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## **Aqueous Solutions Exposed to Electric and Magnetic Fields: Spectroscopic and Biochemical Analysis**

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### **Abstract**

*Aqueous solutions in apparatuses equipped with electrodes and permanent magnets can be subjected to electric and magnetic fields. By collecting the liquid in the vicinity of the electrodes, it is possible to obtain fractions of water with different pH and ionic content. The effect of electric and magnetic fields on the bulk properties of water is still under scrutiny.*

*At room temperature, water molecules are usually linked together by hydrogen bonding, and surround dissolved ions to form solvation nuclei. Upon exposure to electric and magnetic fields, these organized domains might be perturbed. Modifications of micro-environmental and bulk properties can be studied by analyzing the behavior of fluorescence centers, by studying the optical activity of dissolved molecules, as well as by exploring the infrared spectroscopy of total water.*

*We have observed that the fluorescence characteristics of Terbium in water depend on the fraction of water in which it is dissolved. The specific optical activity of ascorbic acid is increased to 33° when dissolved in the one of the fractions. The near-infrared absorption of gels and emulsions is larger around 1400 nm and 1900 nm when they contain low pH treated water. This result is in agreement with what is expected if the treatment increases the activity (i.e. the number of freely oscillating molecules) of water.*

*One could expect that treated water with high activity increases the availability of a solute, which should for instance, achieve the same biochemical action at a lesser concentration. As an example of the technological interest of this treatment, we have measured the antioxidant properties of butylated hydroxy toluene, as well as the antichemotactic activity of caffeine in vitro. When solutions of these two compounds are treated prior to the test, equivalent anti-oxidant or anti-chemotactic activity was achieved at 1.5 to 5-fold lower concentrations.*

### **Introduction**

Bulk and domain properties characterize the behavior of fluids. In fluids composed of polar molecules, directional attraction caused by hydrogen bonds and short-range repulsive forces contribute to the overall orientation of neighboring molecules. The anomalous kinetic and thermodynamic properties of water have been interpreted in terms of underlying

structural causes. Water molecules can form clusters in the vapor phase (1) and can interact to achieve ordered structures on hydrophobic surfaces (2). Relatively mild alterations of environmental conditions, such as temperature, allow one to observe modifications in some physical-chemical properties of water: for instance total freezable and non-freezable (also called “structured”) water can be characterized by Nuclear Magnetic Resonance in concentrated protein solutions by lowering the temperature to around 273 °K (3). It has been suggested that the bulk behavior of total water can be influenced by the behavior of domains of “structured” water (3). It was also suggested that the equilibrium of H<sub>3</sub>O<sup>+</sup> and OH<sup>-</sup> ions in water could subsist as long as no ordering electric field was applied to the sample (4,5). Mathematical models have been developed to describe the observed effects on water of external electric fields on solvated ion mobility and size (6,7, 8).

This paper describes some observations relative to the physical and biochemical properties of solutes in aqueous solutions subjected to electric and magnetic fields and separated into an alkaline and acidic fractions using technologies similar to previously described ones (9).

## Material and Methods

### Treatment of water with electric and magnetic fields.

The experimental apparatus consists substantially of a voltammeter in the form of a parallelepiped ( 30 cm x 15 cm x 35 cm), equipped with two cotton twills (Testfabrics, West Pittston, Pennsylvania) symmetrically arranged relative to the axis of the segment joining the two electrodes. Thus the parallelepiped is divided in three volumes, one containing the anode, the other containing the cathode and the third being the one delimited by the two twills. The potential difference between the electrodes was fixed at V= 28 V. Feed water enters the apparatus *via* an opening located at the center of the bottom face of the apparatus itself. Before entering the apparatus, feed water is circulated in a constant magnetic field of 190 Gauss (0.019 Tesla). The flow rate is of 20 liter/hour.

Water was withdrawn from two outlets located in the vicinity of the electrodes. For the sake of brevity we call the water collected in the vicinity of the anode “I-water”, and “S-water” the fraction collected in the vicinity of the cathode (10). A solute can be added to feed-water, and its concentration can be measured in I- and in S-water.

