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Aquagrams: Water Spectral Pattern as Characterization of Hydrogenated Nanomaterial

Aquaphotomics is a novel approach in science to water and aqueous solutions investigation. It is based on near infrared spectroscopy (NIR), which in our current research is used for the analysis of interaction of water and hydrogenated nanomaterial. Infrared spectroscopy, with a new developed concept that of aquaphotomics, is used to investigate the organization of matter as a mixture of harmonized hydrogenated fullerene (nano-harmonized substance-NHS) and pure water. Composition of matter follows a harmonized form by Fibonacci law (Φ/ϕ). The effect of dilution on nano-harmonized substance is analyzed and the results of near infrared spectra are presented in the form of aquagrams. The presence of NHS in biological tissues is a driving force of natural self-assembly process, which is capable of restoration of damaged functions of biomolecules.

Keywords: fullerenes, nanomedical engineering, nano-harmonized substance, water, NIR spectroscopy, aquaphotomics, aquagram.

1. INTRODUCTION

Water comprises approximately 60 % of human organism, and is one of the five structures essential for life.

Hydrogen bonding is of immense importance for the major biomolecules. In case that natural hydrogen bond of biomolecule becomes weakened or broken, conformation state of molecule is changed. Usually, conformation change violates molecular functionality, and leads for example to protein folding and misfolding, which is a direct consequence of hydrogen bonding. Therefore, the hydrogen bonds being direct link between normal and abnormal protein states make the crucial difference between healthy, normal state of the organism and dysfunctional, unhealthy organism.

Because all biomolecules are dissolved in water (aqueous systems), the solution to the problem may lie in controlling and forcing dysfunctional biomolecule to oscillate under its natural state law by using hydrogen bonds of water around the molecule [1]. In order to develop new technology, which will mimic biomolecules, it is necessary to overcome some problems such as material, design, logic, self-assembly and control system on a molecular level [2].

It is well known that four very important biomolecules, DNA, microtubules, clathrin and collagen, are structured by Fibonacci sequences, the Golden Mean law [3,4]. The discovery of fullerene material (Fig. 1) in 1985 by Kroto/Smalley research team [5], brought new possibilities in science and technology. One of them was establishing the relationship between the symmetry operation and the energy state of fullerene C_{60} ordered by the Golden

Mean rule [6]. The C_{60} became a very promising candidate for developing new nanomaterials with self-assembly properties for molecular nanotechnology.

In the last fifteen years molecule C_{60} has been used in research and development for many applications including biomedicine, but the safety and toxicity has been a question of debate.

The molecule C_{60} (Fig. 1), which generates beneficial vibration by the golden Mean law, is toxic in concentrations higher than 10 ppb. This is the ultimate paradox that the same material is beneficial from physical (vibrational) point of view, but toxic from chemical point of view. However, after addition of hydroxyl groups (Fig. 1), it becomes water soluble and concentration for nontoxic effect increases to $10^4 - 10^6$ ppb. However, the most beneficial effect of hydrogenated fullerenes to biomolecules and tissues is from $10^6 - 10^9$ ppb. To overcome this problem, water stabilizing belt has been made around carbon hydroxyl molecule (Fig. 1) and in the range of $10^6 - 10^9$ ppb it becomes nontoxic [7]. Having hydroxyl groups and nanolayering water shell around fullerene, for the first time it is considered safe and beneficial for cosmetic and medical use [7]. The presence of water based nano-harmonized substance (wNHS) in biological tissues presents the Golden Mean driving force of natural self-assembly process, which is capable of restoration of damaged functions of biomolecules [8].

Energetically stabilized nano-harmonized substance could be considered biomimetic nanomaterial, which mostly in its symmetrical and energetical properties resembles clathrin – a protein with a critical role in intracellular trafficking.

To explore the properties of designed nano-harmonized substance, a new approach called Aquaphotomics based on the light – matter interaction is employed in this study.

Aquaphotomics [9] is a new term recently introduced to describe the concept in which water as

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multi-element system could be well described by its multi-dimensional spectra. For dynamic, non-invasive studies, visible infrared (VIS-NIR) spectroscopy has proved to be a powerful tool and source of information and it facilitates the establishment of Aquaphotomics [9].

