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To cite this article: A De Ninno and A Congiu Castellano 2011 *J. Phys.: Conf. Ser.* **329** 012025

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On the Effect of Weak Magnetic Field on Solutions of Glutamic Acid: the Function of Water

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Abstract. Even though a wide literature is available on the effects of weak Extremely Low Frequency ElectroMagnetic Fields (ELF-EMF) on the biochemical reactions, nevertheless the physical nature of these effects is largely unknown. The main challenge is the mismatch between the infinitesimal amount of the energy carried by the perturbation and the entity of the response of the system. More than 10 years ago, it was claimed that a weak current can be induced in a aqueous solution of Glutamic acid exposed to a weak magnetic field having a certain frequency. We have checked the influence of ELF-EMF on Glutamic acid using the FTIR-ATR spectroscopy in order to observe structural changes induced by the exposure. Samples have been exposed for 10, 20 or 30 minutes to a magnetic field generated by Helmholtz coils and then placed into the spectrometer. After the exposure solutions having different pH values always shown a shift toward the de-protonated species. The effect lasts for minutes after the exposure before the native configuration is restored.

1. Introduction

In the early 1990s, at the Institute of Cell Biophysics in Russia, a cycle of experiments was carried out on the action on aqueous solutions of amino acids of weak low-frequency AC magnetic fields combined with a parallel DC magnetic field. Glutamic acid was selected because it afforded the most evident effect, even if several aminoacids were tested, in that it was observed that a weak current, in order of tens of nA, could be detected in a solution exposed to a combination of a static magnetic field of 50 μ T and alternating magnetic field 1000 times weaker, whenever the frequency matched the cyclotron frequency of the Glutamic acid in its ionic form [1]. Experiments such as this followed the discovery in 1985 of an unusual resonance effect caused by a simultaneous weak DC and low frequency AC magnetic field in concentrations of calcium in the nervous tissue [2]. Although controversial, the unexpected Zhadin effect has been confirmed by a number of independent researchers [3, 4, 5]

Two main problems arise in trying to provide physical insights of this phenomenon: 1) the very small amount of energy carried by these EMFs is much lower (by as much as an order of magnitude) than $k_B T$ (k_B being the Boltzmann constant and T room temperature) and thus any information carried is hidden in thermal noise, this is known as the KT paradox; and 2) even if an excited state is induced by the perturbation, according to the general accepted models of molecular dynamics, it should rapidly disappear as a result of the thermal collisions that resume when the perturbation is removed.

Furthermore, the attempt of explaining the role of the cyclotron frequency $\Omega = \frac{1}{2} \frac{e}{m} B$, i.e. the characteristic frequency due to the Lorentz force acting on an ion having charge e and mass m in presence of a magnetic field B , have to face the problem arising from the large cyclotron orbits in comparison with the mean free path of the molecules at room temperature.

In 2002, E. Del Giudice et al. [6] proposed that the peculiar structure of water, depicted with a two-fluids model according to the predictions of Quantum Electrodynamics (QED), may account for the Zhadin effect. In such a picture ions in a static magnetic field move in special orbits around the coherence domains (CD) of water, the radius of these orbits being that of the water CD. The motion of these ions is protected by an energy gap, thus removing the disruptive actions of the thermal motion on the trajectory. The addition of a weak alternating magnetic field of frequency ω adds sideband to the fundamental rotational frequency having frequencies $\Omega - n\omega$. The "Zhadin condition" corresponds to $n=1$ and provides the essential mechanism for extracting ions from the cyclotronic orbits.

A complementary experimental technique, such as the vibrational spectroscopy, may provide useful information on the amino acid structure with a high sensitivity and allows to gain a powerful insight on the modifications of the chemical chains involved in the reactions that occur in the changing environment. We therefore selected FT-IR spectroscopy in order to investigate the mechanisms of interaction between weak fields and Glutamic acid using a very simple experimental layout, in order to minimise the effects of other spurious experimental factors.