### Physical Chemical measurements

Fluorescence spectra were recorded with a pulsed laser fluorescence spectrophotometer. A Kr-F excimer laser (Compex 110, Lambda Physik, Fort Lauderdale, Florida ) was utilized to excite Terbium ( $\lambda_{\text{ex}} = 248 \text{ nm}$ ) in feed water, I-water or S-water. This laser was selected because it is able to emit coherent radiation at 248 nm, a wavelength corresponding to one of the strong transitions of Terbium in LiYF<sub>4</sub> matrices ( $\sim 39,000$  and  $\sim 47,000 \text{ cm}^{-1}$ ) (11). The sample was irradiated in a quartz cuvette (section: 1 cm x 1 cm ) and the emitted fluorescence was collected at 90° relative to the incident beam. Fused silica optical fibers were used to connect a 0.25 m, 300 lines/mm grating spectrograph model 01-002 AD (PTI, Lawrenceville, New Jersey). Spectra were recorded on an optical multichannel analyzer system OMA III system model 1406 (EG&G, Wellesley, Massachusetts) equipped with an EG&G PARC model 1420 UV intensified photodiode array detector. Wavelength calibration of the system was performed with a low pressure Hg lamp.

Atomic absorption spectrometry of samples was performed by Schwartzkopf Microanalytical Laboratory Inc. (Woodside, N.Y.).

The optical rotary dispersion of ascorbic acid at 25 °C was measured for the Sodium D line with a fully automatic Schmidt and Haensch saccharimeter (Topac Instrumentation, Hingham MA).

Raman spectra were obtained by irradiating the samples with blue light (488 nm) from an Ar laser and scanning the scattered radiation in the range between 2800 cm<sup>-1</sup> and 3800 cm<sup>-1</sup>. The measurements were performed with a Raman spectrometer in the laboratory of Dr. Dang Vinh Luu, Institut Mediterranéen de Documentation, d'Enseignement et de Recherche sur les Plantes Medicinales (IMDERPM) Montpellier, France.

Infrared absorption measurements were performed with a Bio-Rad Dynamic Alignement FT-IR spectrometer (Cambridge, Massachusetts). Near-infrared reflectivity was measured with a NIR system Pharma Model 5000 (Perstorp Analytical, Silver Spring, Maryland) equipped with a smart probe module in a 0.5 mm cuvette. The gels for infrared spectroscopy absorption were prepared by mixing water (I- or S- or distilled) with 0.5% carbopol (BF Goodrich Company; Specialty Polymers & Chemicals Division, Cleveland, Ohio) in the presence of 0.15% amino-methyl propane diol. The emulsions for near-infrared reflectivity measurements were prepared by replacing distilled water with I-or S-water in a commercially available skin-care cream.

### **Biochemical measurements**

The antioxidant activity of butylated hydroxy-toluene (BHT) was measured as described (12, 13) Briefly, Phosphate Buffered Saline (PBS) was prepared in either distilled water, feed water, I-water, S-water or mixtures thereof. Phosphatidylcholine in ethanol (with or without 0.003% BHT) was injected into PBS to form liposomes. Liposomes were exposed to UVC radiation from a germicidal lamp (Atlantic Ultraviolet, Bay Shores, New York) for two hours at room temperature. The fluence (2.8 mW/cm<sup>2</sup>) was measured with a UVX radiometer (Ultra Violet Products, San Gabriel, California). Lipid peroxidation was determined by reacting the samples with thio-barbituric acid and measuring the absorption at 532 nm. Experiments were performed in quadruplicate.

The antichemotactic activity of caffeine was measured as described (14, 15, 16). Briefly, the assay measures the movement of polymorphonuclear leukocytes (PMN) toward fMetLeuPhe (a chemo-attractant). The assay is performed using the Boyden chamber apparatus consisting of two superposed chambers separated by a filter. The upper chamber contains freshly harvested human PMNs. The pores of the filter have a size appropriate for the cells to actively crawl through them, but not large enough for the cells to physically fall through. The lower chamber contains the chemo-attractant. The cells are incubated for an hour in the upper chamber at 37° C in the presence or in the absence of caffeine. At the end of the incubation the filters are removed and stained. The cells having migrated to the leading front into the filter are counted under the microscope. A quantitative estimate of the anti-chemotactic activity (ACA) of a compound is given by

$$ACA = [1 - N(t)/N(c)] \times 100$$

where: N(t) is the number of PMN having migrated in one hour to the leading front in the presence of caffeine and N(c) is the number of cells having migrated to the front line in the control experiment.

