Bioelectromagnetics 21:25–33 (2000)

The Use of Nonlinear Dielectric Spectroscopy to Monitor the Bioelectromagnetic Effects of a Weak Pulsed Magnetic Field in Real Time

Elizabeth Davies,* Andrew Woodward, and Douglas Kell

Pharmacy Department, University of Brighton, East Sussex, United Kingdom, and Institute of Biological Sciences, University of Wales, Aberystwyth, United Kingdom

Nonlinear dielectric spectroscopy (NLDS) was used to detect interaction of a pulsed magnetic field (PMF) with membrane protein dynamics in aggregating *Dictyostelium discoideum* amoebae. In the experiments reported here, a strong nonlinear dielectric response of *Dictyostelium discoideum* cells is shown, and a distinctive nonlinear dielectric response of cells previously exposed to PMF is shown. The method of NLDS is shown to be capable of monitoring and charting the dynamic frequency response of the cell to an electromagnetic field. Bioelectromagnetics 21:25–33, 2000. © 2000 Wiley-Liss, Inc.

Key words: nonlinear dielectric spectroscopy; harmonics; *Dictyostelium discoideum*; cell membrane; membrane enzymes; pulsed magnetic fields

INTRODUCTION

Nonlinear dielectric spectroscopy (NLDS) is a technique developed over the last ten years [Woodward et al., 1996]. The technique monitors the nonlinear dielectric response of cell membranes to an applied weak sinusoidal electric field (typically 350 mV/cm), using a four-terminal gold electrode with computer driven data control, acquisition and multivariate analysis. It records, in real time, the kinetics of polarisable enzymes in the membranes of living cells. It does this by monitoring the harmonics produced at the inner voltage-sensing electrodes while a series of sinusoids at a variety of voltages, frequencies and amplitudes are passed across a cell suspension in rapid succession via the outer electrodes. The technique has so far been shown capable of monitoring metabolic activity of ATPases in yeast cells and erythrocytes, and of membrane electron transport enzymes in bacteria and plant cells [Woodward and Kell, 1990, 1991a, 1991b; McShea et al., 1992].

Whereas traditional dielectric spectroscopy monitors the response of cells to an applied sinusoid at the fundamental frequency, NLDS monitors the response at a range of frequencies other than the fundamental, using fast Fourier transform (FFT) analysis.

MATERIALS AND METHODS

Nonlinear Dielectric Spectroscopy

The NLDS spectrometer is shown diagrammatically in Figure 1. Data in original work was logged using an in-house software programme 'swpspec' [Woodward et al., 1996] at a sampling frequency corresponding to 20 times the fundamental, and spectral analysis was based on a 256-point Fourier transform, giving a 128-point power spectra. In this experiment 256 samples were recorded for each combination of voltage and frequency (3 voltages and 5 frequencies). This comprised one data block. The mean was subtracted from the data block, which was then Blackman windowed, and the FFT power spectra was recorded. In all experiments, 30 data blocks were recorded, processed as above, and the 30 spectra mean averaged to reduce noise. In the early work, using the 'swpspec' programme, the results thus obtained, were filtered, retaining only the first five harmonics (3 voltages, 5 frequencies, and 5 harmonics, totalling 75 variables). The time taken to record 30 blocks of data using a fundamental frequency of 10 Hz was 76.8 s, and the same procedure using a fundamental frequency of 50 Hz took 15.36 s, leading to a total recording time for all frequencies of 2.92 min.

A ''difference'' spectrum was obtained by dividing the sample power spectrum by the spectrum recorded for supernatant alone (sample minus cells,

*Correspondence to: Elizabeth Davies, Pharmacy Department, University of Brighton, Cockroft Building, Moulsecombe, Brighton, East Sussex, BN2 4GJ, United Kingdom. E-mail: E.A. Davies@bton.ac.uk

Received for review 12 May 1998; Final revision received 22 March 1999.

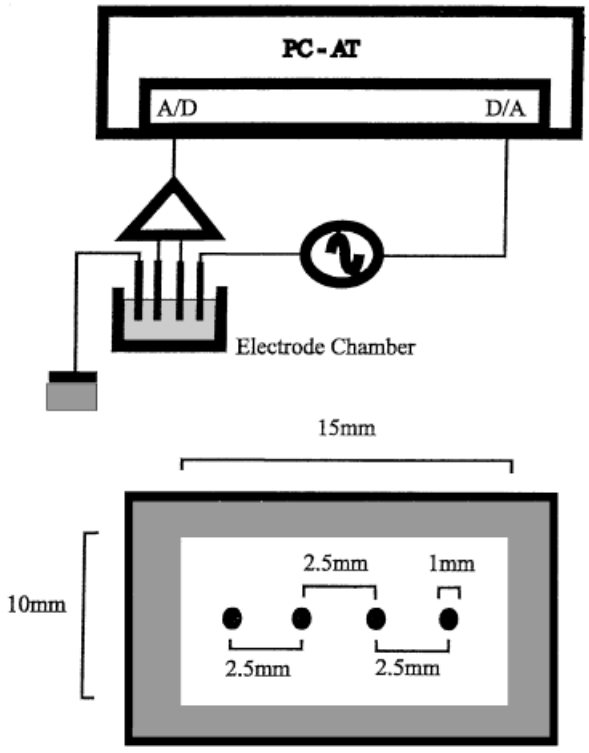

Fig. 1. NLDS spectrometer. The electrode chamber contains four gold electrodes, inner sensing and outer signalling, driven and logged by a DT2823 d/a board.

with conductivity matched by replacing the missing cell volume with distilled water). This deconvolution has been shown to reveal harmonics solely due to cell biology and ideally free of artifactual non-linearities from electrode and medium [Woodward and Kell, 1990]. (See Fig. 2 for example of sample, supernatant and difference spectra, taken with fundamental frequency/voltage combination of 1.5 V and 40 Hz and showing a significant third difference harmonic). The biologically relevant and reliable information in these spectra has been found to reside solely in the harmonics. Therefore in order to reduce the recording time in subsequent longer experiments, (and the inherent problems of time variation of electrode polarisation) interharmonic bins in the spectra were eliminated and data sampling was performed at such a rate that one block contained a whole number of cycles of the fundamental, eliminating the need for windowing. An in-house software program 'swplgmed' was used for this later work. In this program a 16-point FFT is applied to data that was sampled at intervals which involved one cycle per block and 16 samples per cycle, and which placed one harmonic in each successive bin up to the first five harmonics [Woodward and Kell, 1996].

