

## Article

# Research of Water Molecules Cluster Structuring during *Haberlea rhodopensis* Friv. Hydration

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**Abstract:** *Gesneriaceae* plant family is comprised of resurrection species, namely *Boea hygrometrica* and *Paraboea rufescens*, that are native to the Southeast Asia and *Haberlea rhodopensis*, *Ramonda myconi*, and *Ramonda serbica*, which are mainly found in the Balkan Peninsula. *Haberlea rhodopensis* is known to be able to survive extreme and prolonged dehydration. Study was carried out after the dried plant *Haberlea rhodopensis* Friv. had been hydrated and had reached its fresh state. Two juice samples were collected from the plant blossom: The first sample was prepared with 1% filtered water through a patented EVOdrop device. Then the sample was saturated with hydrogen with EVOdrop booster to a concentration of 1.2 ppm, pH = 7.3, ORP = −390 mV. This first sample was prepared with filtered tap water from Sofia, Bulgaria. The second sample, which was a control one, was developed with tap water from Sofia, Bulgaria, consisting of 1% solutions of *Haberlea rhodopensis*. A study revealed that during the drying process in *H. rhodopensis* the number of free water molecules decreases, and water dimers are formed. The aim of our study was to determine the number of water molecules in clusters in 1% solutions of hydrated *H. rhodopensis* plants. Results were analyzed according to the two types of water used in the experiment. The EVOdrop device is equipped with an ultranano membrane and rotating jet nozzle to create a vortex water and saturation thanks to a second device EVObooster to obtain hydrogen-rich water. In the current study Hydrogen-rich water is referred to as Hydrogen EVOdrop Water (HEW). Research was conducted using the following methods—spectral methods non-equilibrium energy spectrum (NES) and differential non-equilibrium energy spectrum (DNES), mathematical models, and study of the distribution of water molecules in water clusters. In a licensed Eurotest Laboratory, the research of tap water before and after flowing through the EVOdrop device was proven. Studies have been carried out on the structuring of water molecule clusters after change of hydrogen bond energies. The restructuring comes with rearrangement of water molecules by the energy levels of hydrogen bonds. Local extrema can be observed in the spectrum with largest amount of water molecules. The structural changes were tested using the NES and DNES spectral methods. The conducted research proved that the application of EVOdrop device and EVObooster changes the parameters of water to benefit hydration and health.

**Keywords:** EVOdrop device; hydrogen-rich water; NES; DNES; *Haberlea rhodopensis* Friv.

## 1. Introduction

*Haberlea rhodopensis* Friv. is a Balkan endemic plant primarily found in the Rhodope Mountains in Bulgaria. It is known for its drought resistance and biosis–anabiosis–biosis cycle [1]. It is a protected species under the Law on Biological Diversity in Bulgaria [2]. The plant is listed in the Red Book of Bulgaria in the category of rare species, Balkan endemic [3]. The biosis–anabiosis–biosis cycle may unveil new properties of the water. A study revealed that, during the drying process in *H. rhodopensis*, the number of free water molecules decreases, and water dimers are formed (Kuroki, et al., 2019) [4]. After contact with water, *H. rhodopensis* plants straighten up. However, the reasons for this phenomenon are not fully known. We consider it to be possible to evaluate hydration with NES and DNES methods and based on water properties when applied after dehydration of *H. rhodopensis*.

The equipment used for these studies is the EVOdrop device with an ultranano membrane [5] and rotating jet nozzle for vortex water [6]. The ultranano membrane is a competitor to the reverse osmosis membrane. The rotating jet nozzle for vortex water [7] is equipped with three injection nozzles according to the golden ratio and algorithm. Some research has been conducted on the effects of magnetic field on water [8–10] and nanomembranes [11,12].