## Results and Discussion

### Physical-Chemical Data

#### Fluorescence

The water collected at the outlets in the vicinity of the cathode and anode are called S-water and I-water respectively. The electrolytic properties of these waters depend of the composition of feed water. In **Table 1** it is shown that the properties of one particular feed water are modified upon treatment and are compared with the properties of distilled water. One interesting observation is that one of the consequences of the treatment is the change in conductivity, which does not exactly match the measured modifications in ionic composition. When feed water contains Terbium at a concentration of 12 ppm, the treatment yields I- and S- waters containing 3 and 9 ppm of Terbium respectively (Terbium was added in the form of Terbium Chloride). The fluorescence of Terbium is reported in **Figure 1**.

**Table 1.** Electrolytic properties of water subjected to electric and magnetic fields Conductivity, pH and cationic composition of Feed Water, I- and S- water, and distilled water. In this case, feed water contained Ca<sup>++</sup>, Mg<sup>++</sup>, Na<sup>+</sup> K<sup>+</sup> and the corresponding counterions with an overall ionic strength  $I = 4.04 \times 10^{-3}$ .

Sample	Conductivity (μS)	pH	Ionic composition(ppm)			
			Ca <sup>++</sup>	K <sup>+</sup>	Mg <sup>++</sup>	Na <sup>+</sup>
Feed water	425	7,03	22	11	5.3	33
I-water	1870	2,3	2.2	0.3	1.2	6.3
S-water	1100	11,2	31	13	0.12	48
Distilled water	9	5,35				

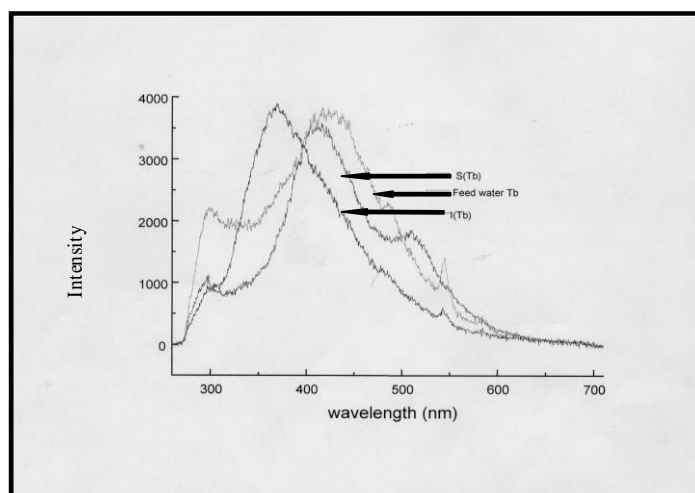


Figure 1

**Figure 1.** Fluorescence of Terbium in water.

A 12 ppm solution of Terbium in feed water was subjected to electrolytic treatment under magnetic field and the collected I-water and S-water contained 3 and 9 ppm of Terbium respectively.

Solutions were exposed to coherent radiation from a KrF excimer laser ( $\lambda_{ex}$  248 nm).

The spectra are subtracted of the background noise, generated by the three kinds of water without Terbium, but are not corrected for the concentration of Terbium.

The arrows point to the fluorescence spectra of Terbium in S-water [S(Tb)], of Terbium in feed water (Feed water Tb) and of Terbium in I-water [I(Tb)].