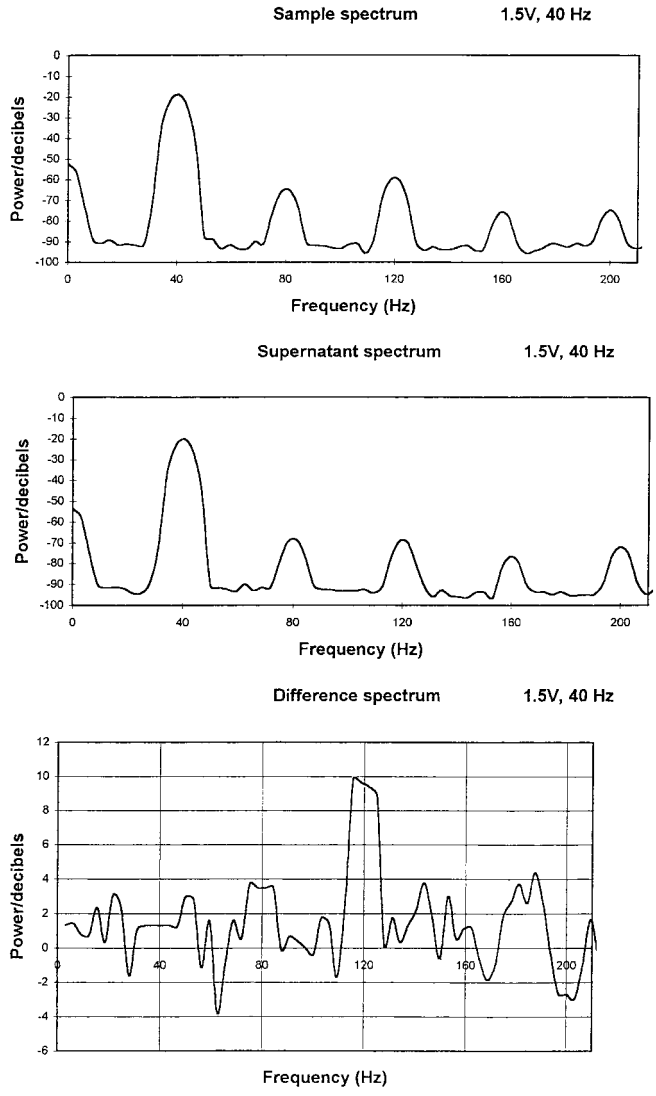

Fig. 2. Sample, supernatant and difference spectra for *Dictyostelium discoideum* cells, (10^7 cells ml^{-1} in KK_2 buffer) at 1.5 V and 40 Hz, showing a characteristic difference in the amplitude of the third harmonic, as well as a very small difference in the second harmonic.

Pulsed Magnetic Fields

It has been argued in a previous publication [Woodward and Kell, 1990] that the type of electroconformational energy transduction evidenced by NLDS may help to explain the many reports of the ability of very weak electromagnetic fields to affect biological activity. In these experiments, the concern was to test whether NLDS was capable of detecting the subtle metabolic changes induced by a Pulsed magnetic field (PMF) in living cells.

The two electromagnetic fields chosen were PMFs similar to those used orthopaedically to stimulate non-unions [Bassett, 1989]. The magnetic

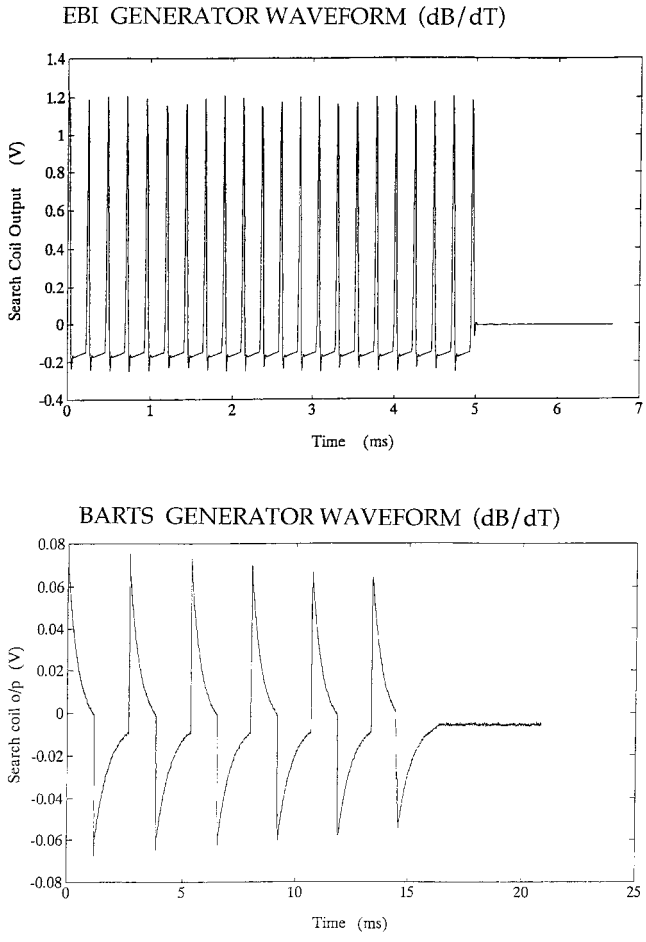

Fig. 3. Pulse waveforms showing the rate of change in magnetic flux with time, (dB/dT) for EBI (upper) and Barts (lower) magnetic fields, as detected by search coil.