Hydroxyl groups (-OH) in H<sub>2</sub>O molecules are polar. A covalent bond of water molecules is a chemical bond that involves the sharing of electron pairs between O and H atoms. Between H<sub>2</sub>O molecules, there are electromagnetic hydrogen bonds. Hydrogen bonds are weaker than covalent bonds. The water molecules could be bonded into complex intermolecular clusters, described by a general formula (H<sub>2</sub>O)<sub>n</sub>. The NES and DNES spectral methods are related to the research of parameters of hydrogen bonds, with estimation of the effects in a 1% solution (*v/v*) *H. rhodopensis* in filtered water, prepared with a patented EVOdrop device and saturated with hydrogen with an EVOdrop booster. As a control, 1% *H. rhodopensis* with tap water was used.

Research by Smith et al. and Keutsch and Saykally showed water clusters with 3 to 50 water molecules [13,14]. Different water cluster models are also described in the investigations conducted by Fowler et al. [15], Shu et al. [16], Chaplin [17], Sykes [18], Liu, Cruzan and Saykally [19], Choi and Jordan [20], Loboda and Goncharuk [21] and Timothy and Zwier [22].

The following methods have been generally used to study water clusters—<sup>1</sup>H NMR [23,24], far-infrared [25], vibration–rotation–tunneling (VRT) [19], neutron diffraction [26], and the SCC-DFTB Method [18,27]. A cluster model at ( $E = -0.1387$  eV) ( $\lambda = 8.95$   $\mu\text{m}$ ) ( $\tilde{\nu} = 1117$   $\text{cm}^{-1}$ ) has been proposed with 20 water molecules in a dodecahedral structure with diameter of the circumscribed sphere equal to 0.822 nm [28–30]. The basis of this research is the NES and DNES methods of Antonov et al. [31–33].

It is accepted that the aqueous solutions can undergo autoprotolysis, i.e., the H<sup>+</sup> proton is released from the H<sub>2</sub>O molecule and then transferred and accepted by the neighboring H<sub>2</sub>O molecule, resulting in the formation of hydronium ions as H<sub>3</sub>O<sup>+</sup>, H<sub>5</sub>O<sub>2</sub><sup>+</sup>, H<sub>7</sub>O<sub>3</sub><sup>+</sup>, H<sub>9</sub>O<sub>4</sub><sup>+</sup>, etc. Thus, water should be considered as an associated liquid composed of a set of individual H<sub>2</sub>O molecules, linked together by hydrogen bonds and weak intermolecular van der Waals forces [19]. The simplest example of such a complex can be a water dimer.

The research has shown that 1% solution of *H. rhodopensis* in water filtered by EVOdrop with hydrogen has the highest hydrogen bonds energies among water molecules at (−0.1112 eV; 11.3  $\mu\text{m}$ ; 887  $\text{cm}^{-1}$ ). The EVOdrop device has a nano membrane and rotating jet nozzle for vortex water and saturation, thanks to a second device EVObooster for hydrogen-rich water. There is an increase in the local extremum in the spectrum compared to the control sample with 1% solution of *H. rhodopensis* in tap water filtered by EVOdrop with hydrogen.

The local extremum at (−0.1112 eV; 11.3  $\mu\text{m}$ ; 887  $\text{cm}^{-1}$ ) is related to calcium conductivity [32,34]. Some studies reveal that signaling-related genes encoding a calcium channel protein are activated after hydration of *H. rhodopensis* [34]. Calcium is involved in the

regulation of DT mechanisms [35]. The calcium ions in xyloglucan enhance strength and flexibility [36].

The aim of the study was to show that water filtered by EVOdrop with hydrogen has better hydration properties and structuring of water molecules into clusters.

The expected applications are for positive effects on human health.

## 2. Results and Discussion

The reported results are average values between the results of the application of the device to test 10 different water samples after treatment with the EVOdrop devices and 10 control water samples. For each sample, 10 measurements were performed. There was a statistically significant difference between the results of the two groups of samples and the control samples according to Student's *t* test with  $p < 0.05$ .