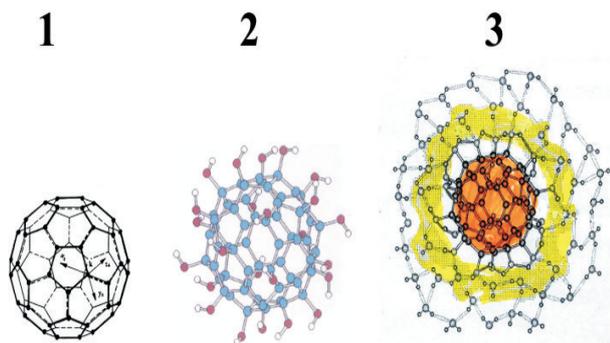


Figure 1. Molecule C_{60} (left), hydrogen bonded $C_{60}(OH)_x$ nanostructure (middle) and energetically stabilized nano-harmonized substance $C_{60}(OH)_x@(H_2O)_y$ [1,7]

Aquaphotomics is based on visible-near infrared spectroscopy (VIS - NIR) and multivariate analysis. It discovers new water hydrogen bonds in biological systems under various perturbations and relates water absorbance patterns to respective biofunctionalities.

Based on years of experience in VIS-NIR spectroscopy research of water and different aqueous systems under various perturbations, 12 characteristic wavelength ranges (6 - 20 nm width each) have been identified in the area of the first overtone of the water NIR spectra, where despite the type of perturbation the observed systems showed predictable spectral variations. To visualize these changes of water absorbance pattern a star chart named “Aquagram” [14] is used. Aquagram displays normalized absorbance values at several water bands on the axis originating from the center of the graph. Absorbance values at specific water absorbance bands were placed on the respective radial axes. Aquagrams were used in studying aqueous and biological systems to obtain more information of the molecules present in the water. In this approach, water is used as a “mirror”, which can reflect functionality of the present structures [14].

Aquaphotomics has been successfully applied in various fields from water characterization, food quality control to early diagnostics in medicine [9-12].

2. MATERIAL AND METHODS

The samples used were Millipore water (Milli Systems Co., USA) and Nano-Harmonized Substance. Nano-Harmonized substance is a mixture of 50 $\mu\text{g/l}$ of $[C_{60}(OH)_{24}@72 H_2O]^{Phi/Phi}$ and 18.2 M Ω water (Millipore water). A volume of 10 ml of nano-harmonized substance was diluted in the ratios of 1:2, 1:5 and 1:10, and so prepared solutions were further diluted using serial dilution technique in increments according to scheme presented in Fig. 2.

2.1. Near-infrared spectral analysis

NIR transmission spectra of water samples in the 400-2500 nm region was recorded using NIR Systems 6500

spectrophotometer (FOSS-NIR Systems) in transmittance mode, with a resolution of 2 nm at a constant temperature of 37°C. A quartz liquid sample cell with 1mm path length is used as a container. For each sample, three consecutive spectra were recorded and 378 spectra in total were obtained. Only the region of the first overtone of water was used for aquagrams making.