fields were of peak flux densities 0.4 mT and 3 mT respectively (measured using a Hall probe, constructed in-house, comprising a linear Hall effect Integrated Circuit, and calibrated using a 992 turn solenoid producing a field of 30.6 gauss/amp centre coil), and were generated via air cored coils in trains of 2 ms pulses gated at 20 ms (Barts) (Dept. Medical Electronics, St. Bartholomew's Hospital, London) (Dixey and Rein, 1982), and 0.3 ms gated at 60 ms (EBI) (Electrobiology Inc. USA) [Sharrard, 1988]. Figure 3 shows the pulse waveforms (monitored using a search coil, 97 turns, area 2.2 cm^2). Figure 4 shows the exposure set-up. For experiments conducted with the Barts PMF, control samples were placed in a dummy field, consisting of an identical set of counterwound coils, producing identical heating effect, but a marginal magnetic field that is decreased by nearly two orders of magnitude (0.01 mT). The EBI coils produced negligible heating effect in the sample; heating was at the 0.1 °C detection limit, as determined by matched

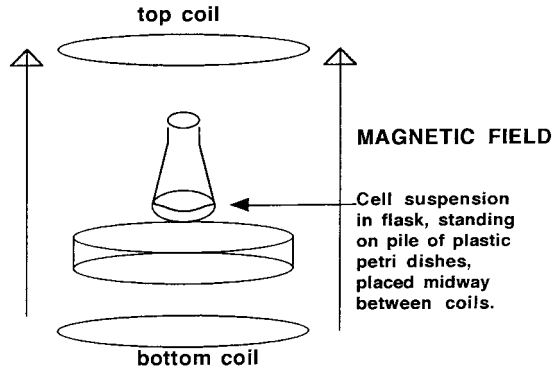

Fig. 4. Magnetic field exposure system, showing the position of the conical flask containing the sample (volume 20 ml, depth 13 mm) between the coils (12 cm diameter, 12.5 cm apart). The peak strength of the magnetic field, measured by Hall probe, was 3 mT, and the rms current induced in cell suspension across the base of the flask by the magnetic field (measured by stainless steel point electrodes and Maplin digital precision gold multimeter, frequency range 45-500 Hz, resolution 100 nA) was 0.8 μA.

thermocouples. The ambient rms AC magnetic field was 0.0001 mT, as measured by Emdex Mate fluxmeter (Enertech).

Dictyostelium discoideum

The cell system was *Dictyostelium discoideum*, a unicellular organism in its early aggregation stage. It is ideal as a model for mammalian cells in the endocrine and immune system, as it is phagocytic and secretory and has an amplified adenylate cyclase activity. During early aggregation cAMP is produced autocatalytically, acting as first and second messenger molecule in a regular oscillatory cycle lasting 5–7 min. This cycle has been shown to correlate with light scattering at 350–500 nm and with calcium and potassium fluxes [Bumann et al., 1986]. Moreover it has been shown to be sensitive to PMF modulation. It has previously been been shown that the Barts PMF damps nucleotide oscillations in aggregating *Dictyostelium discoideum* and that the EBI PMF modulates the kinetic activity of membrane bound phosphodiesterase in *Dictyostelium discoideum* [Davies et al., 1994].

Culture of *Dictyostelium discoideum*

It was first established that NLDS was capable of monitoring metabolic activity in *Dictyostelium discoideum* cells. Cells of the strain V12 were grown and prepared as follows: A loopful of spores was transferred aseptically to a sterile petri dish and mixed in 5 ml sterile distilled water. Log phase *E. coli*, kept on agar slopes at 4 °C, was transferred by means of a sterile loop to the dish as the bacterial food supply. This suspension was well mixed, and 0.3 ml was spread aseptically onto petri dishes containing solid

agar. The agar was constituted as follows: D-glucose, 10 g, bacteriological peptone, 10 g, yeast 1 g, $MgSO_4$ $7H_2O$, 1 g, Na_2HPO_4, 1 g; agar (Oxoid, Technical No. 3), 18 g, dissolved and autoclaved in one litre of double distilled water. The cultures were grown for 46 h at 22 °C in an incubator. After 46 h growth, when small round clear plaques (the signs of bacterial lawn clearing) were apparent on the agar plates, the plates were removed from the incubator, and harvested by flooding them with double distilled water at 22 °C, and scraping and pipetting the suspended amoebae and bacteria into centrifuge tubes. The mixture was centrifuged at 4 °C for 1 min at 3000 rpm and the bacteria decanted with the supernatant. The amoebal pellet was resuspended in potassium phosphate buffer (KK_2) at 4 °C consisting of KH_2PO_4, 0.02 M; K_2HPO_4, 0.004 M; $MgSO_4$, 0.001 M; pH 6.3, and the centrifugation process repeated 5 times, until the supernatant was clear of bacteria. The amoebae were then resuspended in KK_2 buffer at 22 °C, mixed thoroughly in a vortex mixer, and counted under a microscope using a haemocytometer. The cell count was then adjusted to 1×10^7 cells ml^{-1}. The cells were allowed to equilibrate in a flask for 30 min before testing.

NLDS Analysis of *Dictyostelium discoideum*

One milliliter of cell suspension was then placed in the electrode well for analysis. Initially the cells were excited at a range of fundamental frequencies from 10–1000 Hz and the response scanned using FFT analysis, to establish any key area of harmonic response. Similarly a range of voltages from 0.5–1.5 V was used, in order to establish a possible threshold voltage for any harmonic response.