2. Materials and Methods

Glutamic acid belongs to the group of amino acids that have a COOH group as a functional group. At low pH values, both the carboxylic and the amine groups are protonated, and deprotonated species appear with increasing pH. The acid dissociation constant of the functional COOH group, pK_a is 2.2 while the acid dissociation constant of side chain carboxylic group, pK_R is 4.3, and the amino group dissociation constant is $pK_a=9.7$. Above this value, the amino acid is fully deprotonated as can be seen in Fig.1. The values of the assignments of the spectral parameters, such as band position, band width, and absorption coefficient, in different protonation states are widely available [7], and it is therefore possible to detect the modifications induced by the applied perturbation quite clearly.

Several samples of 0.06M solutions of Glutamic acid in water were prepared. The concentration in double distilled water was 9 mg/ml, and the pH was adjusted by adding HCl or NaOH. Each sample was subdivided into two and each subsample decanted into safe-lock Eppendorf polypropylene tubes, each of 1.5 ml, to prevent contamination by air during the measurements. One tube was used as a control and measured as prepared, and the other was exposed to the ELF-EMF for 10, 20 and 30 minutes, removed from the field and then inserted into an IR spectrometer. During the exposure, the control tube was kept far away from the EMF.

The spectra were recorded using an FTIR/410 Jasco Fourier Transform IR spectrometer.

The samples were exposed to the EMF generated by Helmholtz coils that were oriented vertically in order to produce a horizontal field that was almost perpendicular to the local geomagnetic field B_0 . The amplitude of the field was adjusted to 45 μ T in order to match the amplitude of the geomagnetic field as measured with a Bell-Sypris gaussmeter having a precision of 1 μ Tesla. The coils were kept in the open air and the space within them was maintained at room temperature $T=21\pm 1$ °C, as was the desk where the control samples were stored.

In order to test the hypothesis that the effect was related to the cyclotron frequency, part of the test were done with DC current but also sinusoidal signals were applied to the coils whose frequency was determined by the cyclotron frequency $\omega_c = \frac{1}{2\pi} \frac{q}{m} B_0$. Some test have been also performed using a 50Hz frequency magnetic field.

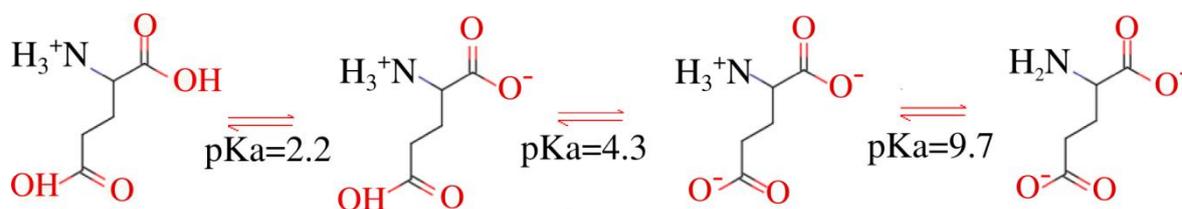


Fig.1 Speciation of Glutamic acid

The electric charge ranges from +1 in the fully protonated form to -2 according to the speciation scheme.

3. Results

The effect of the exposure was checked at different values of pH. The low-pH solution consisted mainly of fully protonated species, whereas the high pH solution mostly contained fully deprotonated species.

The IR spectra at low pH (< 3.2) contain the clear signature of the stretching and bending bands of the protonated COOH especially in the broad band absorption centered around 1235 cm^{-1} which is attributable to the coupled C-O stretch and O-H bend of the COOH group. Figure 2 shows a comparison between the spectra of the pH=1.53 Glutamic acid as prepared and after 10 and 20 min of exposure to the EMF. A subsequent measure repeated after 30 min of exposure shows no further modifications with respect to the spectrum acquired after 20 min, and it can therefore be assumed that the modifications of interest take place within 20 min. The overall effect of the field is the modification of the spectra in the regions of absorption of COOH and of the NH_3^+ head group. However, because of the complexity of the spectra, a simple comparison of the spectra of exposed with those of unexposed samples may be misleading, a kinetic analysis using the ratio of the protonated/deprotonated forms is not straightforward and a more sophisticated statistical treatment such as the Principal Component Analysis is needed in order to verify the statistical difference between exposed and not exposed samples. Another interesting effect of the exposure is the splitting of the band centred around 1630 cm^{-1} . Such a shift witnesses a change in the C-O vibrations which are sensitive to H-bonds.