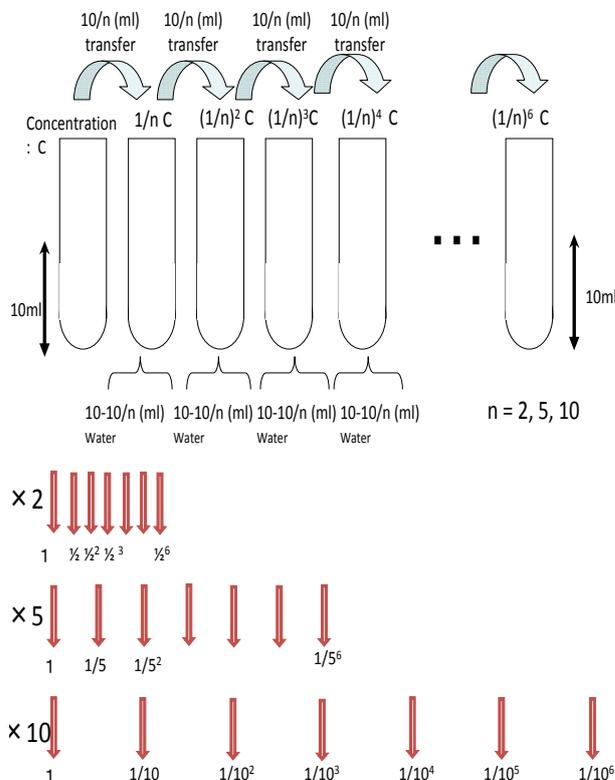


Figure 2. Serial dilution of nano-harmonized substance – experimental scheme

2.2. Multivariate analysis

The whole multivariate spectral analysis was carried out by Pirouette ver. 4.0 (Infometrics, USA) software program. Prior to analysis the spectra were transformed using: 1) multiple scatter correction, 2) autoscaling for each wavelength, and 3) averaging of 3 consecutive spectra. The spectra this way transformed, were used in further analysis – principal component analysis and for obtaining aquagrams.

Principal component analysis (PCA) is a well-known statistical method for reducing the dimensionality of data sets. Its operation can be considered to be revealing the internal structure of the data in a way that best explains the variance in the data. The new dimensions, principal components – PCs – are built taking into account the maximum variance of data and the requirements about an orthogonal space. The results of a PCA in terms of component scores and loadings are discussed.

3. RESULTS AND DISCUSSION

In this study, we have applied NIR spectral analysis combined with multivariate analysis and aquaphotomics approach to investigate the influence of dilution on

nano-harmonized substance. This is a way to investigate whether water molecules truly bind to the nanomaterial and what the nature of this binding is.

The results of principal component analysis are presented through scores plots and corresponding loadings plot for undiluted nano-harmonized substance and by the comparison with the results of analysis for nano-harmonized substance diluted in various concentrations.

Water molecular species in undiluted and diluted solutions are presented by aquagrams [14], circular diagrams having 12 characteristic wavelength ranges selected from the region of a first overtone of water in its near infrared spectra, which was found to be of importance to biological aqueous systems functioning. The absorbance pattern presented through these 12 wavelength ranges (WAMACS [9]) in aquagram shows dominant water species present in the analyzed systems.

3.1. Principal component analysis

The results of principal component analysis for spectra of undiluted solutions and water, in a form of scores plot and loadings plot, are presented in Fig. 3 and Fig. 4, respectively. In Fig. 3 one can notice that the multivariate analysis of the NIR spectral data classifies samples of water and samples of NHS into two distinctive groups. It should be noted that the number designations next to the scores of NHS and water refer only to samples prepared for dissolution, but are still not dissolved, and all scores presented on this scores plot represent only undiluted water or undiluted NHS.

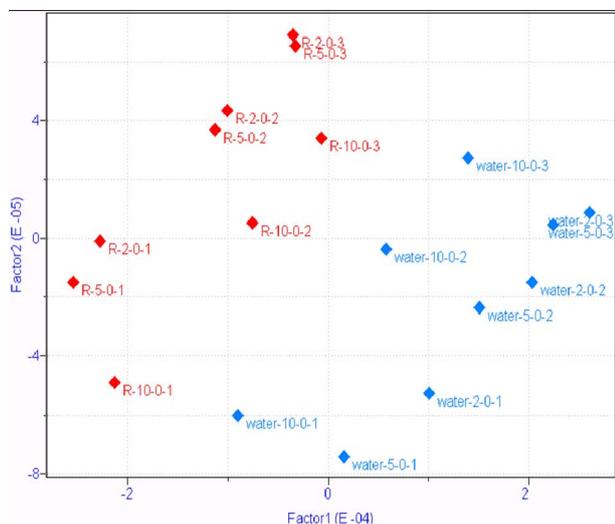


Figure 3. Principal component analysis of pure water and undiluted nano-harmonized substance. Spectral data presented on scores plot PC1- PC2. Scores plot illustrates good separation of classes: water (blue diamond) and undiluted nano-harmonized substance (red diamond)

From the scores plot one can notice that the scores for NHS are shifted on the left side of PC1 factor compared to water scores, and they are located entirely on the negative side of the PC1.

The first two principal components explain 91 % and 6 % of variance in data, respectively, and in total explain 97 % of variance. The first vector PC1, related to water, distinguishes NHS scores from water scores, and its loading is presented in Fig. 4 (below).

The main features of PC1 loading are absorbance at 1386 nm, and 1414 nm. Its main negative feature-absorbance at 1414 nm indicates freer, unbound water (S_0 – free water molecules, [9]).