The most effective frequency range for producing coherent and detectable harmonics was discerned to be 10–50 Hz, and the optimum voltage range was from 1.2–1.4 V, 1.4 V being the maximum advisable voltage to avoid electrode fouling [Woodward and Kell, 1990]. It should be emphasised that any voltage applied is amplified 1000-fold by the membrane. In subsequent experiments, using coated electrodes [Denyer et al., 1993], the voltage range was increased to 1.25–1.75 V, since the coating reduced sensitivity, such that a larger applied voltage passed less current through the interfacial layer, albeit increasing stability. Therefore five frequencies, 10, 20, 30, 40, and 50 Hz, and three voltages: first, 1.2, 1.3, 1.4 V and later, 1.25, 1.5, and 1.75 V were selected for the signal sweep. This original work with *Dictyostelium* used a 'swpspec' program to record data, as previously described. Since the only biologically relevent information was previously found to be contained in the harmonics generated, inter-bin noise was zeroed to improve display legibility [Woodward et al., 1996]. A difference spectrum was obtained for each cell suspension, as previously indicated, by dividing the sample power spectrum by the supernatant power spectrum.

Analysis of Spectral Data

Figure 2 shows the sample, supernatant and difference spectrum at a specific voltage and frequency combination. The supernatant spectrum shows harmonics due to electrode and medium characteristics, without the presence of cells. When the supernatant spectrum is deconvolved from another supernatant spectrum, no harmonics are present, showing that electrode effects are nullified by taking a difference spectrum. This makes the NLDS difference spectrum, which is a deconvolution of cell sample and supernatant, significant, since this difference signal is solely dependent on the presence of cells. The difference in third harmonic between sample and supernatant is, therefore, a small but significant indicator of cell biology.

A 3-dimensional plot of amplitude of harmonic (decibels) as a function of harmonic number and applied frequency, allowed visualisation of an NLDS spectrum peculiar to *Dictyostelium*, at three specific voltages, as seen in Figure 5.

Further programming allowed information to be displayed from repeated sampling over 20 min, showing the temporal variation of a harmonic during 20 min of biological activity, during which *Dictyostelium discoideum* amoebae are actively engaged in their oscillatory chemotactic signalling cycle, for the whole range of voltage and frequency combinations. Cell samples were prepared as described above, and 10 ml aliquots placed in flasks in control and EBI PMF test positions simultaneously. 1 ml samples were taken alternately from control and PMF test flasks over a period of 20 min, scanned in the NLDS spectrometer, analysed, and the difference spectra recorded. Figure 4 shows the position of the *Dictyostelium discoideum* samples with respect to the magnetic field exposure system. Figure 6 shows the effect of EBI PMF on the second harmonic over 20 min. From this latter time series it can be appreciated that the number of variables potentially modulated by PMF is enormous. (The x-axis shows frequency and voltage combinations for the second harmonic; 1.2 V: 10, 20, 30, 40, 50 Hz; 1.3 V: 10, 20, 30, 40, 50 Hz; 1.4 V: 10, 20, 30, 40, 50 Hz; with frequency windows at 10 Hz: 1.3 and 1.4 V for both the control and MF samples, with an additional window at 20 Hz: 1.3 and 1.4 V for the control samples). Although it was possible to detect some consistent differences relating to treatment in a particular frequency/voltage amplitude of a particular

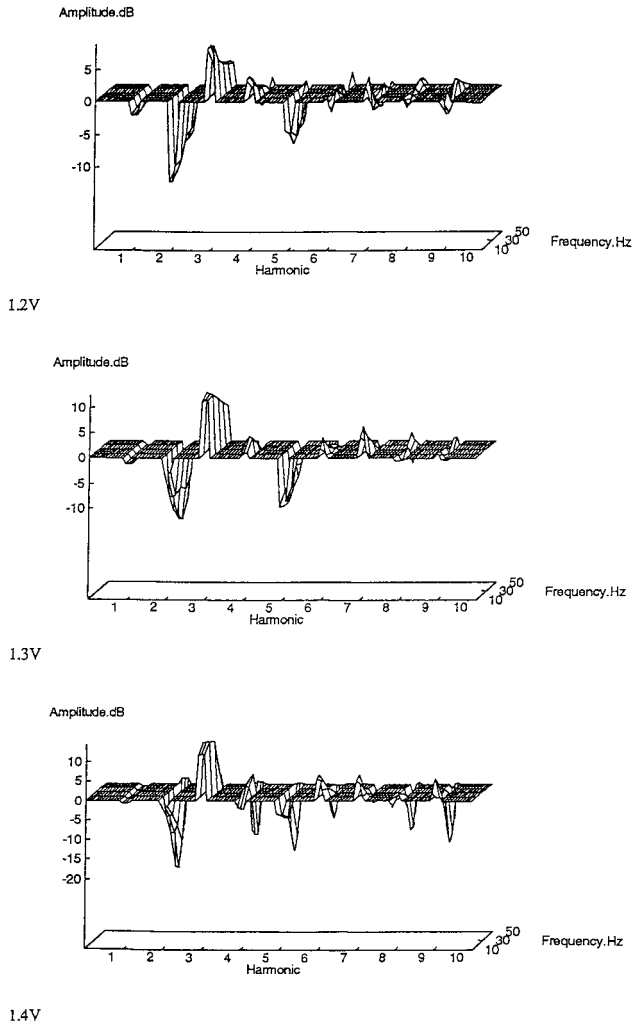

Fig. 5. NLDS harmonic profile vs. frequency of aggregating *Dictyostelium discoideum* amoebae (suspension of 10^7 cells ml^{-1} in KK_2 buffer) at 1.2 V, 1.3 and 1.4 V zero-to peak showing characteristic second and third harmonics, coherent over five different fundamental frequencies (Y axis) and showing characteristic frequency windows of these harmonics.

harmonic after hours of sifting through plots and tables, such variations are often too small to detect by inspection of graphical data and too time consuming without computer analysis. It may be seen from Figure 6 that no harmonics are generated by the supernatant/supernatant difference spectra, showing that the cells themselves are responsible for harmonics in these spectra and not the suspending medium.