### 2.1. Mathematical Models of Clusters in a 1% Solution of *Haberlea rhodopensis* Friv. Blossom Extract

A mathematical model of the number of water molecules [37–39] according to the energy of hydrogen bonds in a 1% solution of *H. rhodopensis* blossom extract has been developed (Table 1; Figure 1).

**Table 1.** Distribution of the number of water (H<sub>2</sub>O) molecules in a 1% solution of *Haberlea rhodopensis* Friv. blossom extract in EVOdrop-filtered tap water saturated with Hydrogen (H<sub>2</sub>) and in the control sample of tap water from Sofia, Bulgaria according to the energy of hydrogen bonds.

–E(eV) x-Axis	Number of Water Molecules		–E(eV) x-Axis	Number of Water Molecules	
	1% Solution <i>H. rhodopensis</i> (Sample)	1% Solution <i>H. rhodopensis</i> (Control Sample)		1% Solution <i>H. rhodopensis</i> (Sample)	1% Solution <i>H. rhodopensis</i> (Control Sample)
0.0912	2	2	0.1162	0	6
0.0937	4	5	0.1187	4	8
0.0962	2	7	0.1212	9 <sup>2</sup>	4 <sup>2</sup>
0.0987	4	5	0.1237	5	4
0.1012	6	3	0.1262	4	6
0.1037	3	8	0.1287	4	6
0.1062	5	4	0.1312	6	4
0.1087	6	6	0.1337	6	7
0.1112	15 <sup>1</sup>	3 <sup>1</sup>	0.1362	6	5
0.1137	1	5	0.1387	8 <sup>3</sup>	2 <sup>3</sup>

Notes: <sup>1</sup>  $E = -0.1112$  eV or ( $\lambda = 11.3$   $\mu\text{m}$ ;  $\tilde{\nu} = 887$   $\text{cm}^{-1}$ ) is the local extremum for calcium conductivity. <sup>2</sup>  $E = -0.1212$  eV or ( $\lambda = 10.23$   $\mu\text{m}$ ;  $\tilde{\nu} = 978$   $\text{cm}^{-1}$ ) is the local extremum for anti-inflammatory effects. <sup>3</sup>  $E = -0.1387$  eV or ( $\lambda = 8.95$   $\mu\text{m}$ ;  $\tilde{\nu} = 1119$   $\text{cm}^{-1}$ ) is the local extremum for inhibition of development of tumor cells.

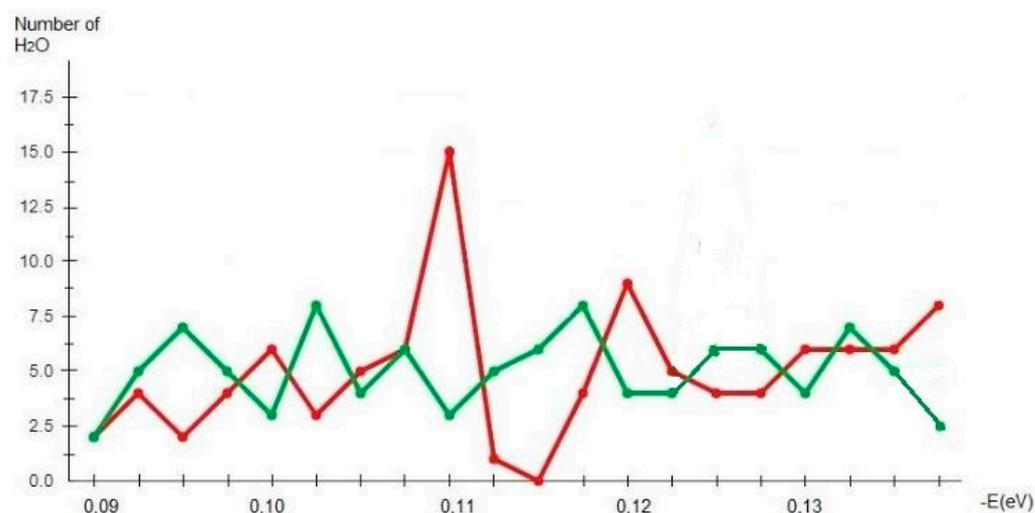
The distribution of the number of water molecules in a 1% solution of *H. rhodopensis* blossom extract in EVOdrop-filtered, hydrogen-saturated tap water and in the control sample of tap water from Sofia, Bulgaria in accordance with the energy of hydrogen bonds is presented in Table 1.