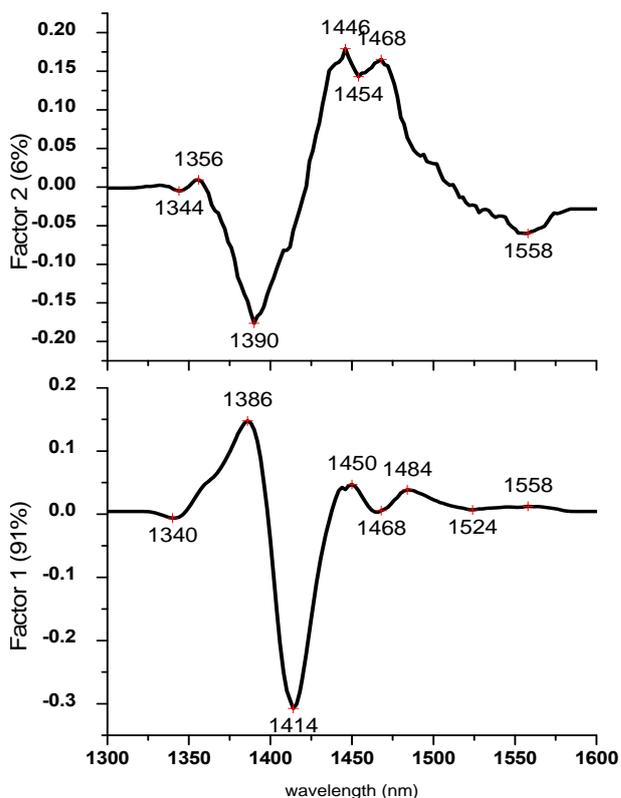


Figure 4. Principal component analysis – Loading plot for undiluted nano-harmonized substance: The first loading vector explains 91 % of variance (down), while the second loading vector explains 6 % of variance. These plots show the importance of certain wavelengths in the respective spectral pattern of each PC

The main features of PC2 loading presented in Fig. 4 are absorbance at 1390 nm and 1446 nm. The majority of scores for NHS are on the positive side of the PC2 vector, suggesting more hydrogen bonded water molecules (absorbance at 1446 nm, 1st overtone DDA symmetric stretch $[OH-(H_2O)_4]$).

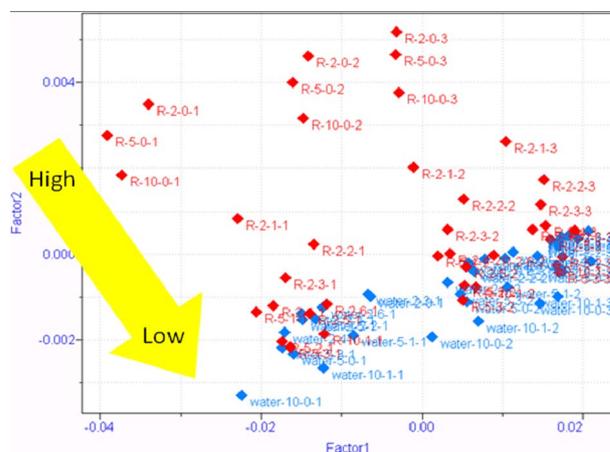


Figure 5. Principal component analysis of diluted water and diluted nano-harmonized substance. Spectral data presented on scores plot PC1- PC2. Scores plot illustrates poor separation of classes: water (blue diamonds) and diluted nano-harmonized substance in various concentration (red diamonds)

Principal component analysis results for spectra of diluted nano-harmonized substance and diluted water as a reference material are shown in Fig. 5 and Fig. 6, respectively.

The scores plot in Fig. 5 reveals poorer separation of classes. Corresponding loadings plots in Fig. 6 reveal that PC1 loading vector explains 97 %, and PC2 loading vector only 1 % of variance, giving in total for the two first loading vectors 98 % of explained variations in spectral data.

The first loading vector, presented in Fig. 6, shows two characteristic absorbencies, on 1412 nm and 1494 nm. According to Stegnan et al. the two major spectral changes of water around 1412 nm and 1491 nm, are a function of temperature [13], and in this case, irradiation. The intensity of these changes occurs in phase with each other in opposite directions, which was proved in the mentioned study. This is used as a proof for two-state mixture model, where the two states represent weaker and stronger H-bonding at 1412 and 1491 nm, respectively, and these two species are perfectly correlated; i.e. the concentration of one species increases by the decreasing of the concentration of the other, when the temperature is changed.

