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A Study on Broadband Electrical Properties of *E. Coli* Bacteria Suspension for Varied Concentration

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Abstract

In this paper, an experimental study is conducted to assess the electrical properties of *E. Coli* bacteria suspended in phosphate-buffered saline. A direct broadband dielectric spectroscopy utilizing a two-wire sensor is applied to derive permittivity data which depends on the bacteria concentration. In such approach, the bacteria suspension constitutes full the propagation medium while the dielectric properties are averaged within a larger (sub-ml) volume of liquid which is favourable for heterogenous mixtures. A set of bacteria samples of known concentrations was prepared for the experiment along with a dedicated sensor optimized for measuring liquids of high permittivity and loss being developed. A measurement setup was assembled, and measurements were carried out with controlled and stable environment conditions. The obtained results are crucial for the development of novel highly sensitive and cost-effective microwave biosensors.

1. Introduction

Escherichia coli is a bacterium commonly found in the lower intestine of warm-blooded organisms [1]. These bacteria are mostly harmless or even beneficial to humans, however some serotypes are pathogenic, causing serious food poisoning in their hosts [2]. It is transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products, raw milk, and contaminated raw vegetables and sprouts [3]. It must be underlined pathogenic *E. coli* bacteria can cause a wide variety of diseases due to the toxins they produce, leading even to death. In such a case rapid diagnosis and treatment of infectors is considered crucial for global public health protection.

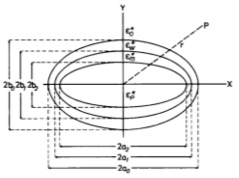
Traditional microbial culture-based tests are the most common methodologies used currently [4]. Usually these methods involve cell culture, cell counts, and cell enrichment, however, this process is time consuming and laborious. Moreover, the results obtained by these techniques can only help to make the circle of search narrower. It must be underlined that they do not guarantee that the strain has been precisely identified. Therefore, it is crucial to develop new diagnostic methods allowing for fast, reliable and precise microorganism identification.

Recently biosensors [5]-[9] are of great interest for bacteria detection, among which microwave biosensors feature a good trade-of between high sensitivity to bacteria and cost of sensing element production. Nevertheless, there is a strong need to investigate novel microwave biosensors realizations allowing to increase sensitivity on significantly low number of bacteria featuring concentration of 100 Colony Forming Unit per milliliter (CFU/ml) and below [10].

In this paper, an experimental study is conducted to determine how the dielectric properties of *E. coli* suspended in Phosphate buffered saline (PBS) change dependent on the bacteria concentration. Importantly, PBS is a buffer solution, which is used to preserve bacteria for longer time for further evaluation. Thus, to sense the bacteria using a microwave biosensor, one must first evaporate the liquid first which is yet another pre-processing step that could potentially be skipped. On the other hand, since PBS is a water-based salt solution it features high permittivity and losses, and so low concentration suspensions and solutions are difficult to measure. Therefore, a dedicated sensor was designed, optimized for measurement of liquids featuring high permittivity well suited for heterogenous mixtures. A two-wire (TW) sensor based on the direct dielectric spectroscopy (BDS) technique is used to derive data, which are used to determine permittivity dependent on bacteria concentration. A set of bacteria samples of known concentrations was prepared and measured. The obtained results show that various concentration of *E. coli* suspended in PBS feature very similar properties to the ones of pure PBS yet can be differentiated based on the measurement of sensors reflection coefficient in a wide frequency range due to fairy high power level and low dynamic range of changes.

2. Properties of *E. coli* and suspension

The *E. coli* bacterium cell has a rod shape with a diameter of 0.68 μm and length of 2.79 μm [11]. Its electrical properties can be evaluated based on a three-shell structure model shown in Fig.1 composed of protoplasm, membrane and cell wall. Along, the relative permittivity values and bulk conductivity [12] are listed. The model is defined at lower frequencies around 1 MHz, thus the dielectric properties of *E. coli* at higher frequencies might deviate from the quasi-static ones. Nevertheless, the low frequency effective permittivity of a single bacteria is in the range of 60 with relatively high losses resulting from fairly high conductivity.



(a)

	Relative permittivity	Bulk conductivity (Sm^{-1})
Protoplasm	67	0.17
Membrane	9.5	5e−8
Cell wall	60	0.73

(b)

Figure 1. Three-shell model of an *E. coli* bacteria [12] (a) with relative permittivity and bulk conductivity values: ε_p^* complex dielectric constant of protoplasm, ε_m^* complex dielectric constant of ε_w^* complex dielectric constant of cell wall (b).