It may be appreciated that the significant harmonics generated by cell biology are small in comparison with the noise and are also fluctuating, due to dynamic cell metabolism. It should be stressed that the largest peaks in a spectrum may not be the most significantly varying in correlation against the required effect. Therefore a very sensitive mathematical modelling system capable of detecting small but consistent changes over a large dataset is required in order to analyse the spectral data obtained.

Multivariate Analysis of Spectral Data

It was only with the use of multivariate analysis that it was possible to show conclusively that PMF had affected the NLDS profile of the cells in a meaningful manner. Principal Components Analysis (PCA) [Martens and Naes, 1989] allows very small data variations, which would otherwise be overwhelmed with noise, to be discerned and charted by means of dimensional reduction of the initially unwieldy multidimensional dataset. In the experiments subsequently described, a dataset of up to 70 samples of 75 variables each, with each variable representing one dimension, was used for analysis and was efficiently projected by PCA onto a few key vectors (refered to as principal components). Thus, each principal component comprises a number of values or ''scores'', one for each spectrum or ''object'' projected onto it. Each ''score'' is determined by the specific variable/dimension from which it derives its value and position in relation to the vector, and this is known as a ''loading''. Thus by observing the loadings on a key principal component, it is possible to observe the harmonic variables that are involved in any distinction of magnetic field effect. Table 1 is the result of such an investigation. If the scores are negatively correllated to a high degree, the statistical significance of the effect will be extremely high.

Chemometric analysis of treated cells required multiple spectra of treated and control suspensions alternately. To speed this data collection, a different 'swplgmed' data collection program was used [Woodward et al., 1996] to record only the harmonic values of interest with very short data blocks and resulting Fourier transforms, as described previously. This program had, in concert with multivariate analysis, proved capable of providing meaningful spectra without the requirement of a reference spectrum, removing the need to derive a supernantant to deconvolve from each suspension sample [Woodward et al., 1996]. A complete spectral voltage/frequency sweep of a sample could be collected in 1.5 min, minimising effects of electrode drift during the collection of a spectral sweep.

In the experiments reported here, it was found that the change in biologically relevant harmonics seen in difference spectra from control and PMF exposed samples, could be discerned from the sample spectra alone; the supernatant spectra from the control and exposed cells did not contain biologically relevant harmonic information. This may be appreciated by