It can be observed that Terbium fluorescence is similar in feed water and in S-water (although the fine structures of the two spectra are somehow different). On the other hand, the fluorescent spectrum of Terbium in I water is dramatically changed as the  $\lambda_{\text{max}}$  of emission shifts from 420nm to nearly 360 nm. When Terbium Chloride is dissolved in I-water (i.e. when I-water is prepared as described and is afterwards added with Terbium Chloride) then its fluorescence spectrum overlaps with the fluorescence spectrum of Terbium in feed water (compare Figure 1 with **Figure 2**). Similarly, when  $\lambda_{\text{ex}}$  is 220 nm, the emission spectra of I- and S- water prepared from Terbium containing feed water are different. They both display a structure characterized by the enhancement of the peak centered at 290 nm. Terbium-containing S-water also displays an enhancement of the emission peak at 520 nm (data not shown).

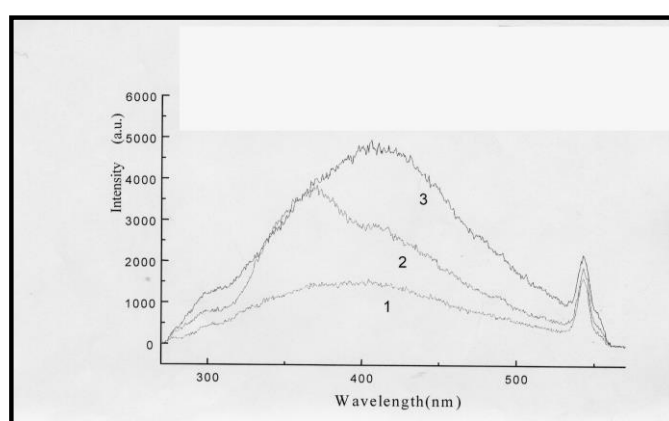


Figure 2

**Figure 2.** Effect of the preparation procedure on the fluorescence of Terbium solutions. Curve 1: emission spectrum of I-water. Curve 2: emission spectrum of I-water prepared from Terbium-containing feed water. Curve 3: emission spectrum of I-water added with terbium ( $\lambda_{\text{ex}} = 248 \text{ nm}$ )

### Optical Activity

Other spectroscopic properties of other solutes are affected when the solution is treated as described. Ascorbic acid is known to be optically active. The specific optical activity of ascorbic acid, for the line D of Sodium at 25 °C in feed water is  $[\alpha] = 21^{\circ} \pm 0.5^{\circ}$ . Ascorbic acid dissolved in S-water has a specific optical activity  $[\alpha] = 33^{\circ} \pm 1^{\circ}$ . The specific optical activity of ascorbic acid dissolved in I-water does not differ from the specific optical activity of ascorbic acid in feed water. It has to be noted that the pH of waters after the addition of 1% ascorbic acid was 2.6 – 2.8, irrespective of the type of water (I or S or feed) to which it was added, so that the result cannot be attributed to a simple pH effect.

### Raman Spectroscopy

The Raman spectra of distilled water, I-water, S-water and feed water and their deconvolution components, are reported in **Figure 3**. No relevant difference can be observed, neither in the shape of the curve nor in the values of the parameters characterizing the deconvolution components. The integral of the curves is different for different waters, the largest being the one relative to distilled water and the smallest being the one relative to feed water. This result can be the consequence of differences in the ionic strength of the four samples and might indicate a smaller probability for water in a solvated state to undergo vibrational transitions.