On the other hand, for biosensor application, *E. coli* is mostly suspended in PBS, which is a water-based solution featuring pH ~ 7.4. Due to the water and salt content such a solution features high permittivity and high losses in the water range. Moreover, due to the random suspension of bacteria, as well as very often small amount of specimen in such heterogenous solutions, fairy high permittivity of the suspension with very little variation is expended. Therefore, most of the microwave sensors, such as industry standard broadband open coaxial probe method, are not suitable for high resolution sensing of volume averaged properties. Thus, a dedicated microwave sensor is tested for this purpose.

3. Microwave Sensor design and fabrication

To evaluate experimentally the *E. coli* bacteria suspensions, an exemplary measurement setup was developed. The volumetric microwave sensor was designed based on the idea presented in [13]. In such a case, the liquid-under-test constitutes fully propagation medium while the dielectric properties are averaged within a larger (sub-ml) volume of liquid which is favorable for heterogenous mixtures. The sensor was realized using a 0.5 mm thick Rogers RO4003C laminate that hosts a 0.8 mm diameter copper-core silver-cladding wire on each side that constitutes the two-wire line. Wire length was set a 10 mm considering that high permittivity-high loss liquids are to be measured, the permittivity range and frequency bandwidth product is directly impacted by the lengths [13]. To provide better field matching in the on-PCB transition region from a double-sided parallel-strip (DSPS) to two-wire (TW) transmission line a taper was added, as shown in Fig. 2. In [13] it was shown that when the wire diameter is much narrower than the DSPS line, a field distribution changes abruptly. Also, a coplanar waveguide section with tapered profile was added to accommodate an end-launch coaxial connector. Considering that a 3D printed seal is to be added, the impedance rise due to a short taper added to the DSPS line is reduced by increasing effective permittivity in the region. Therefore, one of the error sources as discussed in [13] is minimized. To complete the setup, the liquid chamber was designed that has an inner diameter of 6 mm and holds approximately 1.1 mL of LUT. Chamber`s wall thickness of 1.6 mm was set, and each half of the 3D model was split into two subparts at 2 mm height from the PCB interface. The bottom one serves as a compressible seal while the top one is a rigid clamp. A set of screws were used to attach the two halves with a PCB in-between and torqued, so the seal is compressed providing leak-proofing. The PCB was fabricated using the LPKF ProtoLaser U4 system and TW sensor was soldered to anchor points (metal pads close to the PCB cut-out). Chamber parts were 3D printing using the Original Prusa i3 MK4 fused filament fabrication printer out of flexible TPU (Thermoplastic Polyurethane) and rigid PET G (Poly (Ethylene Terephthalate-1, 4-Cylclohexylenedimethylene Terephthalate)) filaments that are non- or hardly reactive with the intended test liquids. Photographs of the manufactured sensing setup are shown in Fig. 3. On top of the above, a THRU standard was fabricated necessary for de-embedding the coaxial-to-TW transitions enabling determination of the liquid-under-test relative permittivity.

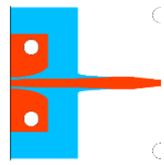
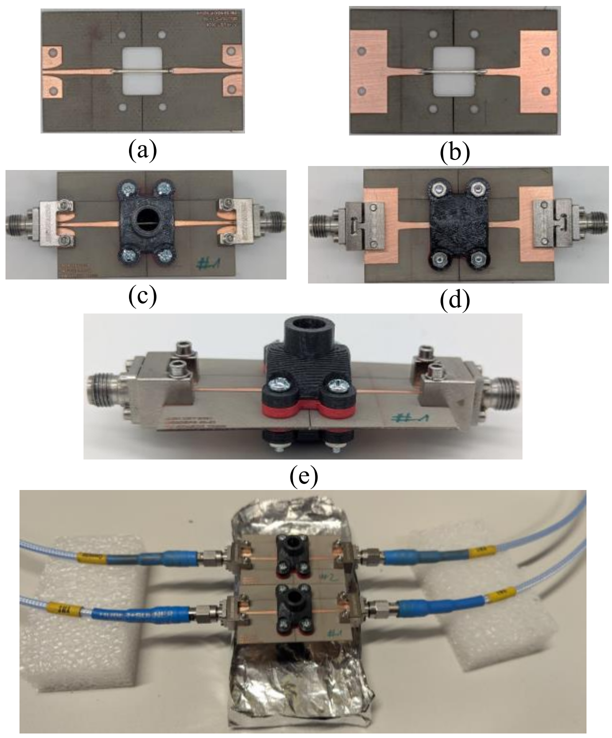


Figure 2. Layout of the coaxial-to-TW transition used to feed the TW sensor.