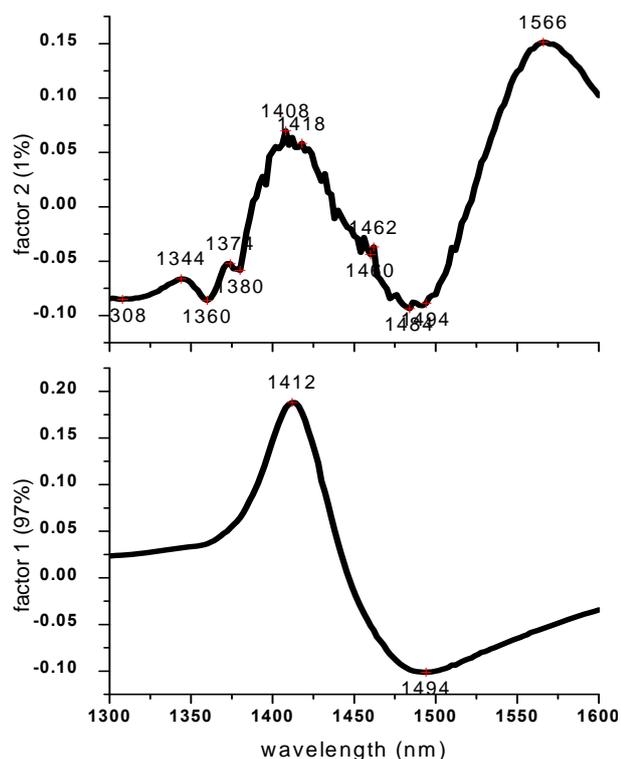


Figure 6. Principal component analysis – Loading plot for nano-harmonized substance: The first loading vector explains 97 % of variance (down), while the second loading vector explains only 1 % of variance. These plots show the importance of certain wavelengths in the respective spectral pattern of each PC, and in this case the first vector shows changes in water due to irradiation, while the second loading vector shows perturbation under the influence of nanoparticles in solution.

However, in this study, the PC1 loading vector, which explains that 97 % of variance in spectral data are due to the water changes under the influence of irradiation, does not provide valuable information regarding the perturbation of water system due to the presence of nanoparticles. Therefore, the second loading

vector PC2, which explains only 1 % of variance among spectral data, gives more information on water molecules organization under the influence of present nanomaterial. This PC2 loading vector is presented in Fig. 6.

The main characteristic of PC2 loading vector is positive absorbances at 1408 nm, 1418 nm and 1566 nm, and negative absorbances at 1360 nm, 1484 and 1494 nm. From the scores plot on Fig. 5, it can be noticed that positive scores on PC2 correspond to spectral data of dissolved nano-harmonized substance in higher concentrations, while negative scores correspond to very diluted solutions of nano-harmonized substance, and water.

3.2 Aquaphotomics of nano-harmonized substance

Aquagrams presented in next figures (Figs. 7,8 and 9) show the characteristic absorbances of analyzed aqueous systems in 12 characteristic wavelength ranges in the region of 1st overtone of water in VIS-NIR region.

The first aquagram (Fig. 7) presents a comparison of absorbance of undiluted nano-harmonized substance and pure water as a reference. From the aquagram, one can see the hydrogen bonds, which nanomaterial builds with the surrounding water molecules. It is clear from aquagram that NHS compared to water has less water molecules organized in water shells, less water molecules with free OH bonds, and also less free water molecules and water dimmers (S1). On the other hand, undiluted nano-harmonized substance shows organization of water molecules in water clusters with 2, 3 and 4 hydrogen bonds (S2, S3 and S4) and bonded water (1518 nm, ν_1 , ν_2).