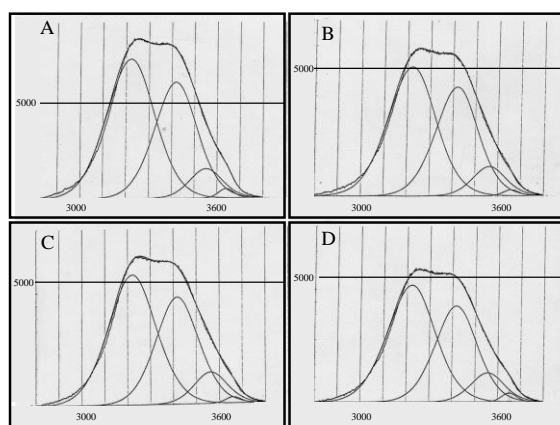


Figure 3

**Figure 3.** Raman spectra of different waters. A: distilled water, B: I-water, C: S-water and D: feed water.

### Infra-red spectroscopy

The changes in luminescence and rotary power of molecules or ions are the consequences of the influence of the environment on their electronic transitions. In order to learn how the treatment of feed water with electric and magnetic fields modifies the environment of dissolved solute molecules (i.e. the population of water molecules) we have investigated the IR spectra of I-water, S-water and feed water. Infrared absorption fails to point out spectral differences between feed water, I-water and S-water, except possibly in the region between  $1000\text{ cm}^{-1}$  and  $1500\text{ cm}^{-1}$  (**Figure 4**). On the other hand, spectral differences can be observed when the waters are mixed with polymers able to bind water and form a gel (**Figure 5**). In this case, the strength of the oscillators at  $1450\text{ nm}$ ,  $2000\text{ nm}$  and  $2400\text{ nm}$  are larger for gels containing I-water than for gels of the same polymer in S- or distilled water. Similar results, although to a lesser extent, are obtained by near infrared reflectivity measurements. **Figure 6** shows the reflectivity spectra of an emulsion prepared with I-water or S-water or distilled water. It can be seen that in I-water the  $\log(1/R)$  is slightly larger (if at all) for the transitions at  $1450\text{ nm}$ ,  $2000\text{ nm}$  and  $2400\text{ nm}$ , in keeping with the results in **Figure 5**. Indeed, where the absorption spectrum presents a maximum, the reflectivity curve is expected to display a minimum, and  $1/R$  is expected to present a maximum.

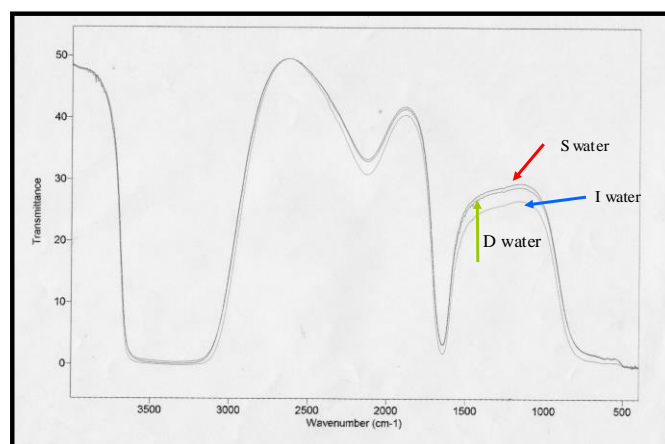
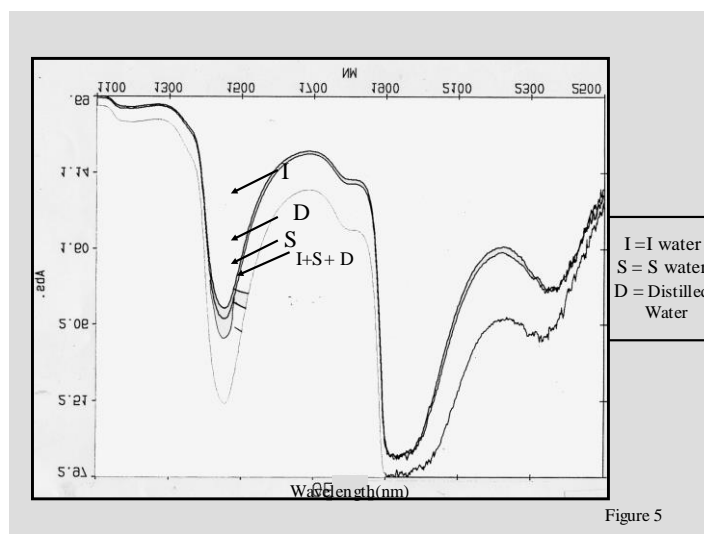
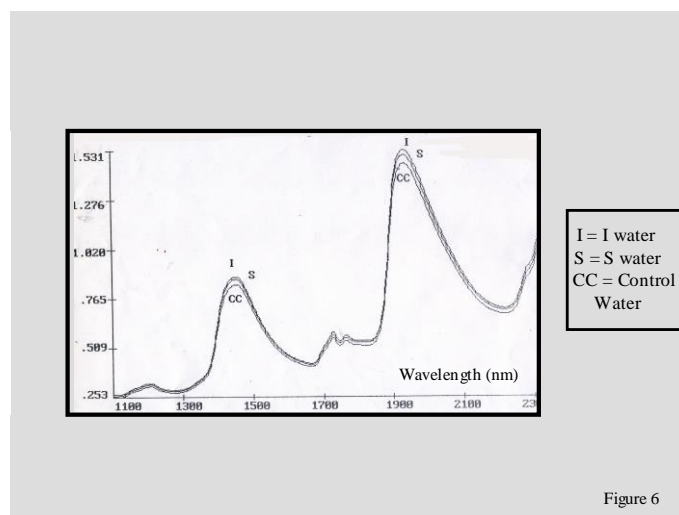


