase angle were found
27 (from 1 kHz to 177.8 kHz) for different contents. The numerical results qualitatively
28 agreed with those in the experimental study. Conductivities of bolus had an effect on
29 detecting its passage.

Conclusions & Inferences: Complex impedance spectroscopy can distinguish between different luminal content within a range of measuring frequencies. The numerical model showed the importance of bolus conductivities for bolus transit studies in those where the bolus is controlled.

Keywords: Impedance spectroscopy, bolus transit, gastrointestinal motility, luminal content.

Key points

- Intraluminal electrical impedance is increasingly used to study bolus movement in the gastrointestinal tract. This article investigates the use of complex impedance spectroscopy to study different luminal content.
- The numerical model of the lumen qualitatively agreed with those in the experimental study. Complex impedance spectroscopy allowed greater differentiation between different luminal contents.
- This work will allow clinical GI researchers to gain insight into the mechanism of electrical impedance and achieve greater quantification in future bolus transit studies.

46 Introduction

47 The electrical impedance spectroscopy (EIS) method has been widely used in
48 biomedical applications ¹, electrochemistry ^{2,3} and food inspection ^{4,5}. The use of
49 impedance measurement in gastrointestinal studies is gaining popularity among
50 clinicians for studying bolus transit, motor patterns, and disorders along the
51 gastrointestinal tract ^{6,7}. Intraluminal impedance has been used in the human
52 oesophagus to record the transit of a controlled bolus, to measure the internal diameter,
53 and to predict risk of aspiration ⁸⁻¹⁰. In addition, it has been shown that impedance
54 measurements can be used to quantify the internal diameter in a rabbit colon in a
55 controlled in-vitro environment ¹¹. However, using impedance recordings to measure
56 transit in the human colon is not straightforward, as the content cannot be controlled and
57 may include liquid, gas, solid or a mixture of these at any one time.

58 Electrical impedance can be obtained at a single frequency but it can also be taken
59 across a range of frequencies. When this is done, it is referred to as impedance
60 spectroscopy. In living tissues, the impedance measurements will not be constant across
61 the range of frequencies. The variation in response is due to the electrolytic and
62 dielectric nature of biological tissue. Biological tissue can be thought of as cells with
63 dielectric membranes and junctions containing conductive fluid floating in a sea of
64 similar conductive extracellular fluid. At low frequencies the electric current
65 predominantly flows through the conductive extracellular liquid whereas at high

frequencies the cellular membranes act as the dielectric in a capacitor allowing current flow through both the extracellular fluid and the cells themselves.

Commercially available equipment (e.g. Solar GI HRM, Laborie/MMS, Canada and InSIGHT GI, Diversatek Healthcare/Sandhill Scientific, USA) being used in clinical environments only measure impedance magnitude and do not provide information on the real and imaginary components of impedance; hence important information of the nature of the gastrointestinal wall and surrounding tissue may be lost. Furthermore, the frequency used for impedance measurement is typically not provided by equipment manufacturers. The first report of gastrointestinal intraluminal impedance measurement by Silny, quoted a measuring frequency of 1 kHz¹². Since then, to our knowledge, frequency has not been reported in any study.

The aim of the present study is to determine whether impedance spectroscopy can be used to differentiate two different forms of luminal content (liquid and gas) in an excised section of guinea pig colon. We report an investigation into the spectral characteristics of intraluminal impedance measurements conducted in an in-vitro organ bath that provides insight into the effect of varying the frequency, and an associated numerical model. We provide recommendations for the best frequencies to use in different situations.

84 Materials and Methods

85 Catheter design

86 A custom designed four-electrode catheter was built for this experiment (Figure 1). Four
87 silver ring electrodes were assembled along a 3.5 mm diameter cylindrical piece of
88 PEEK (Polyether Ether Ketone). The electrodes were 1 mm in length and were
89 separated by 1 mm intervals. The cylinder containing the electrodes was then attached
90 to a length of PTFE (PolyTetraFlouroethelene) tube of 3 mm outer diameter (Figure 1).
91 The surface electrodes were roughed with sandpaper to minimize the tissue/electrolyte
92 interface impedance and to fix the half-cell potential that appear between metallic
93 conductors and biological tissue and which commonly cause impedance measurement
94 errors ¹³.