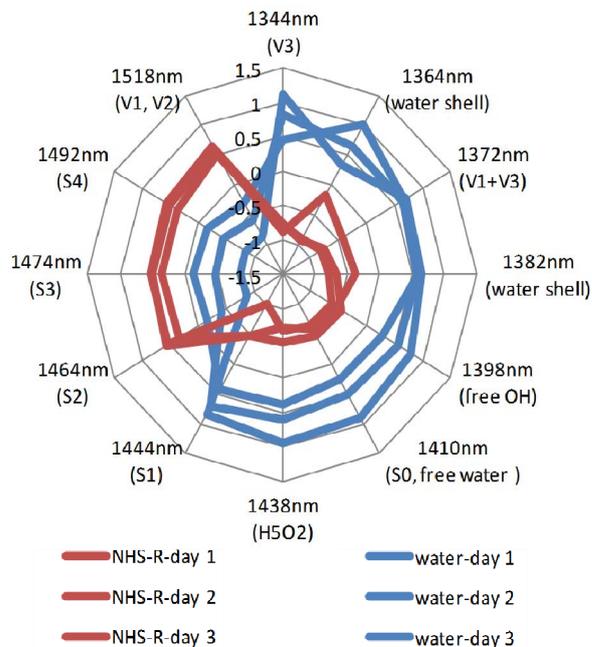


Figure 7. Aquagram: water and undiluted nano-harmonized substance (Transformation of spectra: 1) multiple scatter correction, 2) autoscaling for each wavelength, 3) averaging for three consecutive spectra)

These results suggest that water around nano-harmonized substance has higher degree of organization

as chains or clusters, or it is bonded with present hydrogenated nanomaterial.

Next two aquagrams presented in Fig. 8 and Fig. 9 are showing absorbance pattern of water diluted with Millipore water, a “solution” used as a reference material for comparison, and nano-harmonized substance in water solutions in various concentrations, respectively.

With dilution of water, as can be seen from Fig. 8, water shows very stable absorbance pattern. Slightly higher absorbance can be observed at 1364 nm, which is indicating slightly higher number of water molecules in a water shell.

The aquagram in Fig. 9 presents changes in absorbance pattern in the region of 1st overtone of water spectra of diluted nano-harmonized substance.

The aquagram for solutions of nano-harmonized substance clearly indicates that with dilution, major changes in absorbance occur at 1364 nm, and 1444 nm. Compared to undiluted solution, absorbances at the region of strongly bound water (1464 - 1518 nm) are significantly lower.

In the Fig. 10 and 11 standard deviations are presented for water dilution and nano-harmonized substance dilution. These figures give insight into which significant wavelengths are the most important fluctuations in hydrogen bonds dynamic, when these solutions are perturbed.

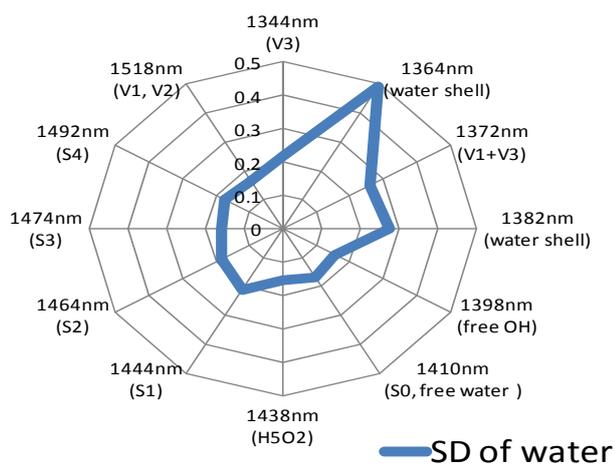


Figure 10. Standard deviation of water solutions (water diluted with water, reference material) shows higher absorbance at water solvation shell – 1364 nm

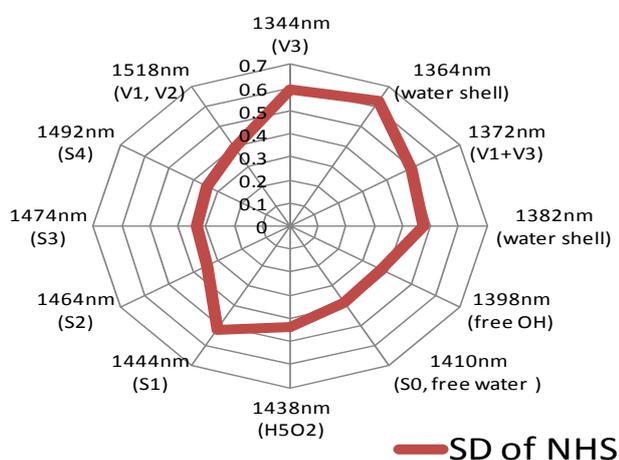


Figure 11. Standard deviation of NHS water solutions shows higher absorbance at 1364 nm

Major standard deviations for water are observed at 1364 nm (Fig. 10), which is the same for nano-harmonized substance solutions (Fig. 11). Major deviations occur at 1382 nm, and 1444 nm, as well as when NHS is diluted. Deviations at these two wavelengths suggest changes in the second (outer) water shells around NHS, and changes in weakly bonded water molecules, respectively.

