

Low-Frequency Dielectric Spectroscopy of Martian Soil Samples

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Abstract—Martian soil simulants and live cell suspensions are under study using low-frequency dielectric spectroscopy (DS) and related techniques, such as nonlinear harmonic response. Such methods hold tremendous potential to develop sensors that can test for subsurface microbial life on Mars and for numerous additional applications. For example, the low-frequency alpha-dispersion, unique to live organisms, leads to enormous dielectric responses that enable a clear distinction between living and inanimate materials. UH researchers measured the low-frequency dielectric properties of soil samples known to be Mars analogues, as well as known live cell suspensions. In this initial study, they tested common soil and JSC Mars-1, a volcanic ash from Hawaii developed for use as a Mars regolith simulant. Biologically active, JSC Mars-1 contains microorganisms and biomolecules equivalent to 10^6 – 10^7 cells/gram, less than common soils. Finally, the UH research team recently discovered resonant-like behavior in the frequency-dependent harmonic responses of live cells. Preliminary evidence suggests that this behavior may result from active molecular motor complexes unique to live organisms. DS may prove to be a life detection tool.

THE ISSUE OF WHETHER OR NOT LIFE ONCE EXISTED ON Mars, or perhaps still exists today,¹ has profound scientific implications for the evolution of life on Earth and the distribution of life in the cosmos. The Viking program made the first serious attempt to detect the presence of living or fossilized organisms in Martian soil and yielded ambiguous results.² However, recent studies of the Martian meteorite Allan Hills 84001 (ALH84001) suggest that microbial life existed on Mars about four billion years ago.³ Perhaps the most compelling evidence is the existence of magnetite (Fe_3O_4) crystals found within carbonate globules and their associated rims in the meteorite.⁴ About one fourth of these tens-of-nanometer sized magnetites are nearly identical to those produced by magnetotactic bacteria on Earth and are not known or expected to be produced by abiotic means. Researchers have argued that these Martian magnetite crystals are in fact magnetofossils, which, if true, would constitute evidence of the oldest life forms known.⁵

Further evidence suggests that subsurface Martian life could potentially survive even today.⁶ There is abundant geological evidence that ice was once deposited in the regolith, where it should still be present above mid-latitudes.⁷ This ice, which probably extends several kilometers below the surface, could be a source of liquid water near magmatic intrusions.⁸ On Earth, the biomass of subterranean organisms may equal

or exceed that at the surface.⁹ These organisms can live in highly saline conditions at temperatures from 115°C to -20°C.^{10,11} Such conditions might prevail beneath the surface in an aquifer or hydrothermal system. For these and other reasons, there is considerable interest in developing new techniques of detecting subsurface life on Mars. Moreover, the likelihood that oceans of liquid water exist below the icy surfaces of Europa and other moons make these exciting candidates for the existence of extraterrestrial life in our solar system. There is a need to develop technologies that could lead to portable devices that could be used in robotic missions or by astronauts for the detection of extant life forms.

Goals of the Project

The goals of this project are to study dielectric spectroscopy^{12,13} and related methods, such as nonlinear harmonic response,¹⁴ as possible techniques for the detection and characterization of live organisms. One objective is to characterize Martian soil simulants using dielectric spectroscopy. A challenge for astrobiological investigation of Mars and other extraterrestrial bodies is to develop in situ instruments capable of distinguishing environmental samples or extracts containing life forms from those that do not. At the same time, the life-detection technology must not be geocentric; that is, it must not be targeted to characteristics that, although specific to life, may be limited to those life forms native to Earth. We are thus investigating dielectric spectroscopy (DS) as a life detection tool, because life throughout the Cosmos, regardless of its biochemistry and the nature of its genetic material, must utilize a variety of complex, charged macromolecules.¹⁵

Results

A material's dielectric constant $\epsilon(\omega)$ represents its linear response to an applied ac electric field at a frequency ω . This property is determined by the motion of free charges inside the live cells, as well as by the way macromolecules polarize in response to the applied field. In our experiments, we employ a parallel plate capacitor configuration for linear response measurements and a four-probe method to measure any nonlinear harmonics produced by changes in the conformational states of macromolecular enzyme complexes. Our setup for linear dielectric response uses a liquid capacitor cell coupled to a Solartron Analytical Model 1260 Impedance Analyzer. This setup enables us to obtain the complex dielectric response, with both the real and imaginary parts, where the imaginary part of the dielectric response is proportional to the conductivity divided by the frequency.