95 Equipment

96 Two different recordings were performed the experiments: impedance spectroscopy and
97 external diameter of segments determined from synchronous digital video imaging. This
98 allowed us to directly measure the bolus transit at different measuring frequencies,
99 compare them and correlate the impedance measurements with the external luminal
100 diameter.

A digital video camera (iPhone 6, Apple, USA), positioned above the preparation was used to record video images to create spatiotemporal maps of changes in diameter, referred to as 'Dmaps'¹⁴.

The four-electrode catheter was connected to a custom made bioimpedance measurement system (BIS), based on a Red Pitaya board (Redpitaya v1.1, Redpitaya, Slovenia), which is capable of measuring impedance from 10 Hz to 1 MHz¹⁵. The unit used a tetrapolar configuration for measuring the complex impedance; an alternating current with a maximum amplitude of 10 μ A was applied between the two outer electrodes at each predetermined measuring frequencies, and simultaneously the voltage drop between the inner electrodes was measured. The complex impedance at five different frequencies (from 1 kHz to 1MHz separated logarithmically) was calculated using the on-board processor of the Red Pitaya unit.; Impedance data was sent in real time to a custom software application built in LabVIEW (National Instruments, USA) to be saved and displayed. The impedance spectrograms were acquired at 10 samples per second which is an adequate sampling rate for oesophageal transit testing, in which bolus transit passes considerably faster than in the human colon, estimated by Bredenoord et al¹⁶.

[In vitro study](#)

A section of a proximal colon from a guinea pig was used. The section was removed from the laboratory animals by methods approved by the Animal Welfare Committee of

Flinders University (Animal Ethics Number: 845/12). The removed section of colon was placed into a beaker containing oxygenated Krebs solution (in mM: NaCl, 118; KCl, 4.7; NaH₂PO₄, 1.0; NaHCO₃, 25; MgCl₂, 1.2; D- Glucose, 11; CaCl₂, 2.5) and bubbled with 95% O₂/ 5% CO₂. The faecal pellets were gently flushed out of the colonic segment with Krebs solution.

The excised section of gut was then placed into an organ bath containing the same Krebs solution warmed to 37°C. Spontaneous gut contractile activities could be present for hours while within the organ bath.

In the organ bath the anal and oral ends of the proximal colon were attached to T-shaped plastic connectors (Figure 2). The catheter was passed through the T-shaped connector at the oral end and the vertical arm of the T-piece was connected to an infusion pump. In the first part of the experiment, warm Krebs (36 - 37°C) solution was infused at 2ml/min through the oral end of the specimen to stimulate contractile activity. In the second part of the experiment, ambient air was infused using the same infusion pump at the same infusion rate. In both instances, the experiment lasted for 60 minutes, and after initial recording of free flow through the gut, the anal end of the segment was blocked causing the gut to swell to large diameters around the four-electrode catheter.

Numerical study

The geometry of a gut segment model for simulating transit of different conductivity content along the segment was designed in Autodesk Inventor 2017 (Autodesk). The

segment model consists of a hollow tube (length = 150 mm) composed of eight regions: luminal content, mucus layer (thickness = 50 μm), mucosa layer (thickness = 900 μm), submucosa layer (thickness = 800 μm), circular muscularis layer (thickness = 450 μm), longitudinal muscularis layer (thickness = 450 μm), serosa layer (thickness = 50 μm) and surrounding tissue (thickness = 7 mm)¹⁷. A catheter (length = 150 mm; outer diameter = 3.5 mm) with four ring electrodes (length = 1 mm; outer diameter = 3.5 mm) was positioned on the axis of the tube. Then, a specific shape bolus (Figure 3.a) with outer diameter of 10 mm was created. Twenty-six different static models were created changing the bolus position by 2.5 mm each time to simulate bolus propulsion and the impedance was simulated for each bolus location using the DC electrical conduction component of the ANSYS Finite Element Method (FEM) software platform (ANSYS Electronics desktop, ANSYS Inc, USA). Each simulation was carried out on 12 CPUs Intel® Xeon® CPU E5 – 2630 v2 running at 2.60 GHz with 128 GB of RAM, with OS Windows 8.1 Enterprise. The models were solved using 3D models method. The meshes used in the FEM simulations varied from model to model, but were composed from 465569 to 6329027 tetrahedral elements, depending on the complexity.