Figure 4

**Figure 4.** Infrared spectrum of different waters. The arrows point to the Infrared spectra of distilled water (D), I-water (I) and S-water (S).



**Figure 5.** Infrared spectrum of water-carbopol gels. IR absorption spectra. The arrows point to the spectra of gels prepared with distilled water (D), I-water (I) S-water (S) and a combination of equal amounts of the three (I+S+D).



**Figure 6.** Infrared reflectivity spectrum of one emulsion. Reflectivity (R) was measured for one emulsion prepared with three types of water, and  $\log(1/R)$  is plotted *versus* wavelength. I: emulsion in I water, S: emulsion in S water, CC: emulsion in distilled water.

## Biochemical Data

### Anti-oxidant Activity of Butylated Hydroxy-Toluene

Liposomes containing BHT were prepared as described in the Materials and Methods section. They were irradiated with UVC radiation at room temperature and the amount of lipid peroxides was determined by quantitative measurements of thio barbituric acid reacting species. The results are displayed in **Table 2**. It appears that in these experimental conditions, BHT affords a 40% protection against UV-induced lipid peroxidation. When the liposomes are prepared with I-water or S- water or with a mixture of the two, the inhibition of the

peroxidation of lipids increases to 50%-60%. The p value is less than 0.05. When in the presence of I-water and/or S-water, the anti-oxidant activity of BHT is therefore increased by a factor 1.25 to 1.5, and this increase is statistically significant.

**Table 2.** Enhancement of anti-oxidant activity of Butylated Hydroxy Toluene  
Butylated Hydroxytoluene mixed with phosphatidylcholine in ethanol was injected into Phosphate Buffered Saline prepared with distilled water, I-water, S-water or a 60%-40% mixture of I-water and S-water. Upon exposure to UVC, the lipid peroxidation was measured.

Sample	Inhibition of peroxidation (%)
BHT in distilled water	40
Distilled water	0
BHT in S-water	46
S-water	0
BHT in I-water	51
I-water	10
BHT in 60% I-water 40% S-water	58%
60% I-water + 40% S-water	0

#### **Anti-chemotactic Activity of Caffeine.**

The phenomenon of chemotaxis is essential in all the inflammatory processes, where immune cells follow the gradient of signaling molecules to reach the object to scavenge. This physiological phenomenon can be mimicked *in vitro* because, when in the presence of fMetLeuPhe, human polymorphonuclear cells (PMN) move against its concentration gradient. Several anti-irritants and anti-inflammatory agents such as caffeine act by interfering with PMN chemotaxis. Apparatuses like Boyden chambers allow one to measure both the fraction of PMN migrating against a concentration gradient, as well as the distance migrated. In **Table 3** the data are reported relative to the movement of human PMN exposed to fMetLeuPhe in the presence of caffeine in feed water or in a mixture of I- and S- waters. Mixtures of I- and S- waters were used mainly to overcome the need to change the buffer concentration, which is necessary when only one type of water (I- or S-) is used. 60% I-water and 40% S-water allowed us to maintain the pH of the medium, thus adding no extra variable in the experimental setting. It appears that a mixture of 60% I-water + 40% S-water is able to reduce the chemotactic movement of PMN and that caffeine dissolved in this mixture is a better antichemotactic agent than caffeine in feed water. This kind of experiment has been repeated in four independent occasions. The antichemotactic activity of caffeine was assessed by measuring the migration distance, as well as the number of cell in the migrating front. In every set of experiments we have observed that the migration of the PMN was inhibited 25-50% by mixtures of I- and S- waters. Moreover, the antichemotactic effect of caffeine at concentrations between 0.01 % and 1 % was enhanced up to three-fold when caffeine was previously diluted in a mixture of 60% I-water and 40% S-water, without affecting the number of cells in the migrating front.