Several dispersions (or relaxations), termed a -, b -, and g -dispersions, can be measure in the linear dielectric responses of biological cell suspensions and tissues over the frequency range 1 Hz-10 GHz. The a -dispersion, which appears below several kHz, is unique to living organisms and has been found to correlate with the cellular membrane potential.¹⁶ The b -dispersion, typically observed at MHz frequencies, is due to interfacial polarization, and is mainly attributed to the insulating plasma membrane surrounding each cell. The g -dispersion, which lies above 1 GHz,¹⁷ results from reorientation of

water and biological macromolecules. This project primarily focuses on the low-frequency a - and b - responses. In addition, we employ related methods, such as nonlinear harmonic response, to probe signals produced by active molecular motors that are unique to live organisms, using a Stanford Research SR 780 Vector Signal Analyzer.

We tested common soil and JSC Mars-1, a volcanic ash from Hawaii, developed for use as a Mars regolith simulant.¹⁸ Biologically active, JSC Mars-1 contains microorganisms and biomolecules equivalent to 10^6 - 10^7 cells/gram,¹⁹ less than common soils (which can contain quantities of up to 10^9 cells/gram). Portions of each environmental sample were left untreated, while other portions were sterilized: autoclaved for 60 minutes at 121°C, at 2 atm, then heated in an oven at 220°C for 3 hours followed by exposure to ultraviolet light for 16 hours. Water extractions were then performed on sterilized and untreated soil and JSC Mars-1 samples. Extracts of untreated soil and JSC Mars-1 yielded multiple microbial strains when incubated on Luria-Bertani (LB) agar for 24 hours at three temperatures: 23°C, 30°C, and 37°C. Extracts of sterilized soil and JSC Mars-1 showed no growth at any of these temperatures, indicating that the sterilization protocol had indeed destroyed all living forms within the environmental samples.

When DS was conducted on extracts, the dielectric constant and conductivity were found to be higher for sterilized samples as compared with untreated samples. We hypothesize that the sterilization protocol results in increased dielectric constant and conductivity due to lysis of cells and the consequent release of charged molecules. However, the values obtained for unsterilized samples may be attributed not only to the presence of charged molecules, but also to membrane potentials of living cells. Samples containing living cells may thus be distinguishable from those containing only macromolecules by performing DS at variable temperatures.

Thus at two temperatures, 4°C and 37°C, we tested a suspension of the bacterium, *E. coli* (2.5×10^9 cells/ml, see Fig. 1), as well as three examples of large, charged biomolecules: deoxyribonucleic acid (DNA), hemoglobin (Hb), and bovine serum albumin (BSA). At 10 Hz, dielectric constant for *E. coli* suspension increased by 70% at 37°C as compared to 4°C, while dielectric constants for DNA, Hb, and BSA increased by 28%, 17%, and 49% respectively. However, the DNA, Hb, and BSA used in this preliminary study were then found to be contaminated with microorganisms. As in the case of *E. coli*, the effects of temperature on life may be the reason the dielectric differences measured for these compounds. We therefore used fetal bovine serum (FBS) (containing proteins, cell wall lipids, and other compounds), specially treated to eliminate all known or suspected life forms, including the controversial entities known as nanobacteria.^{20,21} Results showed that at 10 Hz the dielectric constant for sterile FBS increases by only approximately 6.5% for the FBS at 37°C vs. 4°C (see Fig. 1).

More recently, we have discovered resonant-like peaks in the frequency-dependent nonlinear harmonic responses of live cells. Preliminary evidence suggests that this behavior may result from active molecular motor complexes unique to live organisms, thus providing another tool for detecting life