Figure 1 presents the distribution of the number of water (H<sub>2</sub>O) molecules in EVOdrop filtered tap water and saturated with Hydrogen (H<sub>2</sub>) (sample) and tap water (control sample) according to the energy of hydrogen bonds. The model shows the number of water molecules and their structuring in clusters.

The function  $f(E)$  is a distribution spectrum according to energies. The non-equilibrium energy spectrum (NES) is measured in  $\text{eV}^{-1}$ .

The local extremum at  $E = -0.11$  eV;  $\lambda = 11.3$   $\mu\text{m}$  ( $\tilde{\nu} = 887$   $\text{cm}^{-1}$ ) is specific to calcium carbonate ions [32,40]. The local extremum at  $E = -0.1112$  eV or ( $\lambda = 11.3$   $\mu\text{m}$ ;  $\tilde{\nu} = 887$   $\text{cm}^{-1}$ ) is typical for calcium conductivity [41,42].

In 2016, Kostainova and co-authors performed in vitro research on keratinocytes. They found that *H. rhodopensis* extracts affect the cell periphery of these cells. The keratinocytes were cultured under standard conditions and supplemented with additional calcium ions ( $\text{Ca}^{2+}$ ) in order to stimulate tight junction formation, thereby suppressing proliferative activity [43].



**Figure 1.** Distribution of the number of water ( $\text{H}_2\text{O}$ ) molecules in EVOdrop water and tap water according to the energy of hydrogen bonds.

## 2.2. Results from Spectral Analysis of EVOdrop Water with NES and DNES Methods

Measurements with the NES and DNES spectral methods show a significant difference between EVOdrop water and the control sample.

The test sample consisted of 1% *H. rhodopensis* blossom extract in filtered water, obtained with the patented EVOdrop device and saturated with hydrogen with the EVOdrop booster. The control sample was of 1% *H. rhodopensis* blossom extract in deionized water.

The result for the test sample in the NES-spectrum was  $-0.1203$  eV, while for the control sample it was  $-0.1151$  eV. The values of  $\Delta E$  for EVOdrop water, measured by the DNES method, were in the interval ( $-0.0052$  eV). The highest number of water clusters in the sample was 15 ( $-0.1112$  eV;  $11.3$   $\mu\text{m}$ ;  $887$   $\text{cm}^{-1}$ ). The number of water molecules in the control sample was 2.

The difference is considerable and shows a higher-level structuring of water clusters in comparison with the control sample. Recently, Ignatov et al. [39] found that in water, clusters with different numbers of  $\text{H}_2\text{O}$  molecules are formed due to the formation of hydrogen bonds. The average hydrogen bonds energy (HBE) increases with the number of  $\text{H}_2\text{O}$  molecules in the clusters, and with the evaporation of water droplets. According Mehandjiev et al. [44], in bulk water, at the beginning of evaporation, the maximum number of clusters has an average HBE of  $(-E) = 0.1162$  eV and contains 12–13  $\text{H}_2\text{O}$  molecules. Discrete changes in HBE of water clusters have the same value and are based on the formation of clusters with different numbers of water molecules.