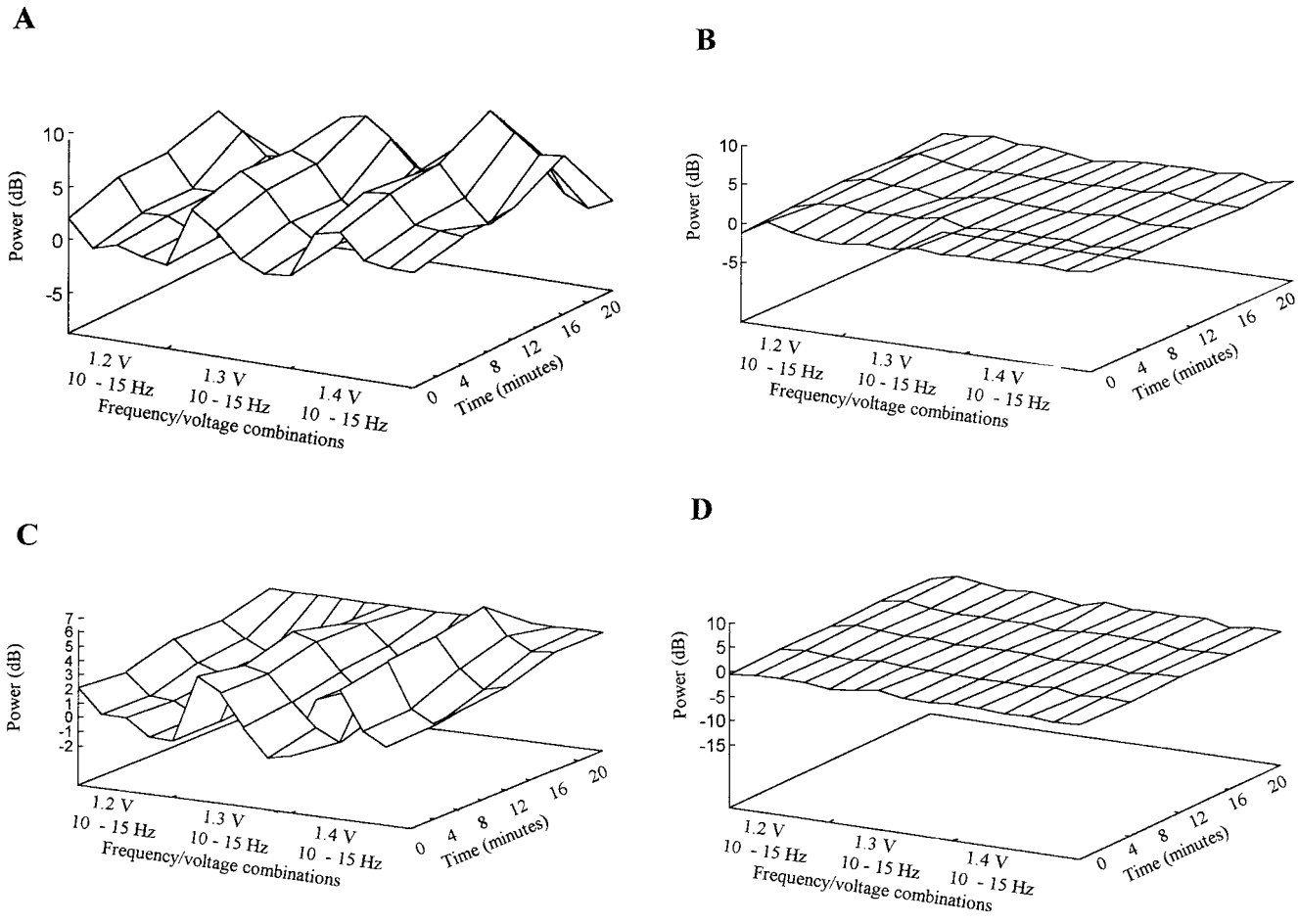

Fig. 6. NLDS temporal harmonic surfaces of aggregating *Dictyostelium discoideum* amoebae, as above, showing second harmonics at 15 different combinations of voltage and fundamental frequency. The x-axis shows frequency/voltage (F/V) combinations for the second harmonic; 1.2 V: 10, 20, 30, 40, 50 Hz; 1.3 V: 10, 20, 30, 40, 50 Hz; 1.4 V: 10, 20, 30, 40, 50 Hz over 20 min (z axis), spectral sweeps having been taken at 5 minute intervals. The different profiles are: (a) control, (b) control supernatant, (c) EBI PMF, and (d) EBI PMF supernatant. This method represented an attempt to capture any harmonic changes due to the naturally occuring metabolic oscillation in *Dictyostelium discoideum* during its chemotactic signalling cycle over a 20 min period. Harmonic frequency windows may be seen at 20 Hz: 1.3 and 1.4 V for both the control and MF samples, with an additional window at 30 Hz: 1.3 and 1.4 V for the control samples. The harmonics can be seen to alter smoothly over the 20 min study period, indicating a slow metabolic change.

perusing Figure 7, in which the difference spectra for control and MF-exposed *Dictystelium discoideum* cells are shown in the first two plots. Beneath them, in the third plot, the difference spectrum of their control and MF exposed supernatants is shown at one fundamental frequency and voltage combination. It may be seen that there is a difference in amplitude of harmonics between control and Barts MF sample difference spectra. Such a difference is used in NLDS as a parameter for monitoring biological changes in the cells. As the difference spectrum of control and MF supernatants contains no harmonics, showing them to be identical, it may be deduced that any difference in harmonics seen between the control and Barts MF difference spectra is cellular in origin and that the Barts field had no significant effect on the supernatant or on the electrode/supernatant interface. Experiments showed this to apply equally to the EBI magnetic field. This is an important observation, as it gives credence to the statement that any changes in the NLDS spectra of exposed cells are due to changes in the cell biology and not to changes in the medium, electrode drift or inhomogeneity.

In the experiments reported here, a conductive polymer coating [Denyer et al., 1993] applied to the electodes helped to stabilise fluctuating interfacial

TABLE 1. Key frequencies and harmonic numbers characterising altered nonlinear dielectric response of exposed *Dictyostelium discoideum* amoebae during early aggregation

F (Hz)	harmonic	EBI PMF			BARTS PMF		
		V1*	V2	V3	V1	V2	V3
10							
	2				+**		
	3	+					+
	4			+			
	5		+	+	+	++	−
20							
	2			+	−		+
	3				−	−	
	4			+			−
	5				−		++
30							
	2				−	−	
	3	+					
	4	+			−		
	5		+		+		−
40							
	2						
	3		++	+			
	4		+	+		+	
	5		+		+	+	
50							
	2						
	3	+	+	+			
	4	+	+			−	

*V1 = 1.25 V, V2 = 1.5 V, V3 = 1.75 V.
**+ = higher amplitude of harmonic in control sample ($P \ll 0.05$) in one of the two full duplicate experiments. ++ = higher in both experiments; − = lower amplitude of harmonic in control sample ($P \ll 0.05$) in one of two experiments. −− = lower in both experiments