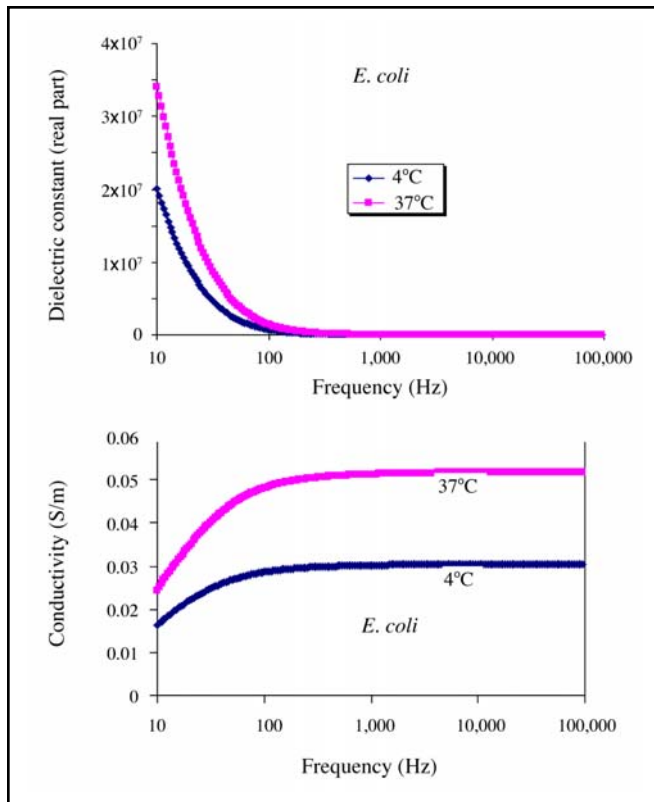


Figure 1. Relative dielectric constants (top, real part) and conductivities (bottom) of a suspension of *E. coli* (2.5×10^9 cells/ml) at 4°C and 37°C.

forms and for fundamental research in biophysics. Nonlinear response is currently probed by measuring induced higher harmonics using a four-electrode probe suspended in the cell suspension, as shown in Fig. 2, in order to study the intrinsic response of the medium and reduce electrode polarization effects. A sinusoidal voltage is applied to the outer electrodes, and the cell response across the inner pair of electrodes is measured as a function of frequency, showing plots of the induced harmonics, using the SR780 signal analyzer. Nonlinear dielectric spectroscopy is an extremely sensitive technique by which the spectra are influenced by the type of organism, its metabolic state, and changes in the conformational states of proteins.

Figure 3 shows the magnitudes of the induced harmonics (across the inner two electrodes) vs. applied fundamental frequency, for an applied voltage amplitude of 5 V across the outer two electrodes. Note that two peaks, centered around 5 kHz and 12 kHz, appear to “grow” out of the background as the cell concentration is increased. In addition, we find that potassium cyanide suppresses the observed peaks. Potassium cyanide (KCN) is a known respiratory inhibitor that binds to the cytochrome c oxidase complex. This enzyme is the fourth complex of the electron transport chain, which pumps protons (H^+ ions) across the mitochondrial inner membrane against the concentration gradient. As it does so, it oxidizes the electron carrier cytochrome c, and is responsible for 90 percent of the oxygen consumption by all living organisms on the plan-

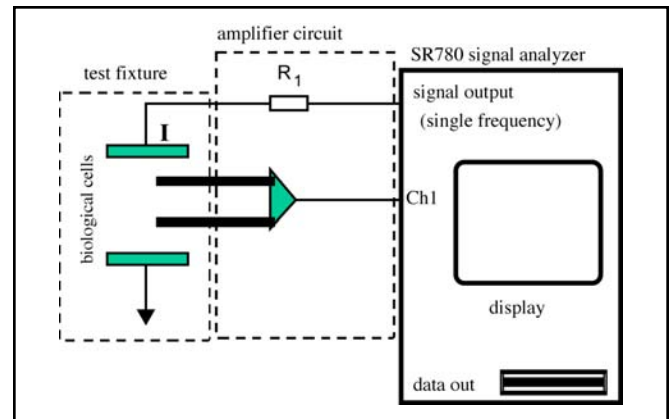


Figure 2. Setup used to measure the nonlinear harmonic response of a live cell suspension using a four-electrode technique, with a 1-cm spacing between the outer two electrodes.

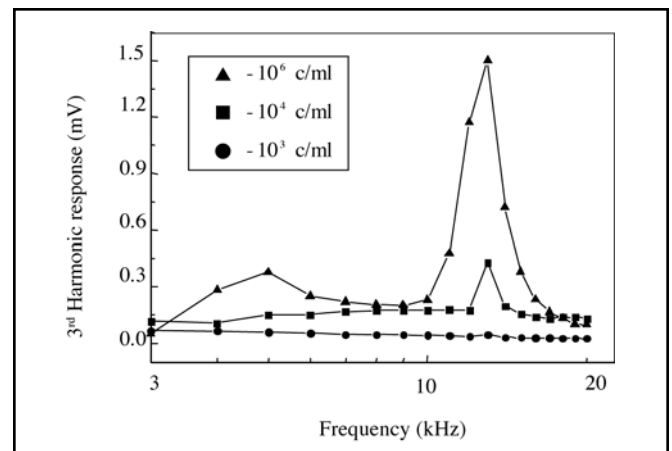


Figure 3. Induced 3rd harmonic amplitude vs. applied fundamental frequency (5-V applied amplitude) for three different concentrations of *S. cerevisiae* (budding yeast). Note that two peaks, centered around 5 kHz and 12 kHz, appear to “grow” out of the background as cell concentration is increased.