*H. rhodopensis*, tested in our research, is a “resuscitating” plant with unique properties, a Bulgarian endemite. This is the only plant that recovers after a long drying time and has an ability to survive up to 31 months dried in an herbarium [44]. It is the only one in which Kuroki et al. [6] proved the presence of clusters of two water molecules in a dry state. There are no proven cluster formations in other plants. When *H. rhodopensis* dries out, dimers of water molecules are formed [6]. When being watered the dry plant turns green. After dehydration of *H. rhodopensis*, the degree of hydration can be estimated with the NES and DNES methods depending on the water quality.

Our results with *H. rhodopensis* show that after drying, the formation of clusters of water molecules depends on the type of water. The experiments were conducted with

deionized and filtered EVODrop water. The EVODrop device enables the formation of clusters in water. The structuring of the water molecules was established, which are analyzed with NES and DNES methods.

In recent years, *H. rhodopensis* has drawn the attention of researchers due to its beneficial effects on human and animal health. The extract of the plant contains high levels of flavonoid antioxidants [45,46]. We used such an extract in the present study. The plant has a tonic and anti-aging effect. In folk medicine, it is used to cleanse the stomach, liver, kidneys, and blood vessels [47,48]. Aqueous and alcoholic extracts of *H. rhodopensis* have shown unique medical and pharmaceutical potential, related to their antioxidant, radio-protective, antimicrobial, antimutagenic, immunostimulatory, anticancer, and anti-aging effects. The extract could be used in phytotherapy, human and veterinary medicine and cosmetics [49,50]. Our results show that its unique useful properties can be enhanced using EVODrop technology.

### 3. Materials and Methods

#### 3.1. Plant *H. rhodopensis* Friv.

Ethanol extract from the *H. rhodopensis* leaves and blossom were used in our study (Figure 2). *H. rhodopensis* Friv. oil has the following chemical composition (Table 2) [51]:



Figure 2. *Haberlea rhodopensis* blossom extract.

Table 2. Chemical composition of *H. rhodopensis* oil.

Compounds	$\mu\text{g}\cdot\text{g}^{-1}$ DW
flavonoids	
Luteolin	2730.18
Hesperidin	928.56
Kaempferol	578.52
Phenolic Acids	
Ferulic acid	630.48
Sinapic acid	580.80

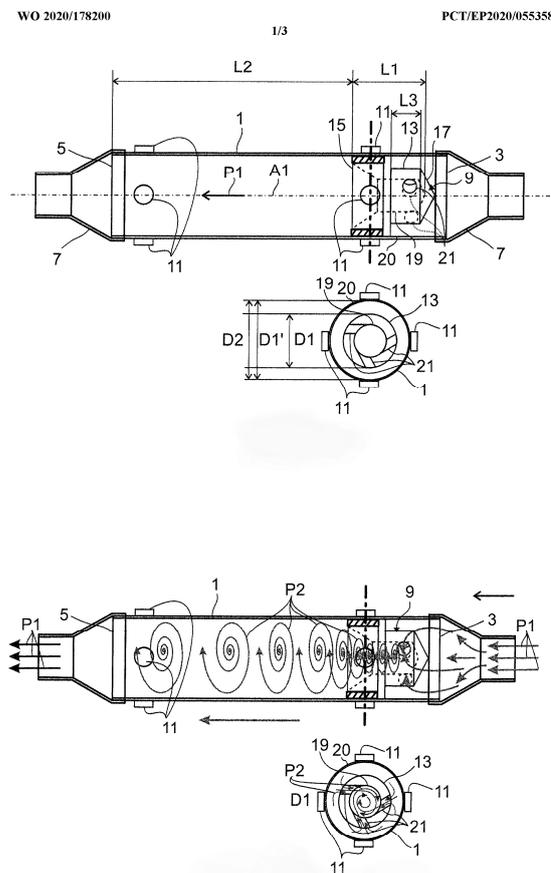
Figure 3 illustrates flowers of *H. rhodopensis*.