The numerical values for electrical properties of different layers of the tissue at 1 kHz were selected from¹⁷, and electrical properties for the catheter tube and electrodes were selected from the Material Library provided in ANSYS Electronics. The properties of the layers are: surrounding tissue ($\sigma = 0.148 \text{ S/m}$ $\epsilon_r = 1.97 \times 10^5$), serosa ($\sigma = 0.383 \text{ S/m}$

$\epsilon_r = 3.03 \times 10^5$), longitudinal muscularis ($\sigma = 0.5 \text{ S/m}$ $\epsilon_r = 1 \times 10^6$), circular muscularis ($\sigma = 0.321 \text{ S/m}$ $\epsilon_r = 4.35 \times 10^5$), submucosa ($\sigma = 0.383 \text{ S/m}$ $\epsilon_r = 3.03 \times 10^5$), mucosa ($\sigma = 0.532 \text{ S/m}$ $\epsilon_r = 2.64 \times 10^5$), and mucus ($\sigma = 1.1 \text{ S/m}$ $\epsilon_r = 72$). Four different conductivity luminal contents from 0.1 S/m to 1.44 S/m for conductivity (0.1 S/m, 0.5 S/m, 1 S/m and 1.44 S/m) and 1 to 80 for relative permittivity (1, 80, 80 and 80), respectively were used, to simulate content ranging from mixed of humid gas with mucus to liquid.

An excitation current of 10 μA was modeled between the outer electrodes, representing the maximum current that the bioimpedance measurement system can provide, and, for the calculations below, the voltage difference between the inner electrodes was measured.

The sensitivity distribution of the tetrapolar electrode system was also simulated within the lumen to define the region of measurement when lumen is empty and when a bolus is passing through. Sensitivity is define as the contribution of a small volume to the total measured impedance. Hence the sensitivity and the total measured impedance is given as: ¹⁸,

$$S = \frac{\vec{J}_1 \cdot \vec{J}_2}{I^2} \quad \text{and} \quad Z = \int_V \rho \cdot S \, dv$$

Where J_1 is the current density vector when current I is injected between the outer electrodes, J_2 is the current density vector when current I is injected between the inner electrodes, and ρ is the resistivity of the medium. Sensitivity can be positive, zero or

negative depending on the dot product of the two vectors (J_1 and J_2). If the two vectors have an angle between them lower than 90 degrees, then S is positive, if the angle is bigger than 90 degrees it is negative, and if it is equal to 90 degrees then S is zero. A positive S means that an increased in resistivity of the small volume will have an increment of the total measured impedance, whereas, a negative S means that an increment of the resistivity will decrease the total measured impedance. A zero S has no influence on the total measured impedance. The higher the absolute value of S , the greater the influence on the total measured impedance.

Statistical analysis

A set of 1500 samples of free flow –which corresponds about 5 minutes of recording data – through the gut for each content were used for statistics. Examination of the normality was tested by Shapiro-Wilks test. A non-parametric Wilcoxon test was used to compare mean difference between contents for impedance magnitude data phase angle at five different measuring frequencies (1 kHz, 5.6 kHz, 31.6 kHz, 177.18 kHz and 1MHz). Correlation between the whole recordings of impedance magnitude and phase angle against luminal diameter were compared using Pearson's correlation coefficient (PCC). In all instances, p -values were less than 0.05 indicating a strong correlation existed. Statistical analysis were carried out using SPSS Statistics, version 23 (IBM Corp., Armonk, NY).

199 Results

200 Impedance Sensitivity

201 Figure 3b and c shows the sensitivity distribution of the catheter within the simulated
202 lumen when is empty and when bolus is passing through, respectively. In both
203 distributions negative sensitivity were found between the outer and inner electrodes, and
204 positive sensitivity were found between inner electrodes. This accords with the
205 simulated tetrapolar system model from Grimnes¹⁸. When the lumen is empty, positive
206 sensitivity reaches to the mucosa and submucosa whereas when the bolus is passing
207 through the positive sensitivity reaches about 2 cm (not getting to the mucosa in this
208 case).