et. Importantly, yeast cells are not necessarily killed by cyanide, and they remain capable of fermentation.

The large H^+ concentration gradient across the membrane results in a transmembrane potential that can be as high as 250 mV and drives a remarkable molecular turbine, known as ATP-synthase. Since the proton gradient and transmembrane potential are suppressed when cyanide blocks the cytochrome c oxidase complex, the operation of ATP-synthase is also, indirectly, inhibited by cyanide. More recent experiments on naked uncoupled mitochondria suggest that the observed lower frequency peak in Fig. 3 may be produced by the cytochrome c oxidase complex and/or other components of the mitochondrial electron transport chain, or, perhaps, molecular motors in the cytoplasm that rely on ATP for their operation.

Ongoing and planned studies will employ a variety of inhibitors that bind to different enzymes, to determine the precise

origins of the observed features. Additional results in our lab suggest that the higher frequency peak may either result from a remarkable molecular turbine—the F_0 unit of ATP synthase—or from molecular motors in the cytoplasm driven by ATP.

Recently, Elston *et al.*²² have proposed a model of energy transduction by the F_0 unit of ATP-synthase. A proton on the high concentration side of the mitochondrial inner membrane enters through a channel onto the F_0 rotor, and is then deflected through electrostatic repulsion by the positively charged arg1 unit on the “stator,” thus providing a torque on the rotor. The number of discrete rotational steps of the F_0 rotor depends on the number of subunits, many eucaryotic mitochondria having F_0 rotors with 12 subunits, but the number being smaller in many bacteria. Eventually, after passing through nearly one complete revolution, the proton leaves through another channel on the low concentration side of the membrane. The Brownian ratchet has become a paradigm for representing a wide variety of molecular motors, both rotary and linear, and can be modeled as a particle in an asymmetric saw tooth potential undergoing random thermal excitations. The stochastic excitation of the particle out of each well, together with the asymmetry, results in a net Brownian motion with an average dc component along a preferred direction. Alternatively, an ac excitation at a specific frequency can also induce motion along the preferred direction, especially when the applied frequency correlates with the washboard frequency. This effect has thus come to be called the “correlated ratchet.”

Our results indicate that dielectric spectroscopy, at variable temperatures, and nonlinear harmonic response may be useful both for *in situ* astrobiology studies on the surface of Mars and for study of the liquid ocean beneath the ice of Europa.

References

¹B. M. Jakosky and E. L. Shock, “The Biological Potential of Mars, the Early Earth, and Europa,” *J. Geophys. Res.* 103 (1998): 19,359-64.

²L. Margulis, P. Mazur, E. S. Barghoorn, H. O. Halvorson, T. H. Jukes, and I. R. Kaplan, “The Viking Mission: Implications for life on Mars,” *J. Mol. Evol.* 14 (1979): 223-32.

³D. S. McKay, E. K. Gibson Jr., K. L. Thomas-Keprta, H. Vali, C. S. Romanek, S. J. Clemett, X. D. F. Chillier, C. R. Maechling, and R. N. Zare, “Search for Past Life on Mars: Possible Relic Biogenic Activity in Martian Meteorite ALH84001,” *Science* 273 (1996): 924-30.

⁴K. L. Thomas-Keprta, S. J. Clemett, D. A. Bazylinksi, J. L. Kirschvink, D. S. McKay, S. J. Wentworth, H. Vali, E. K. Gibson, Jr., M. F. McKay, and C. S. Romanek, “Truncated Hexa-Octahedral Magnetite Crystals in ALH84001: Presumptive Biosignatures,” *Proc., Nat. Acad. Sci. USA* 98 (2001): 2164-69.