**Figure 3.** Flowers of *Haberlea rhodopensis*.

### 3.2. The EVOdrop Turbine Water Purifier

The proprietary operating principle and developed geometry of the EVOdrop turbine (Figure 4) allow for highly efficient treatment. Incoming water passes through the rotating turbine, driving it with its pressure, which in turn makes water pass through the rotating device. Specific outcomes of this treatment are based on magnetohydrodynamic forces [4,5]. Figure 4 shows EVOdrop’s turbine operation principle.



**Figure 4.** The EVOdrop turbine operation principle.

Figure 5 illustrates the EVOdrop filter.

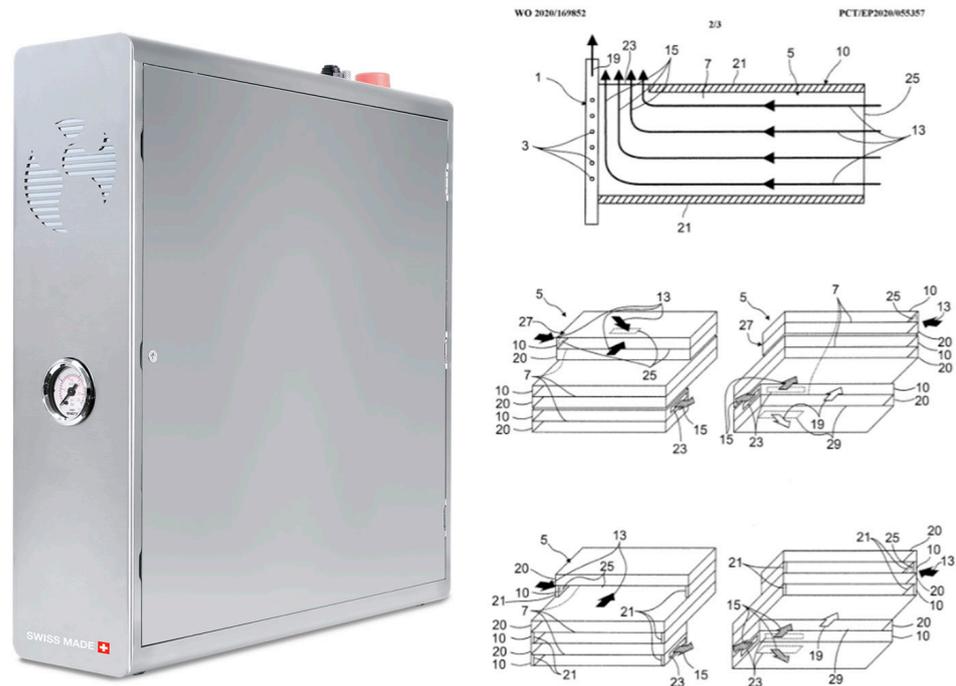


Figure 5. The EVOdrop filter.

### 3.3. The EVOdrop Booster for Hydrogen-Rich Water

The EVOdrop Booster produces hydrogen-rich water. EVOdrop hydrogen water has a concentration of hydrogen (Figure 6).