At intermediate values of pH, the solutions were characterized by very complex IR spectra in which many of the bands were broader and overlapped and the attribution of absorptions to single modes is also more difficult at these pH values.

A straightforward evaluation of the effect is possible at high pH values. Fig.3 shows a comparison between the unexposed and exposed spectra at pH=11.86, at which pH the Glutamic acid should be fully deprotonated; however, the presence of a small band centred around 1650 cm^{-1} reveals the presence of the contribution of the deformation of NH_3^+ in addition to the deformation of NH_2 and the stretching of C=O. Such a band is severely restricted by exposure signalling the shift towards the deprotonated specie.

Similar effects were detected for both the cyclotron frequency of Glutamic acid (i.e., 4.7 and 9.4 Hz respectively for the acidic and basic forms), 50 Hz and DC field. Hence, the hypothesis of the existence of a resonant effect is not supported by these results.

The reversibility of the effect was also investigated by measuring the samples 1 hour after exposure. In all cases the spectra returned to their original, unexposed conditions, which shows that the effect of the EMF is reversible.

A detailed description of the Glutamic acid preparation and measurement together with comprehensive discussion of its IR spectra has been published by the Authors elsewhere [8].

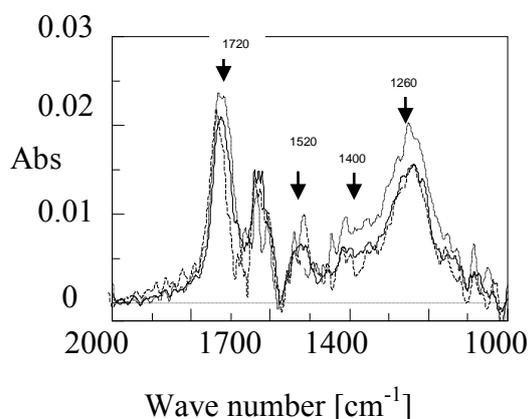


Fig.2 IR spectra of pH=1.53 Glutamic acid in water. Comparison among samples unperturbed (solid line), exposed to the magnetic field for 10 min (dotted) and 20 min (dashed). The major changes in spectra show the absorption for the stretching mode of C=O (1720 cm^{-1}), C-O (1260 cm^{-1}) and of carboxylate (1400 cm^{-1}) and for the bending of NH_3^+ (1520 cm^{-1}).

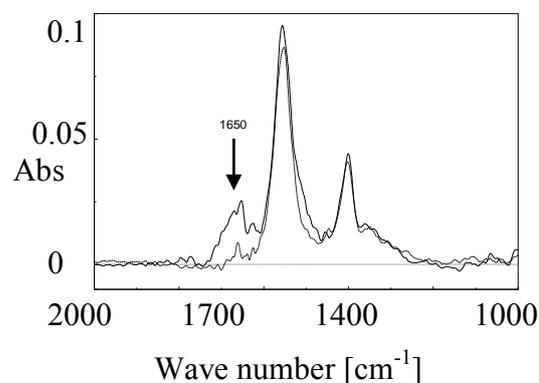


Fig.3. IR spectra of pH=11.86 Glutamic acid in water. Comparison between unperturbed samples (solid line) and samples exposed to magnetic field for 20 min (dotted line).

4. Discussion

The most remarkable characteristic of this effect is the evidence of the existence of a long living state induced by the perturbation, i.e. the effect induced survive to the removal of the perturbation for very long times compared to the characteristic relaxation times of the molecular excited states. The modification of the protonated vs deprotonated population of Glutamic acid in aqueous solution is lasting enough to be revealed by the IR spectroscopy in spite of the very feeble amount of the energy carried by the perturbation.