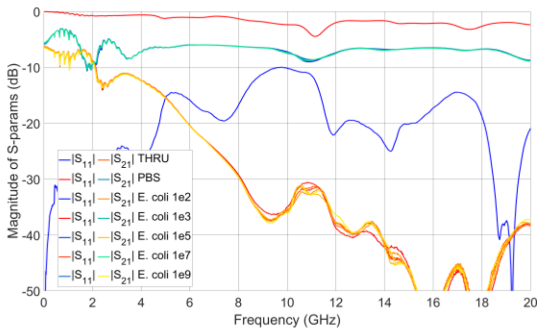


(a) (b) (c) (d) (e) (f)

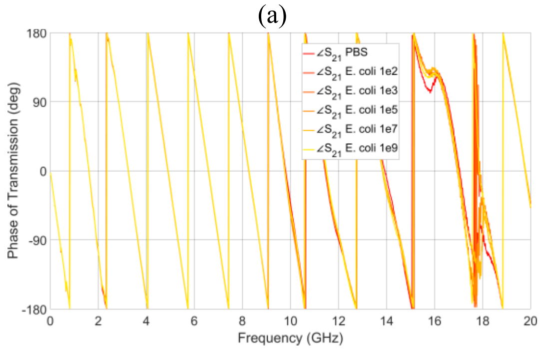
Figure 3. Photographs of the manufactured sensor with consecutive assembly steps: top view (a), (c) and bottom view (b), (d). Side view of the sensor (e) and overview of the experimental setup (f).

4. Experimental results

For the sake of this study, a bacteria PBS suspension test set having well-defined *E. coli* concentrations of 0 CFU/ml (pure PBS), 10^2 CFU/ml, 10^3 CFU/ml, 10^5 CFU/ml and 10^7 CFU/ml was used. The samples were prepared in laboratory conditions, their concertation was established using reference method and strict protocol was followed regarding their transportation, storage and use in the experiment. Preparation, sensor exposition to bacteria and measurements were carried out in laboratory setting with all safety measures in hand. All steps were done at room temperature. The electrical response of the TW sensor was measured using the Agilent PNA N224A vector network analyser (VNA) in the 10 MHz to 20 GHz frequency range.



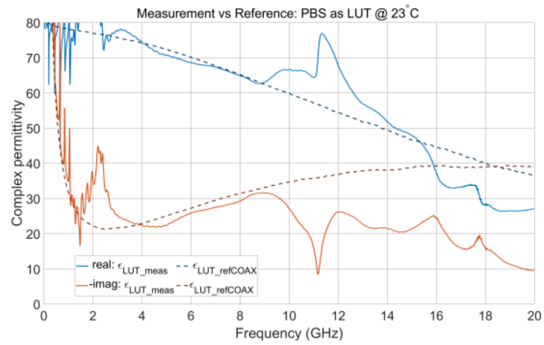
(a)



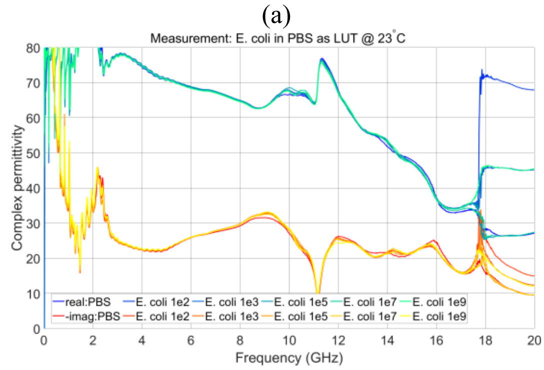
(b)

Figure 4. Magnitude (a) and phase (b) of S-parameters of the embedded sensor filled with PBS suspension of various *E. coli* concentrations.

