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Article *in* Journal of Physics Conference Series · April 2013

DOI: 10.1088/1742-6596/429/1/012016

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2013 J. Phys.: Conf. Ser. 429 012016

(http://iopscience.iop.org/1742-6596/429/1/012016)

Biocompatibility and cytotoxicity study of nanophotonic rigid gas permeable contact lens material

M Tomić[1], J Munćan[1], D Stamenković[2], M Jokanović[3] and L Matija[1]
[1]NanoLab, Biomedical Engineering, Faculty of Mechanical Engineering, Kraljice Marije 16, Belgrade, Serbia
[2]Optix, Oračka 13, Zemun, Serbia
[3]Academy of Sciences and Arts of the Republic of Srpska, Bana Lazarevića 1, Banja Luka, Republic of Srpska

E-mail: mmtomic@mas.bg.ac.rs

Abstract. Since materials on nanoscale have different characteristics from materials on macro scale their biocompatibility should be precisely and specifically investigated. Fullerenes, the third carbon allotrope, are one of the most used nanomaterials. The least stable and the most common is fullerene C_{60}. One of the main disadvantages of fullerene is its low solubility in water. In order to make it soluble, it must be functionalized with polar groups such as –OH and –COOH. From all the water soluble fullerenes the most important ones are those with –OH groups attached named fullerols. We have developed new materials for contact lenses by adding fullerene (C_{60}) and fullerol ($C_{60}(OH)_{24}$) into PMMA. The aim of our investigation was to compare the influences of those materials on aqueous solutions similar to tear film. For the analysis of the solutions we used opto-magnetic imaging and IR spectroscopy. The acquired spectrums were commented and compared with the standard contact lens material, which was analyzed by the same methods. The ISO 10993 cytotoxicity test on extract of nanophotonic material with incorporated C_{60} was done as well. This research contributes to better understanding of the biocompatibility of new rigid gas permeable contact lens materials.

1. Introduction

Due to more often application of nanomaterials, the studies of their interactions with tissue have become very important. Clinical applications of biomaterials shouldn't make any unwanted reactions in the organism and shouldn't compromise the life of a patient therefore every material must be biocompatible. Biocompatibility of nanomaterials should be specifically tested since they have different characteristics from the bulk materials and due to the size of the particles their penetration into tissues is easier.

When designing a contact lens few things should be considered, but probably the most important one is biocompatibility. A biocompatible contact lens is the one that doesn’t damage surrounding ocular tissue during contact. The contact lens should permit oxygen from air to the cornea because unlike other tissues the cornea doesn’t get any oxygen from the blood vessels. If the cornea doesn’t get enough oxygen, it would swell and become infected. Another important property is wettability because the lens is in contact with tear film which consists of proteins, lipids, enzymes and other molecules that could be deposited on the contact lens surface.

In this paper we present the investigation of influence of three sorts of contact lenses on different aqueous solutions which are similar to tear film. Opto-magnetic imaging spectroscopy method and IR spectroscopy were used for characterizing the samples. Also the cytotoxicity test on extract of nanophotonic contact lenses with incorporated C_{60} was conducted to evaluate the potential for cytotoxicity.

2. Materials

Fullerenes are a big family of super-atomic three-dimensional molecules. They were discovered by H. W. Kroto, R. F. Curl and R. E. Smalley in 1985, but the gram quantities became available in 1990 when Krätschmer and Huffman set the procedure for their production. Fullerenes consist of sp^2 hybridized carbon atoms distributed in hexagons and pentagons. Geodesic dome in Montreal made by Richard Buckminster Fuller was an inspiration for the name of these molecules, because this dome has the same structure as fullerene cage. The most popular fullerene is C_{60} also known as the *Buckyball* which is shown on figure 1 [1].

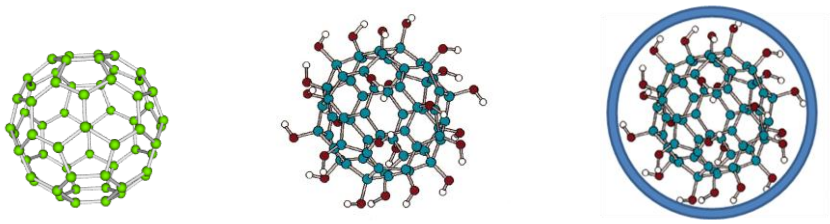

Figure 1. Fullerene C_{60} (left), fullerol $C_{60}(OH)_{24}$ (middle) and NHS-fullerol: $C_{60}(OH)_{24}$ @ $X(H_2O)$ or water shell fullerol (right) [3,4].