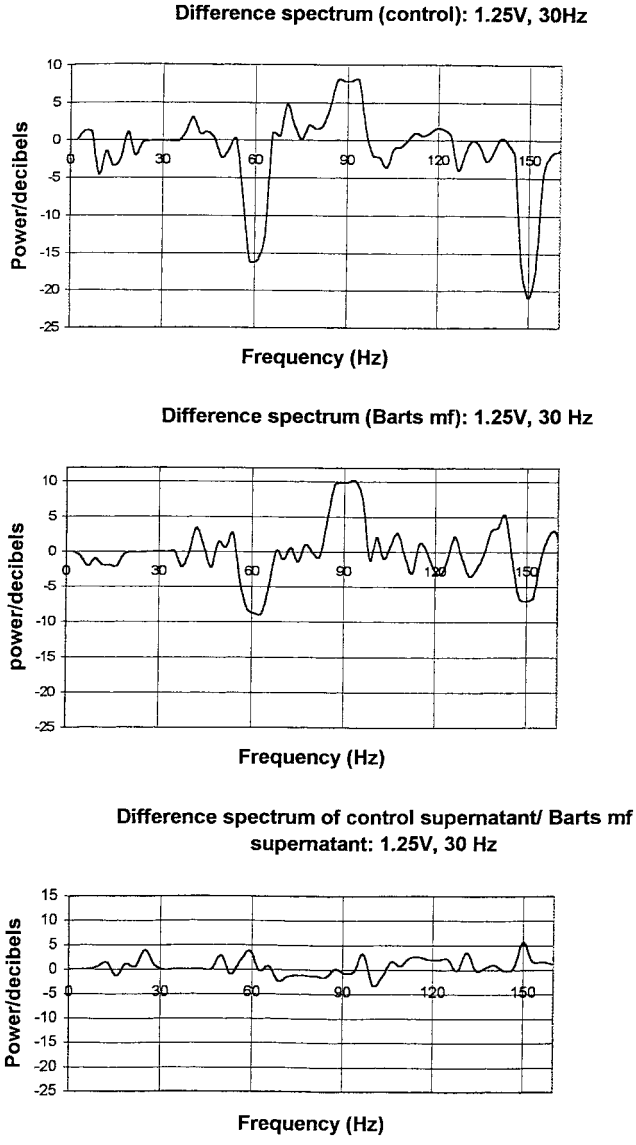

Fig. 7. Difference spectra of *Dictyostelium discoideum* cells, 10^7 cells ml^{-1} in KK_2 buffer, (control and Barts PMF exposed), and supernatants (control/Barts PMF), at 1.25 V, 30Hz. The first two plots show difference spectra between suspensions of cells and supernatants. The third plot shows the comparison of the two respective supernatant spectra. The difference spectra show second and third harmonics. However, the supernatants' difference spectrum shows no harmonics as the result of subtraction of Barts MF supernatant spectrum from the control supernatant spectrum.

impedance and allowed collection of 70 samples of 75 variables each over a 2 h period with minimal interfering electrode drift or instability. Any consistently changing parameters in the NLDS response between control and test sample could be extracted and highlighted and identified.

Protocol for Obtaining Spectra of PMF Exposed *Dictyostelium discoideum* Cells for Multivariate Analysis

Cells were grown and prepared as previously indicated, and 10 ml of cell suspension placed in a flask between coils for PMF exposure (see Fig. 4). An identical flask containing 10 ml from the same suspension of cells was placed simultaneously in an adjacent control position, either between dummy coils for the Barts field, or at the same height adjacent to the coils on the bench for the EBI field. After 30 min, the control flask was gently stirred and a 1 ml aliquot of cell suspension was withdrawn by pipette, placed in the electrode well and scanned. It was then withdrawn from the electrode well by pipette and returned to the flask. The flask was again given a gentle stir, and a fresh aliquot withdrawn for sampling. This procedure was repeated 10 times for the control samples. The same procedure was immediately executed 10 times with the treated sample. The whole procedure was

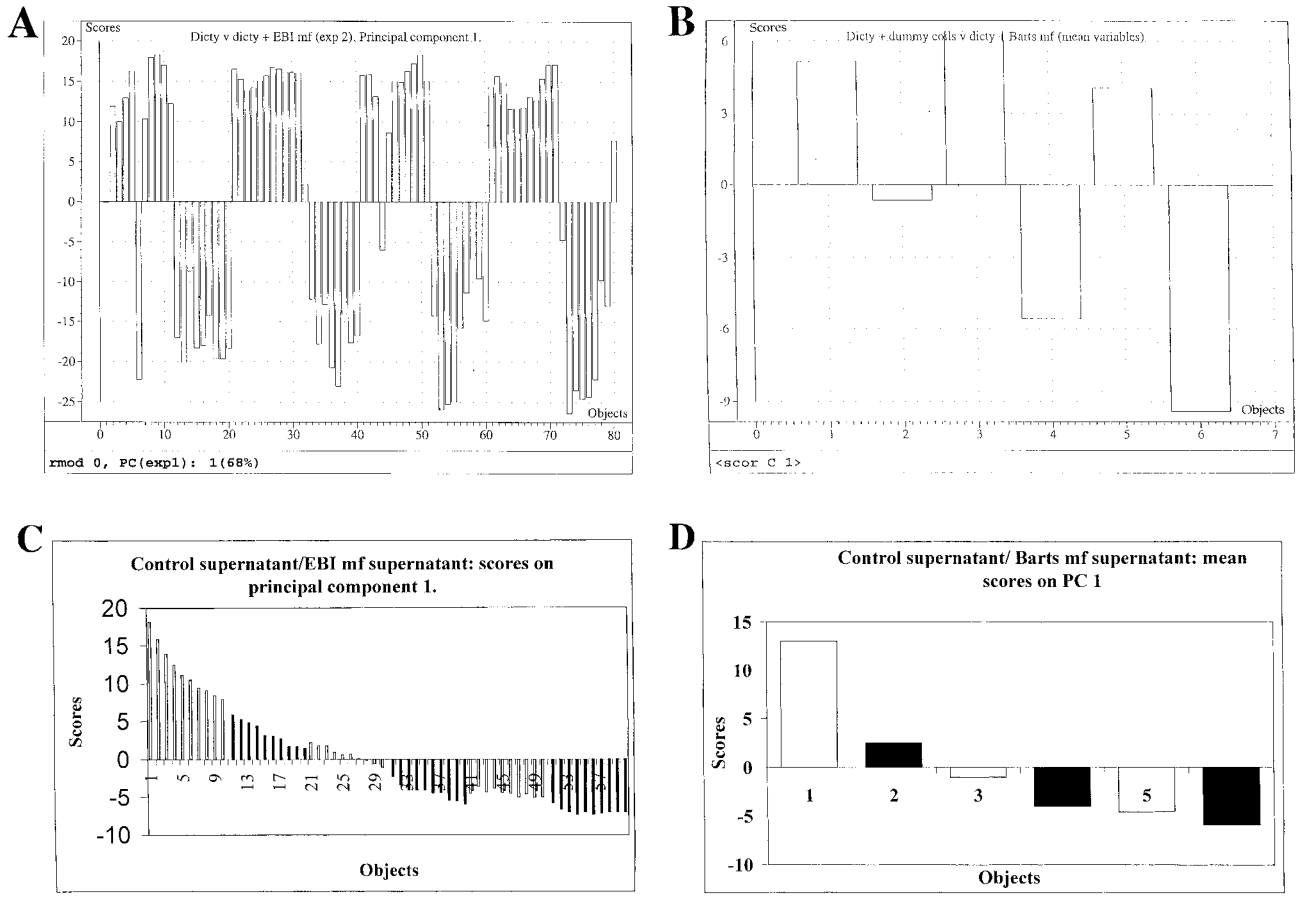

Fig. 8. Plots showing PCA scores of discriminating harmonic parameters. A and B show distinction between control suspension of *Dictyostelium discoideum* cells and (**A**) EBI PMF exposed cells, or (**B**) Barts PMF exposed cells. C and D show distinction between control supernatant and (**C**) EBI exposed supernatant and (**D**) Barts exposed supernatant.

repeated to give between 6 and 8 sets of 10 spectra per set.