#### Current per dissolved concentrations

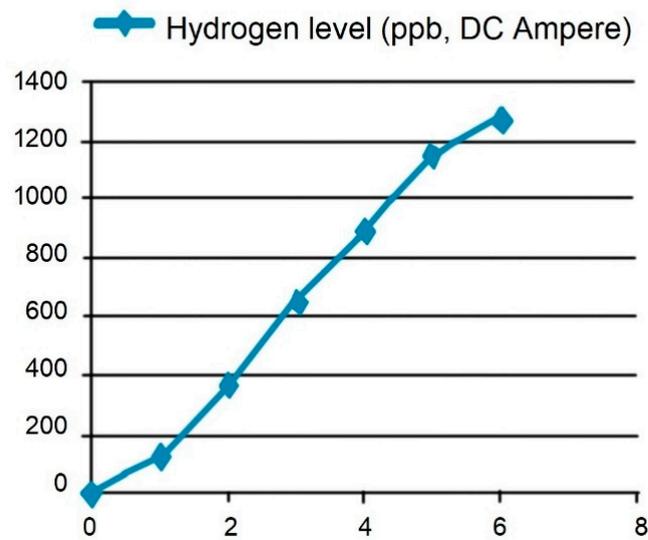


Figure 6. Hydrogen (H<sub>2</sub>) concentration of EVOdrop hydrogen water (1.2 ppm). Correspondence of hydrogen concentration (ppb) of EVOdrop hydrogen water and the DC Current (Ampers).

Figure 7 illustrates the EVObooster device for EVOdrop hydrogen water.

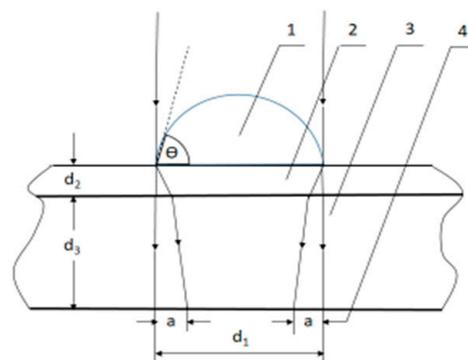


**Figure 7.** The EVObooster device for EVODrop hydrogen water.

The biological effects of hydrogen-rich water at a concentration of 0.08–1.5 ppm are described [17,18]. In the current study, hydrogen-rich water is referred to as Hydrogen EVODrop Water (HEW).

### 3.4. Differential Non-Equilibrium Energy Spectrum (NES) and Differential Non-Equilibrium Energy Spectrum Spectral Analyses (DNES)

The device of the author A. Antonov [52–55] for spectral analysis with NES and DNES methods is based on an optical principle. The evaporation of water drops is performed in a hermetic camera with a glass plate and water-proof transparent pad which consists of thin maylar folio. Evaporation of water drops was performed at a stable temperature of 22 °C. The drops were placed on a BoPET (biaxially oriented polyethylene terephthalate) foil with a 350  $\mu\text{m}$  thicknesses (Figure 8).



**Figure 8.** Operating principle of the method for measurement of wetting angle of liquid drops on a hard surface: 1—drop, 2—thin maylar foil, 3—glass plate, 4—refraction ring width. The wetting angle  $\theta$  is a function of  $a$  and  $d_1$ .

The parameters are as follows:

- Monochromatic filter with wavelength  $\lambda = 580 \pm 7 \text{ nm}$ ;
- Angle of evaporation of water drops from  $72.3^\circ$  to  $0^\circ$ ;
- Energy range of hydrogen bonds among water molecules is  $\lambda = 8.9\text{--}13.8 \mu\text{m}$  or  $E = -0.08\text{--}-0.1387 \text{ eV}$ .

The energy ( $E$ ) of hydrogen bonds among  $\text{H}_2\text{O}$  molecules in the water sample is measured in eV. The function  $f(E)$  is called the energy distribution spectrum. The energy

spectrum of water is characterized by a non-equilibrium process of water droplet evaporation; this is non-equilibrium energy spectrum (NES) and is measured in  $\text{eV}^{-1}$ . DNES is defined as the difference

$$\Delta f(E) = f(\text{samples of water}) - f(\text{control sample of water}),$$

DNES is measured in  $\text{eV}^{-1}$

where  $f(*)$  denotes the evaluated energy [31,32].

### 3.5. Filtration with EVOdrop Filter for Tap Water, Sofia, Bulgaria

Table 3 illustrates the physicochemical parameters of tap water from Sofia, Bulgaria before and after filtration with the EVOdrop device. The certificate No. 10216/21 June 2022 corresponds to the water before filtration, and No. 10217/14 June 2022 after filtration, with the EVOdrop device. The research was performed according to the parameters of Ordinance No. 9/2001, Official State Gazette, issue 30, and Decree No. 178/23 June 2004 regarding the quality of water intended for consumption and domestic uses in the accredited laboratory “Eurotest control” JSC, Sofia, Bulgaria [56].