209 A bigger surface of positive sensitivity can be seen in figures 3b and 3c, and this can be
210 seen in the experimental data and in the simulated lumen since an increase in resistivity
211 there was an increase in the total measured impedance. Although, when different
212 conductive boluses (liquid equals to lower resistivity and gas equals to higher
213 resistivity) pass through there is an increment in total impedance for gas but a decrease
214 for liquid. This could be explained as the resistivity, when lumen is in normal condition,
215 is higher due to the overall resistivity of content, mucus, mucosa and submucosa, and
216 when the bolus passes through the resistivity is lower since the overall resistivity is due
217 to the content.

218 Numerical and in vitro study

219 In steady state, where a continuous flow of Krebs is going through the model and small
220 changes in diameter occur, there is a baseline of impedance magnitude that stays stable
221 with little variance. Figure 4 (top graph) shows the baseline of impedance magnitude of
222 the continuous flow and the passage of the bolus for different content conductivities in
223 the simulation. The passage of the bolus travels from left to right (Figure 3a). The
224 impedance magnitude baseline range (when there is a continuous flow of Krebs) goes
225 from 48.80 ohms to 63.80 ohms for higher conductive content (liquid) to lower
226 conductive content (air moisture), respectively. Similarly, the experimental data of both
227 bolus types (liquid and gas) are shown in the lower graph of figure 4. The impedance
228 magnitude baseline, when continuous flow of Krebs or gas, range for liquid is 34.95
229 ohms and for gas is 45.00 ohms.

230 Impedance spectroscopy

231 Table 1 shows the descriptive and the summary statistics for the difference between
232 impedance magnitude and phase angle between content at each measuring frequency.
233 There were significant differences between content for impedance magnitude and phase
234 angle means except for impedance magnitude at 1 MHz.

235 The impedance magnitude means for liquid content (34.9, 30.5, 28.8, 25.5 and 18.1 at
236 different frequencies from 1 kHz to 1 MHz) were lower than for gas content (48.4, 40.9,
237 37.2, 30.3 and 18.3). The Wilcoxon matched-pairs test showed that the difference

between means of contents at frequency range 1 kHz to 177.81 kHz for impedance magnitude (13.5, 10.4, 8.4 and 4.8 respectively) were significant beyond 0.001 level: asymptotic $p < 0.001$ (two-tailed). The matched-pairs rank biserial correlation are (0.98, 0.98, 0.98 and 0.9, respectively) which are 'large' effect. No significant difference of means in impedance magnitude at 1 MHz between contents was evident (asymptotic $p = 0.485$).

Similarly, the phase angle means for liquid content (-11.5, -5, -5.4, -11.2 and -19.8 at different frequencies from 1 kHz to 1 MHz) were lower than for gas content (-12.1, -6.8, -8.3, -16.8 and -24.5). The Wilcoxon matched-pairs test showed that the difference between means of contents at frequency range 1 kHz 1 MHz for phase angle (0.6, 1.8, 2.9, 5.6 and 4.7 respectively) were significant beyond 0.001 level: asymptotic $p < 0.001$ (two-tailed). The matched-pairs rank biserial correlation are (0.26, 0.98, 1.00, 1.00 and 0.89), which r values between 0.1 and 0.3 have a 'small' effect, and above 0.5 have a 'large' effect.

The difference between median and mean is small for liquid whereas for gas that difference is bigger at lower frequencies and lower at bigger frequencies, table 1. Further, for impedance magnitude measurements, no overlap in interquartile range (IQR) was observed between mediums over the frequency range (1 kHz – 177.81 kHz), however, some overlap was seen at 1 MHz (Figure 5). Standard deviation and IQR for impedance magnitude are smaller for liquid than for gas content.

For phase angle, no difference between median and mean was observed for liquid and gas content, table 1. Conversely, the phase angle measurements did not overlap in the IQR at higher frequencies, but did at the lowest frequency. No overlap in IQR between mediums were observed at the frequency range (5.62 kHz – 1 MHz, Figure 5). Further, no difference between IQR and standard deviation for liquid and gas content were observed. In both instances, for impedance magnitude and phase angle, outliers are present. The causes of these outliers are explained in the discussion section.