Fullerenes show strong affinity to electrons and act like ‘'radical sponges", because they easily enter addition reactions with nucleophiles. Spectroscopic characteristics of fullerenes are in strong relation with their symmetry. Structural information can be acquired from the number of bonds, for example, in IR spectra. Fullerene C_{60} is a good optical limiter but has low solubility and poor transparency. Fullerene materials are a group of new optical filters with such remarkable attributes as easy fabrication, predictable wavelength tuning, and excellent performance stability [2].

One of the main disadvantages in fullerene applications is its low solubility in water. In order to make them soluble, they must be functionalized with polar groups such as –OH and –COOH. From all the water soluble fullerenes the most important ones are those with –OH groups attached, named fullerols or fullerenols. When stable water layers (water shell) are surrounding $C_{60}(OH)_{24}$, in notation $C_{60}(OH)_{24}$ @ $X(H_2O)$, it is named nano harmonized supstance (NHS) (figure 1) [3]. They are free radicals scavengers and have anti-oxidative properties. Modified fullerenes are water soluble because they interact with water through hydrogen bonds. These fullerenes are unstable and can be degraded in contact with chemical agents from the environment. Modified fullerenes can be stabilized in the process of harmonization [3].

The properties of C_{60} can also be modified by its incorporation into polymers making it a material that can be easily handled and produced (figure 2). Therefore, combination of both systems has led to a wide variety of new materials showing appealing features based on the possibility of tuning their properties by modifying the chemical nature of the components or the chemical linkage between them [1].

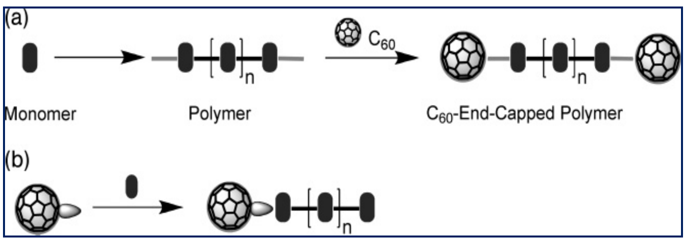

Figure 2. Synthetic strategies for the synthesis of C_{60}-end-capped polymers [2].

In our investigation of new contact lens materials we used two different sorts of nanomaterials: fullerene C_{60} and fullerol $C_{60}(OH)_{24}$. One gram of each nanomaterial was added during the polymerization process to the standard polymethylmethacrylate material, SolekoSP40™. The percentage of nanoparticles was 0.33%, but they were not completely dissolved in methylmethacrylate. Polymerization was homogenous in all samples, so the new nano-photonic material for rigid gas permeable lenses was made. The third polymerization was done without nanomaterials, so the standard Soleko SP40™ RGP material was made and was used as a referent sample.

For biocompatibility investigation we used three sorts of solutions: aqua purificata, saline and drops for dry eyes. Aqua purificata is a drinking water which is purified by double reverse osmosis. This water is completely demineralized. The saline is Natrii Chloridi infundibile 0.9% (Sodium chloride solution). The artificial drops were Refresh Contacts drops that consist of sodium-carboxymethylcellulose of 0.5%, as well as PURITE®. This is the unique preservative which breaks down into natural tear components – sodium and chloride ions, oxygen and water. Since these components are also in natural tears, the risk of irritating the eye or damaging cornea is minimized. Carboxymethylcellulose is a polymer which is ideal cytoprotective lubricant for the ocular surface. It provides long-lasting relief and protection from the symptoms of dry eye. This polymer is a mucoadhesive that shares similar protective qualities against harmful agents as natural ocular mucin.

3. Methods

For the characterization of different aqueous solutions we used two different types of spectroscopy methods: opto-magnetic imaging spectroscopy (OMIS) and IR spectroscopy based on 12 vibration modes, called Aquaphotomics [4].

3.1. Opto-magnetic imaging spectroscopy method (OMIS)

Each type of matter has special, different, angle value of light polarization. When the incident white light is diffuse, the reflected white light is composed of electrical and magnetic components, whereas diffuse incident light that is inclined under certain angle will produce reflected light which contains only electrical component of light (figure 3). This angle is called Brewster's angle and it represents the magnitude of the angle of incidence under which the sample polarizes the incident light. Taking the difference between white light (electromagnetic) and reflected polarized light (electrical) yields magnetic properties of matter based on light-matter interaction. Because such measurement can identify the conformational state and change in tissue on molecular level we named this method the opto-magnetic imaging spectroscopy [5].