The next step in our research should be an attempt to prove that the water solvation shells oscillate under the same law as the harmonized nanomaterial, and to determine the extent to which this vibration mode extends in water molecular matrix. In case this is confirmed, the final objective of our work – developing of nano-controlling device could be achieved. A comparison of clathrine aquagrams (which is a nature’s own harmonized molecule) in solutions with the aquagrams of nano-harmonized substance solutions will be very interesting to explore its (clathrin) potential as a biomimetic nanostructure.

4. CONCLUSION

This study presents the investigation of a new type of nanomaterial, nano-harmonized substance, which is considered to be a suitable candidate for regulation (nano-control) of biomolecular conformation state. The idea of nano-control device is to use NHS whose composition of matter follows a harmonized form (Φ/φ). Conducting its vibrations to water molecules in near surroundings could force biomolecules to recover its natural vibration mode.

We used near infrared spectroscopy with aquaphotomics to investigate whether this hydrogenated harmonized nanomaterial has influence and what kind of influence on nearby water molecules. This method seemed to be a promising choice for the questions we wanted to resolve. The preliminary results showed that water molecules are strongly bounded by undiluted nano-harmonized substance, while the rest of water species have more than one hydrogen bond, i.e. water clusters. Nano-harmonized substance in solution shows that water molecules organization around hydrogenated nanomaterials are mainly solvation shells. Solvation shells or hydration shells are water molecules layers placed around hydrogenated nanomaterial, suggesting that oscillations follow the Golden mean rule. This indicates that oscillations of the harmonized nanomaterial could be transferred to its surrounding layers of water molecules.

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АКВАГРАМ: СПЕКТРАЛНА „ПАУКОВА МРЕЖА“ ВОДЕ КАО МЕТОД КАРАКТЕРИЗАЦИЈЕ ХИДРОГЕНИЗОВАНИХ НАНОМАТЕРИЈАЛА

Лидија Матија, Румјана Ценкова, Мари Мијазаки, Киоко Бамба, Јелена Мунћан

Аквафотомика је нови приступ у науци о одређивању особина воде, водених раствора и присуства у малим концентрацијама биомолекула и наноматеријала у води. Ова метода се заснива на карактеристичним фреквенцијама воде у инфрацрвеном (IR) спектру на основу којих се израђује дијаграм облика „паукове мреже“. Промене у спектралном дијаграму „паукове мреже“ чисте воде, даје информацију о присуству и организацији додате материје у воду.

Интензитет поремећаја спектра је пропорционалан концентрацији и организацији унете материје. Уместо да се идентификују честице (микро, нано) у води, као што је до сада био случај (а што је доста тешко када се ради о малим концентрацијама), у акваграму се идентификују најмање промене матрикса воде на карактеристичним фреквенцијама. Карактер и интензитет тих промена у нашем истраживању омогућио је анализу интеракцију воде и хидрогенизованог фулеренског наноматеријала.

Инфрацрвена спектроскопија, са новим развијеним концептом, „пауковом мрежом“, се користи да се испита организација ново настале супстанце, као мешавина хармонизованог хидрогенираног фулерена (NHS) и деминерализоване воде.

Анализа акваграма показује да се NHS организовао у форму Фибоначијевог низа (Ф/φ) и да преко водоничних веза делује на окружење. Ефекат разблаживања NHS супстанце на воду се такође анализира помоћу акваграма. Како су неки биолошки молекули (микротубуле, колаген, клатрин и др) уређени по Фибоначијевом низу то присуство NHS у биолошким ткивима може постати покретачка снага природног процеса саморепарације, која је у стању да обнови оштећене функције биомолекула.

Ова истраживања отварају могућност развоја наномедицине на бази хидрогенизованих наноматеријала у води који су уређени и способни да генеришу вибрационе модове по Фибоначијевом низу.