Impedance spectroscopy and Dmaps

Table 2 shows the correlations and 95% confident intervals between the impedance magnitude and phase angle against the measured diameter with for the two extreme types of content. All Pearson correlation were all significant different beyond 0.001 level ($p\text{-value} < 0.001$). The impedance magnitude is negatively correlated for liquid (coefficient range: -0.78 – -0.12 being the highest at 5.62 kHz), whereas for gas the correlation is positive (coefficient range: 0.47 – 0.39). Conversely, phase angle correlation for liquid is positive (coefficient range: 0.87 – 0.58 being the highest at 177.81 kHz, Figure 6) and for gas is negative (coefficient range: -0.48 – -0.04). Liquid content gives better correlation coefficients than gas content. The correlation coefficients were stronger for phase angle than impedance magnitude.

276 Discussion

277 This present study has demonstrated, for the first time, that complex impedance
278 spectroscopy can be useful to differentiate between liquid and gas media inside the
279 colon. Differences between mean values between intra-luminal content were found
280 significant (Table 1). For example, the difference between impedance magnitudes of
281 contents is larger at low frequency (1 kHz), and decreases at higher frequencies (up to
282 177.81 kHz). Contrarily, the difference between phase angles of contents is larger at the
283 highest frequency (1 MHz) and decreases at lower frequencies (to 5.6 kHz).

284 In addition, IQR and standard deviation for impedance magnitude of liquid are at least
285 two times smaller than for gas content. This makes sense as the conductivity of the
286 mixed gas varies more than the conductivity of liquid that is constantly flowing through
287 the specimen. Three reasons could explain this: a.) when gas is passing through the
288 specimen, this is mixed with moisture and mucus but these are not homogenously
289 distributed and b.) the catheter may touch the walls due to gravity, affecting the
290 measurement as well, c.) similarly, a few small bubbles were seen in the liquid bolus,
291 which could alter the impedance readings, however this effect was less than seen with
292 gas. This could also be the reason for the outliers in the graphs.

293 The difference in impedance magnitude at different measuring frequencies shows that
294 dielectric materials are involved in the measurements during bolus transit studies. As
295 the content medium (Krebs) is pure electrolytic, this behaviour can only come from the

luminal wall. This decrease in impedance, when the frequency increases, is also seen in a study in pigs for monitoring ischemic injury in the gastric mucosa performed by Cesar Gonzalez¹⁹.

The correlation between impedance magnitude and diameter partially agrees with previous values calculated by Costa et al¹¹, however we cannot directly compare these data due to the unknown measuring frequency used by their system, the differences in the geometry of electrodes, and the two element configuration used, compared to the 4 element configuration used in this work. A strong correlation between changes in phase angle and external diameter was shown.

The numerical model allowed us to further understand the bolus passage with different content media (conductivities). The numerical data were compared with the two big boluses created by clamping the end (liquid and gas bolus diameter were approximately 6 mm and 7.5 respectively mm). Bolus media with high conductivity (1 S/m and 1.44 S/m) had the same trend (a decrease in impedance magnitude) as the liquid bolus from experimental data. Similarly, bolus mediums with low conductivity (0.1 S/m) had the same trend (an increase in impedance magnitude) as the gas bolus from the experimental data, these trends are the same as those shown in paper²⁰. The total change in both instances are lower in the experimental data but as mentioned before the diameter of the bolus did not reach the 10 mm used in the simulation. Furthermore, the

shape of the bolus in the simulation stays constant unlike in the experimental data in which the bolus shapes could vary with the spontaneous propagating contractions.

The impedance magnitude baseline from the numerical data (1.44 S/m had 48 ohms and 0.1 S/m had 61 ohms) were slightly higher than the mean from the experimental data at 1 kHz (mean impedance magnitude for liquid and gas were 34 ohms and 51 ohms, respectively). That could be explained as the temperature effect on conductivity was not included in the simulations. Temperature increases conductivity of materials. For example, the conductivity of 0.9% NaCl at 25 degree is 1.44 S/m but at 37°C, temperature at which the experiments were carried out, is 2 S/m¹³.