The energy transfer mechanism described by the semiclassical model of “hopping” along discrete energy levels cannot account for such a figure. It has been shown [9] that long-lived electronic quantum coherence plays an important part in the energy transfer process in biological systems justifying the extreme energetic efficiency of livings. In example, it is known [10,11] that light-absorbing molecules can funnel energy with a near-unit quantum efficiency across a total distance of 20-100 nm. This implies an high degree of correlation among a great number of molecules; the existence of defined phase relationship among molecules can account for a coupling among molecules stronger than to the environment and explain how the energy can be transferred. Nevertheless, the very fast (nanoseconds) relaxing time observed in those systems cannot explain the present work observations, in fact decoherence time as long as tens of minutes should imply a stable mesoscopic coherent structure in our solutions which needs to be identified.

Therefore a new hypothesis may be suggested: the magnetic field effect is mediated by the water structure.

The existence of water in two different grades of co-ordination population was foreseen in the framework of Quantum Electrodynamics (QED), in which the interaction among atoms mediated by electromagnetic fields is explained from first principles [12, 13]. The electrodynamic attraction due to coupling to the electromagnetic fields that exist in condensed matter is responsible for the long-range

forces that explain the existence of the condensed state. These forces are additive to the short-range static attractions that come into play only after the molecules have been brought sufficiently close together by the attraction of the long-range force.

Since the beginning of the last century, it has been widely accepted that the attractive forces that act between two neutral atoms or molecules separated by a distance that is large in comparison with their dimensions (Van der Waals Forces) are electromagnetic in origin. A theory of long-range attractive forces in condensed matter was first proposed in 1955 by Lifshitz, using Quantum Field Theory. He proposed that "the interaction between bodies is considered to take place through a fluctuating electromagnetic field" [14]. In consideration of these fluctuations, Walther Nernst suggested the possibility of tuning the fluctuations of all the components of a system together in order to produce the appearance of a common phase. Several decades later, QED allowed it to be shown that fluctuating electromagnetic fields induce the appearance of a coherent state among atoms or molecules that co-resonate, thereby giving rise to spatial regions known as Coherence Domains (CD) that are characterised by components triggered by a fluctuating electromagnetic field having the same frequency and phase.

At temperatures other than $T=0$, electrodynamic interaction is counterbalanced by thermal collisions, in which components are extracted from a coherent state, and a dynamical distribution between the two phases F_c , F_{nc} of coherent and non-coherent molecules is thereby established, depending on temperature: $F_c(T) + F_{nc}(T) = 1$. According to this view, each atom or molecule belongs to one of the two fractions in a dynamical sense.

However, it is quite clear that the boundary between the two phases represents an interesting discontinuity surface that merits a more detailed investigation.

The particular case of water deserves special consideration. As shown in [15, 16], the excited state of the coherent oscillation of water molecules (12.06 eV) lies just below the ionisation threshold (12.60 eV). This leads to the appearance of an almost delocalised, quasi-free electron for each molecule, which contributes to an easily excitable reservoir of electrons associated with each CD and accessible from outside the surface, see Fig.4.

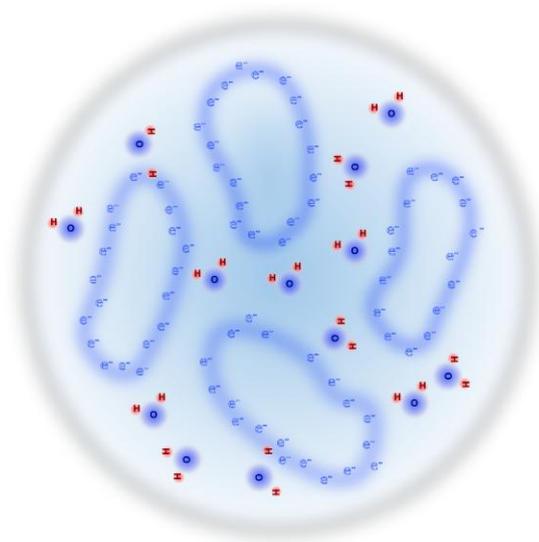


Fig.4 Pictorial representation of the two components model of water. The dimensions of the physical objects shown have just an imaginative meaning.