**Table 3.** Effect of I-water and S water on the antichemotactic activity of caffeine.

<b>Experiment 1</b>		
<b>Caffeine %(w/v)</b>	<b>Solvent</b>	<b>migrated distance</b>
0	feed water	0,6
0,2	feed water	0,05
0,2	60% I-water+40% S-water	0,02
<b>Experiment 2</b>		
<b>Caffeine %(w/v)</b>	<b>Solvent</b>	<b>residual mobility (%)</b>
0	60% I-water+40% S-water	42
0,1	60% I-water+40% S-water	5
1,0	60% I-water+40% S water	7
1,0	feed water	15

### Analysis of the data and conclusion

Several bulk properties of water are characterized by unusual behaviors, the understanding of which has been sometimes elusive. In this paper we have described results relative to the physical-chemical properties of solutions in which the solute was dissolved in water prior to or after treatment with electric and magnetic fields. The experiments led to the observation that some physical-chemical properties of the solutes can be changed by such a treatment. The fluorescence properties of a solution of Terbium in feed water are different from the ones observed after that the same Terbium solution is processed as described and separately collected near the electrodes. A solution of Terbium in I-water (i.e. of Terbium dissolved in I-water) does not have the same fluorescence characteristics as a solution of I-water prepared from Terbium-containing feed water. These results indicate that the treatment of a solution with magnetic and electric fields modifies the optical properties of a solute. The modification of these optical properties might be the consequence of the modification of the distribution of charges and dipoles surrounding the fluorescent chromophore, as it could be, if the organization of the solvation molecules around the Terbium ions were changed. For the sake of brevity, therefore, it is not unreasonable to speak of “structurally modified” water when speaking of water treated as described in the Materials and Methods section.

The structural modifications can be local (e.g. they can lead to the formation of transient fluorescence centers able to modify the overall observed luminescence from Terbium) or could affect the bulk of the solvent. To learn about the extent of these modifications, we have undertaken the measurement of the infrared scattering, absorption and reflectivity spectra of “structurally modified” waters. Not to our surprise, the Raman and the IR spectra of I-water and of S-waters did not significantly differ from the IR spectrum of distilled water. In order to have better chances to observe a difference, we prepared gels and emulsions. The rationale for doing so is that the inferred structural modifications might affect only a subset of the water molecules and that one might increase the fraction of water molecules having undergone an organizational modification by adding polymers able to coordinate large amounts of water. One of the chosen polymers serendipitously allowed us to meet the experimental conditions in which the IR spectrometer could detect some differences in the strength of the oscillators involved in three transitions.

One of the possible interpretations of these results is that the treatment of feed water with electric and magnetic fields increases the number of water molecules which do not bind to the added polymer and are free to resonate with three IR frequencies. One might then tentatively conclude that “structurally modified” water contains a smaller proportion of

molecules trapped in organized domains. This is of course but one of the possible interpretations of these results.

If this is the case, however, one might expect that solutions of biochemically active molecules might have their activity enhanced. Indeed, with more water molecules free to resonate, a solute might be more available to reach and interact with its targets or receptors. We observed that BHT in liposomes prepared with “structurally modified” waters has an increased anti-oxidant activity. Furthermore, caffeine increases its anti-chemotactic activity when the experiments are performed in “structurally modified” water. The interpretation of these biochemical results is complex. Indeed, the presence of “structurally modified” water could affect the redox potential of lipid molecules as well as the crawling capability of PMNs, but other interpretations are possible. Yet, whatever the mechanism, the “structurally modified” waters seem to exhibit puzzling pharmacological potentials.

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