When a high conductivity bolus passes the impedance sensor, the impedance magnitude drops, while for low conductive bolus, the impedance magnitude goes up. The closer the conductivity of the medium gets to the conductivities of the luminal wall the less change occurs. The sensitivity of the overall impedance sensor is related to the conductivity of the content, the higher the conductivity of the content, the higher the sensitivity of the sensor. Hence, where possible it is advisable to use a bolus with the greatest contrast to that of the luminal wall. This is of direct interest to studies where a controlled bolus can be used, except that any bolus used in the oesophagus must also be palatable to the subject.

With regard to this study, some limitations need to be considered. First, only one specimen was used for this preliminary study. The strong agreement with our FEM

simulation gives us confidence in our results. Second, the lack of information on electrical and mechanical properties of faecal content made it impossible to generate a suitable analog material for semi-solid faeces, hence only two contents (liquid and gas) were used in this preliminary study. And lastly, the minimum measuring frequency was limited to 1 kHz as using measuring frequencies below this would affect the sample frequency.

To conclude, we observed that the impedance measurements recorded during the passage of a liquid or gas bolus are frequency dependent due to the electrical properties of the luminal wall. Given the differences observed in complex impedance spectroscopy between liquid and gas, a good frequency range is 5.62 kHz to 177.81 kHz, depending on what parameter (impedance magnitude or phase angle) is used for the analysis. Frequencies closer to 5.62 kHz are recommended for impedance magnitude, closer to 177.81 kHz are recommended for phase angle and/or a median frequency around 100 kHz if both parameters are to be taken into account. Lastly, increasing the conductivity of the bolus content with respect to the conductivity of the mucosa will increase the sensitivity of the impedance sensor.

Author Contribution

AR-V built the catheter and the impedance measurement system, designed the research study, assisted with the experimental study, performed data analysis, created the figures,

354 and wrote the manuscript; RMR carried out the experimental study, proof read the
355 manuscript; AI contributed to design of the catheter and of the impedance measurement
356 system and proof read the manuscript; JWA contributed to the catheter design, designed
357 the research study, assisted with data analysis and interpreting the results, and edited the
358 manuscript.

359 [Conflict of Interest](#)

360 None of the authors have any conflict of interest.

361 Tables

		Liquid				Gas						
	Frequency	Median	IQR	Mean	Std	Median	IQR	Mean	Std	Mean difference	p-values	Effect size
Impedance magnitude	1 kHz	34.3	5	34.9	3.6	44.9	12.5	48.4	10.1	-13.6	p<0.001	r = 0.98
	5.6 kHz	30.2	3.9	30.5	2.8	38.3	8.8	40.9	7.3	-10.3	p<0.001	r = 0.98
	31.6 kHz	28.6	3.4	28.8	2.4	35	7.3	37.2	6.1	-8.4	p<0.001	r = 0.98
	177.8 kHz	25.3	2.6	25.5	1.9	28.7	5.4	30.3	4.6	-4.8	p<0.001	r = 0.90
	1 MHz	17.7	1.5	18.1	1.8	17.2	3.7	18.3	3.1	-0.3	p=0.485	r = 0.02
Phase angle	1 kHz	-11.4	1.6	-11.5	1.1	-11.9	2	-12.1	1.6	0.6	p<0.001	r = 0.26
	5.6 kHz	-4.9	0.7	-5	0.5	-6.6	1.5	-6.8	1	1.8	p<0.001	r = 0.98
	31.6 kHz	-5.4	1.2	-5.4	0.8	-8.3	1.3	-8.3	0.9	2.9	p<0.001	r = 1.00
	177.8 kHz	-11	2	-11.2	1.4	-16.8	1.2	-16.8	0.9	5.6	p<0.001	r = 1.00
	1 MHz	-19.5	3.3	-19.8	3.3	-24.5	2.3	-24.5	2.7	4.7	p<0.001	r = 0.89