The series of sets was thus an alternation between control and treated samples. In each experiment, the treated samples had been exposed to treatment for 45 min prior to the first sampling. To check that electrode characteristics had not changed during the 2 h experiment, a difference spectrum of both standard resting yeast suspension and of *Dictyostelium* cell suspension was taken before and after the experiment, and the stability, position and amplitude of harmonics was noted and compared. In the experiments reported here, the coated electrode proved remarkably stable, giving good standard difference spectra before and after experiments, and showing negligible change in polarisation of the outer exciting electrodes during the experiment, and confirming reliability of the data acquired.

The matrix of 70 samples $\times$ 75 variables thus obtained, was subjected to PCA analysis, using the UNSCRAMBLER 5 software package, (Camo A/S, Olav Tryggvasonsgt. 24, N-7011, Trondheim, Norway).

RESULTS

The plots in Figures 8a and b clearly show that the PCA scores of the samples show strong separation between treated and control cells. This distinction is statistically highly significant ($P < 1 \times 10^{-6}$ by paired-t test). It is clearly possible to distinguish the effect of these two PMFs, EBI (a) and Barts (b), on principal components encompassing a high percentage of the total variation in the dataset. The PCA loadings on the discriminating components reveal the key combinations of voltage, frequency and harmonic number, responsible for the differences. These are given in

Table 1; this Table combines results from two replicate experiments. These differences can only be attributed to the prior effect of the PMF on the cell suspensions, as this constituted the only difference in treatment between the control and exposed cells. The major effect of the PMFs is on *Dictyostelium discoideum* cells themselves, as the plots of PCA scores on supernatant samples, in the absence of cells, show drift over 2 h, and no separation of control from MF-exposed samples in any principal component combinations (see Figures 8c and 8d).

CONCLUSION

The clear separation seen in control and exposed cell samples, and the lack of separation in the respective supernatant samples, shows that the method is not just yielding an overanalysis of noise, which would show up similarly in both supernatant and cellular samples. The cell biology itself is essential for any consistent and meaningful harmonic changes. The fact that such changes show systematic distinction between exposed and control spectra, over a period of 2 h, with respect to cell biology, suggest that this is not due to electrode changes, but to the effect of the magnetic field. If this were due to correlated changes in the electrode noise, the effect would show itself as systematic distinction in the supernatant spectra also.

Thus NLDS seems capable of monitoring and charting the dynamic frequency response of metabolising cells to an electromagnetic field. This is obviously a curtailed account, and only essential and highly condensed information has been given. Further research needs to be undertaken to develop and explore this technique. It may have potential application in the development of electromagnetic therapeutic devices for modulation of biochemical activity.

REFERENCES

Bumann J, Malchow D, Wurster B. 1986. Oscillations of Ca^{++} concentration during the cell differentiation of Dictyostelium discoideum. Differentiation 31:85–91.

Davies E, Olliff C, Wright I. 1994. Pulsed magnetic fields affect oscillations of the adenylate cyclase enzyme system in Dictyostelum discoideum, athermally. Biochemical Society Transactions 22(1) 74-S.

Davies E, Kell DB, Woodward AM. 1995. The use of nonlinear dielectric spectroscopy to monitor the interaction of β-lactam antibiotics with gram-positive bacteria. Journal of Pharmacy and Pharmacology 47:1097.

Dixey R, Rein G. 1988. 3H-noradrenaline release potentiated in a clonal nerve cell line by low-intensity pulsed magnetic fields. Nature 5854:296:253–256.

Denyer SP, Gorman SP, Sussman M, editors. 1993. Microbial biofilms: Formation and control. Blackwell Scientific Publications: Technical Series: 30.

Martens H, Naes T. 1989. Multivariate Calibration, Wiley.

McShea A, Woodward AM, Kell DB. 1992. Nonlinear dielectric properties of Rhodobacter capsulatus. Bioelectrochemistry and Bioenergetics 29:205–214.

Sharrard WJW. 1990. A double blind trial of pulsed electromagnetic fields for delayed union of tibial fractures. J Bone and Joint Surgery 72-B:347–355.

Woodward AM, Kell DB. 1990. On the nonlinear properties of biological systems — *Saccharomyces cerevisae*. Bioelectrochemistry and Bioenergetics 24:83–100.

Woodward AM, Kell DB. 1991a. Dual frequency excitation — a novel method for probing the nonlinear dielectric properties of biological systems, and its application to suspensions of S.cerevisiae. Bioelectrochemistry and Bioenergetics 25: 395–413.

Woodward AM, Kell DB. 1991b. On the relationship between the nonlinear dielectric properties and respiratory activity of the obligately aerobic bacterium Micrococcus luteus. Bioelectrochemistry and Bioenergetics 26:423–439.

Woodward AM, Jones A, Zhang X-Z, Rowland J, Kell DB. 1996. Rapid and non-invasive quantification of metabolic substrates in biological cell suspensions using nonlinear dielectric spectroscopy with multivariate calibration and artificial neural networks. Principles and applications. Bioelectrochemistry and Bioenergetics 40:99–132.