A number of observations, especially those made by biologists, seem to point to the existence of two kinds of water-water bonds that coexist as microdomains throughout the liquid range of temperatures [17, 18]. Furthermore, the dependence of the Raman [19], IR [20] and x-ray absorption spectra [21] on temperature seem also to indicate the existence of HB configurations that are spectrally distinguishable. In particular, IR spectra of water, in Fig. 5, provide a clear evidence for the existence of substructures, each of which may be assigned to a particular kind of water molecule. In particular, the broad band of absorption related to the OH stretching mode (ranging from 2800 to 3800 cm^{-1}) may be satisfactorily decomposed into three Gaussians, as can be seen in Fig.6, each corresponding to a stretching mode of a particular energy. Because the OH bond is sensitive to the environment, these energies may easily be ascribed to different degrees of co-ordination of the molecules concerned. We propose that low energy mode may easily be identified with the coherent phase, while the high energy population coincides with the non-coherent fraction. The intermediate energy mode may be classified as the fraction of water molecules located on the surface of the CD, whose extension is a function of the temperature T [22].

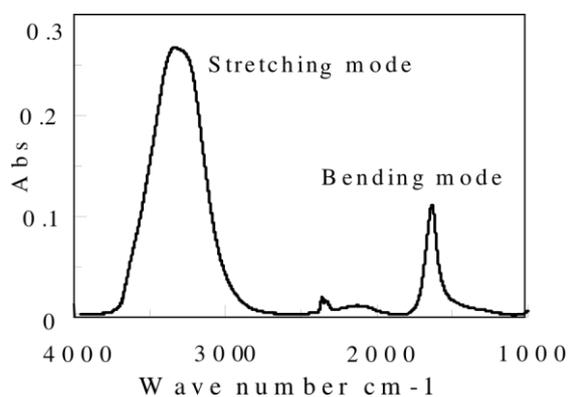


Fig.5 IR spectrum of liquid water at $T=25^\circ\text{C}$.

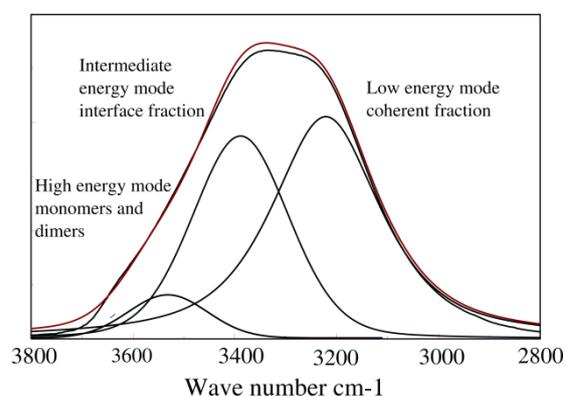


Fig.6 Deconvolution of the stretching mode area. The deconvolution has been done using the Jasco Curve Fitting Analysis software.

It may be noteworthy to ask whether an effect of the magnetic field can be observed also on the structure of liquid water.

We exposed a sample of bi-distilled water to the same EMF used on the Glutamic acid solution using the same experimental procedure. The difference between the spectra of non exposed and exposed samples, even though quite small, is clear and meaningful. In Fig. 7 the spectra are shown together with their difference amplified by a factor of 10 in order to be clearly visible on the same graph. It is interesting to observe that the field affects in a different way the three populations described above. In particular, the larger effect is on the lower energy part of the stretching absorption band, which is related to the coherent population which decrease increasing the fraction of the intermediate population while the “free molecules” seem not to be affected by the field in the range of the errors, see Fig. 8. The effect is very small and just a little over the errors, however we believe that it is useful to be cited as example to show the way to understand the origin of the effect of weak magnetic fields on the water solutions of amino acids and ions. Larger effect, as high as the reduction of 20% in the maximum light scattering intensity, has been observed with a pulsed (1-500Hz) low-frequency field having a magnetic field density 20 times greater than which used in our experiment [23].