**Table 3.** Physicochemical parameters of the tap water from Germany before and after filtration with EVOdrop device.

Controlled Parameter	Measuring Unit	Maximum Limit Value	Before EVOdrop	After EVOdrop
1. pH	pH values	$\geq 6.5$ and $\leq 9.5$	$6.73 \pm 0.11$	$8.88 \pm 0.11$
2. Total hardness	$\text{mg}\cdot\text{L}^{-1}$	12	$1.76 \pm 0.5$	$0.98 \pm 0.24$
3. Calcium ( $\text{Ca}^{2+}$ )	$\text{mg}\cdot\text{L}^{-1}$	150	$12.7 \pm 1.3$	$12.7 \pm 1.3$
4. Magnesium ( $\text{Mg}^{2+}$ )	$\text{mg}\cdot\text{L}^{-1}$	80	$21.2 \pm 2.1$	$4.2 \pm 0.4$
5. Hydrocarbonates ( $\text{HCO}_3^-$ )	$\text{mg}\cdot\text{L}^{-1}$	-	$27.5 \pm 2.8$	$<24.4$
6. Carbonates ( $\text{CO}_3^{2-}$ )	$\text{mg}\cdot\text{L}^{-1}$	-	$<12$	$<12$
7. Sodium ( $\text{Na}^+$ )	$\text{mg}\cdot\text{L}^{-1}$	200	$5.7 \pm 0.9$	$5.7 \pm 0.9$
8. Potassium ( $\text{K}^+$ )	$\text{mg}\cdot\text{L}^{-1}$	-	$1.7 \pm 0.2$	$1.6 \pm 0.2$
9. Manganese	$\mu\text{g}\cdot\text{L}^{-1}$	50	$3.8 \pm 0.4$	$3.6 \pm 0.4$
10. Zinc	$\text{mg}\cdot\text{L}^{-1}$	4	$0.074 \pm 0.07$	$0.02 \pm 0.002$

The research of physicochemical filtration with the EVOdrop filter shows that there is filtration of molecules with bigger sizes, such as hydrocarbonate ions ( $\text{HCO}_3^-$ ). The result for  $\text{HCO}_3^-$  is from  $27.5 \pm 2.8$  to less than  $24.4 \text{ mg}\cdot\text{L}^{-1}$ . There is an increase in pH from acidic value to alkaline—from  $6.73 \pm 0.11$  to  $8.88 \pm 0.11$ . The hardness of tap water after filtration is reduced from  $1.76 \pm 0.5$  to  $0.98 \pm 0.24$ .

## 4. Conclusions

The unique properties of *Haberlea rhodopensis* Friv., related to interaction between the water molecules during the biosis–anabiosis–biosis cycle, were used to investigate EVOdrop nanofiltration technology of tap water and its enrichment with hydrogen in terms of hydration and water molecules clustering. The statistically significant results clearly demonstrated that EVOdrop treatment leads to a shift in the hydrogen bonds energy distribution towards larger values along with the corresponding formation of local maxima. Based on previous results, health benefits of such water treatment can be expected in the areas of malignant growth inhibition and tissue regeneration for humans.

**Author Contributions:** Conceptualization, I.I., T.P.P., Y.K.-K. and R.B.; methodology, I.I., N.N., I.A. and F.H.; validation, F.H. and N.V.; formal analysis, A.I.I. and S.B.; investigation, I.I., T.P.P. and R.B.; resources, M.A. and I.A.; writing—original draft preparation, I.I., F.H. and T.P.P.; writing—review and editing, R.B., N.V., Y.K.-K. and N.N.; visualization, F.H. and A.I.I. All authors have read and agreed to the published version of the manuscript.

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