⁵K. L. Thomas-Keprta, S. J. Clemett, D. A. Bazylinksi, J. L. Kirschvink, D. S. McKay, S. J. Wentworth, H. Vali, E. K. Gibson, Jr., and C. S. Romanek, “Magnetofossils from Ancient Mars: A Robust Biosignature in the Martian Meteorite ALH84001,” *Appl. & Environ. Microb.* 68 (2002): 3663-72.

⁶B. P. Weiss, Y. L. Yung, and K. H. Nealson, “Atmospheric

Energy for Subsurface Life on Mars?” *Proc., Nat. Acad. Sci. USA* 97 (2000): 1395-99.

⁷M. T. Mellon and B. M. Jakosky, “Geographic Variations in the Thermal and Diffusive Stability of Ground Ice on Mars,” *J. Geophys. Res.* 98 (1993): 3345-64.

⁸M. H. Carr, *Water on Mars*. New York: Oxford UP, 1996.

⁹W. B. Whitman, D. C. Coleman, and W. J. Wiebe, “Prokaryotes: The Unseen Majority,” *Proc., Nat. Acad. Sci. USA* 95 (1998): 6578-83.

¹⁰K. H. Nealson, “The Limits of Life on Earth and Searching for Life on Mars,” *J. Geophys. Res.* 102 (1997): 23,675-86.

¹¹J. C. Prisco, C. H. Fritsen, E. E. Adams, S. J. Giovannoni, H. W. Paerl, C. P. McKay, P. T. Doran, D. A. Gordon, B. D. Lanoil, and J. L. Pinckney, “Perennial Antarctic Lake Ice: An Oasis for Life in a Polar Desert,” *Science* 280 (1998): 2095-98.

¹²H. P. Schwan, “Electrical Properties of Tissue and Cell Suspensions,” in *Advances in Biological and Medical Physics*. Vol 5. Eds: J. H. Lawrence and C. A. Tobias. 1957. 147-209.

¹³K. Asami, “Characterization of Biological Cells by Dielectric Spectroscopy,” *J. Non-Crystalline Solids* 305 (2002): 268-77.

¹⁴D. Nawarathna, J. R. Claycomb, J. H. Miller, Jr., and M. J. Benedik, “Nonlinear Dielectric Spectroscopy of Live Cells Using Superconducting Quantum Interference Devices,” *Applied Physics Letters* 86 (2004): 023902-1-3.

¹⁵C. Prodan, “Dielectric Properties of Live Cell Suspensions,” Ph.D. Dissertation, University of Houston, 2003.

¹⁶C. Prodan, F. Mayo, J. R. Claycomb, J. H. Miller, Jr., and M. J. Benedik, “Low-Frequency, Low-Field Dielectric Spectroscopy of Living Cell Suspensions,” *J. Appl. Phys.* 95 (2004): 3754-56.

¹⁷J. B. Hasted, *Aqueous Dielectrics*. London: Chapman and Hall, 1973.

¹⁸C. C. Allen, R. V. Morris, K. M. Jager, D. C. Golden, D. J. Lindstrom, M. M. Lindstrom, and J. P. Lockwood, “Martian Regolith Simulant JSC Mars-1,” 29th Annual Lunar and Planetary Science Conference, NASA Johnson Space Center, Houston, TX, March 16-20, 1998.

¹⁹C. C. Allen, C. Griffin, A. Steele, N. Wainwright, and E. Stansbery, “Microbial Life in Martian Regolith Simulant JSC Mars-1,” 31st Lunar and Planetary Science Conference, Johnson Space Center, Houston, TX, March 13-17, 2000.

²⁰E. O. Kajander, N. Ciftcioglu, K. Aho, and E. Garcia-Cuerpo, “Characteristics of Nanobacteria and their Possible Role in Stone Formation,” *Urol. Res.* 2 (2003): 47-54.

²¹A. P. Sommer, H. I. Hassinen, and E. O. Kajander, “Light-Induced Replication of Nanobacteria: a Preliminary Report,” *J. Clin. Laser Med. Surg.* 5 (2002): 241-44.

²²T. Elston, H. Wang, and G. Oster, “Energy Transduction in ATP Synthase,” *Nature* 391 (1998): 510-13.

Publications

Prodan, C., F. Mayo, J. R. Claycomb, J. H. Miller, Jr., and M. J. Benedik. “Low-Frequency, Low-Field Dielectric Spectroscopy of Living Cell Suspensions,” *J. of App. Physics* 95.7 (2004): 3754-56.