The role of the magnetic field on water seems actually to be that of a “chaotrope” (disorder-maker) agent.

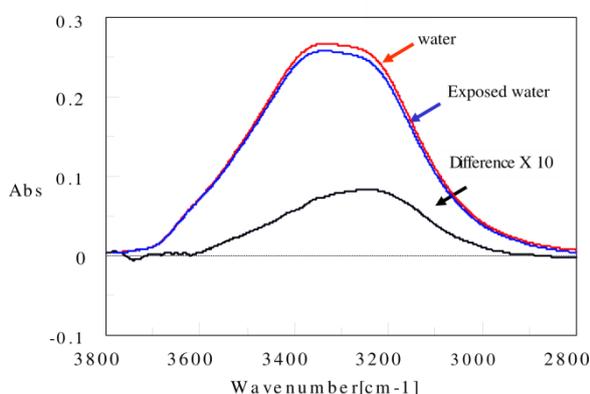


Fig. 7. IR spectra of liquid water, OH stretching band. Comparison between exposed and non-exposed sample (see text for details). The difference is plotted with an amplification factor of 10 in order to be visible on the graph.

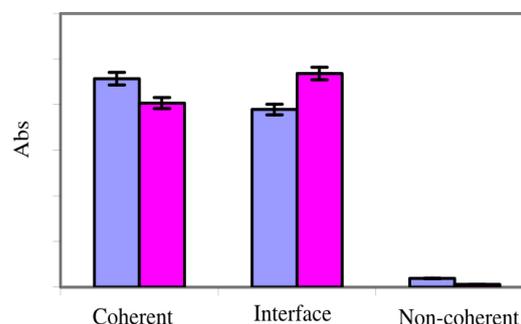


Fig.8. Comparison between the areas below the Gaussians corresponding to the different populations: coherent, intermediate and non-coherent. Blue bars refer to non exposed water; magenta bars to the exposed water

5. Conclusions

The detailed mechanism which leads to the de-protonation of the amino acid is still unclear, however we have observed how the effect of the weak EMF on the solutions of Glutamic acids is mediated by the water: the exposure to the field seems to modify the structure of water in terms of the relative abundance of the coherent and non-coherent fraction.

The amount of water molecules forced out the coherent phase increases the quantity of molecules at the boundary of the CDs, that we have called intermediate population. Such a region is the most reactive place in liquid water because of the abundance of quasi-free electrons and of the existence of the evanescent tail of the electrodynamic field which acts as the source of the ponderomotive force acting on the ions in proportion to the inverse of their masses, thereby inducing the formation of a zwitterion form of water $H \equiv O-H$ [24]. Because of its electrical charges, the zwitterion will be highly soluble in water, in that the hydrogen atoms in the bulk non coherent water immediately surround the negatively charged group of the zwitterion and the oxygen atoms immediately surround the positively charged group of the zwitterion, thereby thoroughly dissolving it. This process could, finally, alters the ionic product of water $[H^+][OH^-]$ and affect the speciation scheme of the Glutamic acid. A detailed exam of the dynamics of this process is needed and will be faced in a next paper.

We have shown that the consequence of a weak EMF on the Glutamic acid has a plausible origin in the disorder-maker result of the field on the coherent structures of water. A fraction of molecules is expelled by the CD under the effect of a EMF and a shift in the quantitative distribution of the Glutamic acid species occur. This provides the essential mechanism for inducing in the solution the temporary injection of ions which may, in due course, give origin to the so called “Zhadin effect”.

Acknowledgments

We wish to tank Emilio Del Giudice for many bright and thought-provoking conversations.

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