362 Table 1. Mean, median, IQR and standard deviation of impedance magnitude and phase angle for liquid and gas bolus.

363

		1 kHz		5.6 kHz		3.16 kHz		177.80 kHz		1 MHz	
Liquid	Magnitude	-0.77	-0.78 -0.76	-0.78	-0.79 -0.77	-0.75	-0.76 -0.74	-0.66	-0.67 -0.65	-0.12	-0.13 -0.11
	Phase	0.83	0.83 0.82	0.82	0.82 0.81	0.74	0.75 0.73	0.87	0.87 0.87	0.55	0.56 0.54
Gas	Magnitude	0.47	0.48 0.46	0.46	0.47 0.45	0.45	0.46 0.44	0.44	0.45 0.43	0.38	0.39 0.37
	Phase	-0.41	-0.42 -0.40	-0.48	-0.49 -0.47	-0.33	-0.34 -0.32	-0.17	-0.18 -0.16	-0.04	-0.05 -0.03

364 Table 2. Pearson correlation coefficients and 95% confidence intervals for magnitude and phase angle of liquid and gas against diameter of the specimen. All p- values for each
365 correlation were found significant (p-value <0.001).

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433

434

Figure 1. 3D model of impedance catheter used for the experiments and numerical study.

Figure 2: Experimental set up.

(a)

(b)

(c)

441

442 **Figure 3. Gastrointestinal model. (a) Lateral cross-section view of the 3D gastrointestinal tract model with**
 443 **electrodes placed in the middle. Bolus travels from left to right mimicking the passage of a bolus along the**
 444 **specimen with all its layers from inside to outside (mucosa, submucosa, circularis muscularis, longitudinal**
 445 **muscularis, serosa and surroundings). (b) and (c) Sensitivity distributions of the catheter in the simulated**

446 lumen when no presence and presence of bolus, respectively.

447

448 **Figure 4. Top graph shows impedance magnitude of bolus passage for four different luminal content (from**
 449 **1.44 S/m to 0.1 S/m, from liquid to a gas content) at 1 kHz. Bottom graph shows the impedance magnitude of**
 450 **bolus passage of liquid content at 1 kHz.**

451

452 **Figure 5. Box plots of impedance magnitude (top) and phase angle (bottom) for different content at different**

453 **measuring frequencies. Star points represent the mean of the data set.**

Figure 6. Correlation graph of phase angle against diameter at 177.81 kHz for a liquid sample.

458 Appendix

459 Normality test

460 Impedance magnitude

Tests of Normality

	Shapiro-Wilk		
	Statistic	df	Sig.
F1_Liquid	0.963	1501	0.000
F1_Gas	0.882	1501	0.000
F2_Liquid	0.966	1501	0.000
F2_Gas	0.880	1501	0.000
F3_Liquid	0.971	1501	0.000
F3_Gas	0.876	1501	0.000
F4_Liquid	0.971	1501	0.000
F4_Gas	0.868	1501	0.000
F5_Liquid	0.724	1501	0.000
F5_Gas	0.802	1501	0.000

461

462 Phase angle

Tests of Normality

	Shapiro-Wilk		
	Statistic	df	Sig.
F1_PA_Liquid	0.964	1501	0.000
F1_PA_Gas	0.958	1501	0.000
F2_PA_Liquid	0.978	1501	0.000
F2_PA_Gas	0.973	1501	0.000
F3_PA_Liquid	0.994	1501	0.000
F3_PA_Gas	0.997	1501	0.004
F4_PA_Liquid	0.979	1501	0.000
F4_PA_Gas	0.998	1501	0.086
F5_PA_Liquid	0.887	1501	0.000
F5_PA_Gas	0.879	1501	0.000

463

464

465 Wilcoxon Signed Ranks Test for impedance magnitude

Ranks

		N	Mean Rank	Sum of Ranks
F1_Gas - F1_Liquid	Negative Ranks	79 ^a	161.59	12766.00
	Positive Ranks	1422 ^b	783.74	1114485.00
	Ties	0 ^c		
	Total	1501		
F2_Gas - F2_Liquid	Negative Ranks	65 ^d	158.00	10270.00
	Positive Ranks	1436 ^e	777.84	1116981.00
	Ties	0 ^f		
	Total	1501		
F3_Gas - F3_Liquid	Negative Ranks	78 ^g	177.42	13839.00
	Positive Ranks	1423 ^h	782.44	1113412.00
	Ties	0 ⁱ		
	Total	1501		
F4_Gas - F4_Liquid	Negative Ranks	166 ^j	344.61	57205.00
	Positive Ranks	1335 ^k	801.53	1070046.00
	Ties	0 ^l		
	Total	1501		
F5_Gas - F5_Liquid	Negative Ranks	859 ^m	669.81	575367.00
	Positive Ranks	642 ⁿ	859.63	551884.00
	Ties	0 ^o		
	Total	1501		