The captured S-parameters for the embedded sensor are shown in Fig. 4. It is seen that due to high loss, the transmission coefficient is attenuated strongly, as expected. Moreover, increased permittivity extraction error is expected below 2 GHz and above 10 GHz due to short sensor length and related errors. The reflection coefficient on the other hand does not vary much over a wide frequency range. Nevertheless, permittivity of pure PBS was derived with no post-processing applied and presented in Fig. 5 with reference data overlayed captured using the Keysight N1501A Dielectric Probe Kit. As seen, the liquid is of high permittivity, high loss nature featuring at 10 GHz real part of 60 and imaginary part of 35 (loss tangent of 0.58). Finally, permittivity of all tested bacteria concentrations was also derived. As seen, very little variability is observed and data is similar to one of pure PBS. Even though at higher frequencies the determined permittivity is erroneous, the focus here is put of resolving different concentration and so relative values are of more importance and so the collected data is useful.



(a)



(b)

Figure 5. Complex relative permittivity of pure PBS at 23 °C derived using the proposed setup (solid lines) and reference coaxial method (dashed lines) (a). Complex relative permittivity of *E. coli* bacteria of various concentrations suspended in PBS (b). Percentage permittivity deviation from the reference value for pure PBS (c).

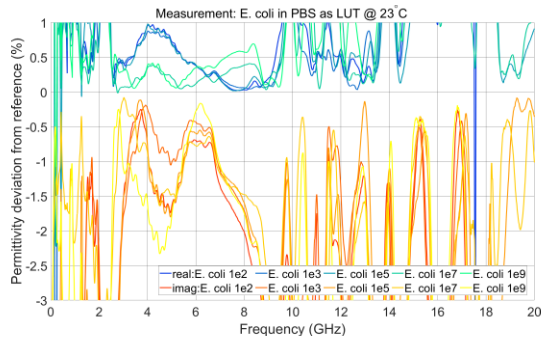


Figure 6. Percentage permittivity deviation from the reference value for pure PBS due to various bacteria concentration in the suspension.

Considering that the liquid chamber holds approximately 1 ml of liquid and assuming uniform bacteria distribution, as low as 1000 bacteria is expected to be within the TW sensor associated EM field. Such small concentrations of bacteria in PBS are difficult to differentiate from pure PBS, thus instead of assessing their permittivity, the PBS referenced percentage permittivity deviation was considered further provided in Fig. 6. Within the 3 GHz to 9 GHz band deviation in the range of fractions to a couple of percent is visible. Interestingly, greater difference is visible on the imaginary part of permittivity that is associated with losses. This means that conductivity of the bacteria plays the major role rather than its effective permittivity. Around 4.6 GHz for example, an almost monotonic trend is observed for the imaginary part of complex permittivity difference where -2.28%, -1.48%, - 1.74%, 1.70 % and -1.05 % deviation is observed for 1e9, 1e7, 1e5, 1e3, and 1e2 CFU/ml, respectively. One must note however, that the distribution of bacteria around the senor`s wires in not necessary uniform and repeatable and so may impact the trend as observed here. Moreover, the changes are comparable with method uncertainty, yet serve a great deal for further studies.

The following conclusions can be drawn from the above presented study to better direct further microwave biosensors development towards more efficient and sensitive designs:
- Bacteria is relatively conductive and so obtaining high sensitivity on losses improves quantitative sensing of bacteria.
- When sensing bacteria suspension, the high permittivity buffer liquid might overdrive and mask the sensors response as the bacteria driven permittivity deviation is relatively small.
- Considering the above sensors requiring rather low volume of the suspension are more preferred so that a strong electric field interacts with the floating bacteria.
- Sensing bacteria alone is much easier than sensing bacteria suspension, as there percentage deviation from air is much higher than from a given buffer liquid, however at the expense of requiring more pre-processing steps.
- Obtaining selectivity on a given bacteria type through a biofunctionalization layers is possible when bacteria alone are sensed.

5. Conclusion

An experimental study was conducted to asses the electrical properties of *E. coli* suspended in a buffer solution for various bacteria content. A highly-sensitive broadband volumetric microwave sensor was developed and used for measurements. It was shown that permittivity of the suspension is very similar to one of the buffer liquid, here PBS with a deviation being in the range of fraction to a couple of percent depending on concentration. Interestingly, the presence of bacteria increases losses more that the permittivity which is an interesting finding for further microwave biosensor development.

6. Acknowledgements

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