a. F1_Gas < F1_Liquid

b. F1_Gas > F1_Liquid

c. F1_Gas = F1_Liquid

d. F2_Gas < F2_Liquid

e. F2_Gas > F2_Liquid

f. F2_Gas = F2_Liquid

g. F3_Gas < F3_Liquid

h. F3_Gas > F3_Liquid

i. F3_Gas = F3_Liquid

j. F4_Gas < F4_Liquid

k. F4_Gas > F4_Liquid

l. F4_Gas = F4_Liquid

m. F5_Gas < F5_Liquid

n. F5_Gas > F5_Liquid

o. F5_Gas = F5_Liquid

466

467

Test Statistics^a

	F1_Gas - F1_Liquid	F2_Gas - F2_Liquid	F3_Gas - F3_Liquid	F4_Gas - F4_Liquid	F5_Gas - F5_Liquid
Z	-32.798 ^b	-32.946 ^b	-32.734 ^b	-30.152 ^b	-.699 ^c
Asymp. Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.485

a. Wilcoxon Signed Ranks Test

b. Based on negative ranks.

c. Based on positive ranks.

468

469 Wilcoxon Signed Ranks Test for phase angle

Ranks

		N	Mean Rank	Sum of Ranks
F1_PA_Gas - F1_PA_Liquid	Negative Ranks	872 ^a	818.56	713781.00
	Positive Ranks	629 ^b	657.34	413470.00
	Ties	0 ^c		
	Total	1501		
F2_PA_Gas - F2_PA_Liquid	Negative Ranks	1436 ^d	779.67	1119603.00
	Positive Ranks	65 ^e	117.66	7648.00
	Ties	0 ^f		
	Total	1501		
F3_PA_Gas - F3_PA_Liquid	Negative Ranks	1487 ^g	757.84	1126914.00
	Positive Ranks	14 ^h	24.07	337.00
	Ties	0 ⁱ		
	Total	1501		
F4_PA_Gas - F4_PA_Liquid	Negative Ranks	1500 ^j	751.50	1127250.00
	Positive Ranks	1 ^k	1.00	1.00
	Ties	0 ^l		
	Total	1501		
F5_PA_Gas - F5_PA_Liquid	Negative Ranks	1366 ^m	781.28	1067234.00
	Positive Ranks	135 ⁿ	444.57	60017.00
	Ties	0 ^o		
	Total	1501		

a. F1_PA_Gas < F1_PA_Liquid

b. F1_PA_Gas > F1_PA_Liquid

c. F1_PA_Gas = F1_PA_Liquid

d. F2_PA_Gas < F2_PA_Liquid

e. F2_PA_Gas > F2_PA_Liquid

f. F2_PA_Gas = F2_PA_Liquid

g. F3_PA_Gas < F3_PA_Liquid

- h. F3_PA_Gas > F3_PA_Liquid
- i. F3_PA_Gas = F3_PA_Liquid
- j. F4_PA_Gas < F4_PA_Liquid
- k. F4_PA_Gas > F4_PA_Liquid
- l. F4_PA_Gas = F4_PA_Liquid
- m. F5_PA_Gas < F5_PA_Liquid
- n. F5_PA_Gas > F5_PA_Liquid
- o. F5_PA_Gas = F5_PA_Liquid

470

Test Statistics^a

	F1_PA_Gas - F1_PA_Liquid	F2_PA_Gas - F2_PA_Liquid	F3_PA_Gas - F3_PA_Liquid	F4_PA_Gas - F4_PA_Liquid	F5_PA_Gas - F5_PA_Liquid
Z	-8.940 ^b	-33.102 ^b	-33.538 ^b	-33.558 ^b	-29.984 ^b
Asymp. Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000

a. Wilcoxon Signed Ranks Test

b. Based on positive ranks.

471