The equipment for recording was a Canon digital camera, model IXUS 105, 12.1 MP. The light solution was accomplished by diffuse white diode and a lighting composition at Brewster's angle (three LED set at angle of 53° in regard to vertical axes). The recording region is circular with diameter of about 25 mm. In addition to customized camera, a software solution is used for analyzing the obtained images, yielding a characteristic diagram – diagnostic result – showing the intensities of light in correspondence with wavelength difference. Since this light is polarized by the sample that means that the character of polarization describes the character of the material. In this way, by characterizing the reflected light we can actually characterize the properties of the sample. This method is very sensitive since it detects magnetic properties on the basis of the response to visible light excitation which is relatively low in energy [5].

In this part of the research we used three samples of three types of contact lenses: standard SP40, SP40 with C_{60} incorporated and SP40 with $C_{60}(OH)_{24}$ incorporated. The solutions, aqua purificata, saline and eye drops, were poured into cups of 20 ml, and the contact lenses were put into those solutions. The solutions with contact lenses were left for 72 hours. After 72 hours, the contact lenses were taken out of the solutions, and the solutions were poured in other cups for taking pictures. Every sample was shot 10 times, with white and reflected polarized light. The pictures of solutions, without influence of contact lenses, were taken as well, for comparison.

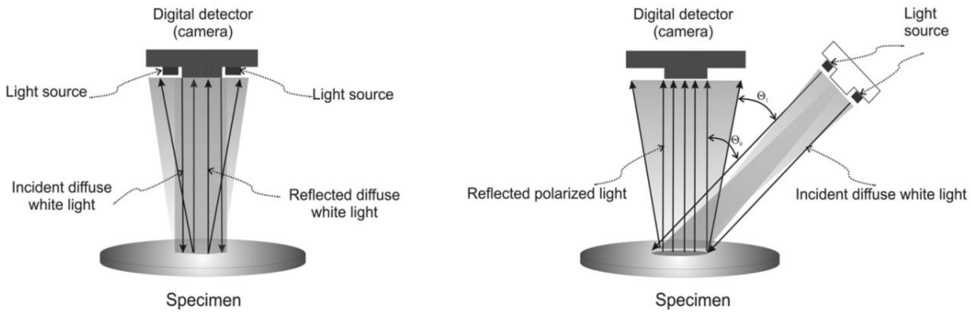

Figure 3. Operational principle of opto-magnetic imaging spectroscopy method [5].

3.2. Set of 12 infrared spectroscopic modes - Aquaphotomics

Aquaphotomics is a term, recently introduced to describe the concept in which water as multi-element system could be well described by its multi-dimensional spectra. For dynamic, non-invasive studies, vis-NIR spectroscopy has proved to be a powerful tool and source of information and it facilitates the establishment of Aquaphotomics. It discovers new water hydrogen bonds in biological systems under various perturbations and relates water absorbance patterns to respective biofunctionalities. To visualize the changes of water absorbance pattern a star chart named "Aquagram" is used. Aquaphotomics has been successfully applied in various fields from water characterization, food quality control to early diagnostics in medicine [5].

For this part of the research we used two types of rigid gas permeable contact lenses with incorporated nanomaterials and a standard SP40 contact lens. For investigating the behaviour of nanophotonic materials in aqueous solution we used saline (Natrii Chloridi infundibile 0.9%) and as a reference sample we used purified water (aqua purificata). First of all the spectrums of pure aqua purificata and pure saline were acquired by infrared spectrometer. Then all three sorts of contact lenses were left in saline. The spectrums were acquired after 1h and 40 minutes, after two days and finally after five days.

4. Results and discussion

4.1. Opto-magnetic imaging spectroscopy method results

The diagrams acquired by opto-magnetic imaging spectroscopy method show the differences and similarities for the same class of samples as well as for different classes. From the average spectrum diagram for aqua purificata and for aqua purificata under influence of SP40 contact lens we can see there are four peaks, two positive and two negative ones.

For aqua purificata average wavelengths and amplitudes for the first and the second positive peak are 111.71/100.728 and 120.71/72.91, respectively, and for the first and the second negative peak they are 110.7/-121.95 and 121.91/-68.49. On the other hand, those peaks under the influence of standard contact lens are at 113.57/85.05 and 119.22/52.91, and negative ones at 112.28/-103.05 and 120.68/-64.69, which is shown on figure 4.

For aqua purificata under influence of contact lens with incorporated C_{60} and $C_{60}(OH)_{24}$, there are three peaks, two negative and one positive. The wavelengths are slightly different (approximately 0.6 nm of difference) however the intensities (amplitudes) of the peaks are very different (the differences are up to 77 units). For aqua purificata under influence of contact lens with incorporated C_{60} the wavelength and amplitude of the positive peak are 115.9/164.2, and for the first and the second negative peak are 114.99/-63.54 and 116.73/-131.25, respectively. The same peak for the case with incorporated $C_{60}(OH)_{24}$ is at 115.29/86.38, and for the negative ones at 114.32/-38.11 and 116.26/-111.81.

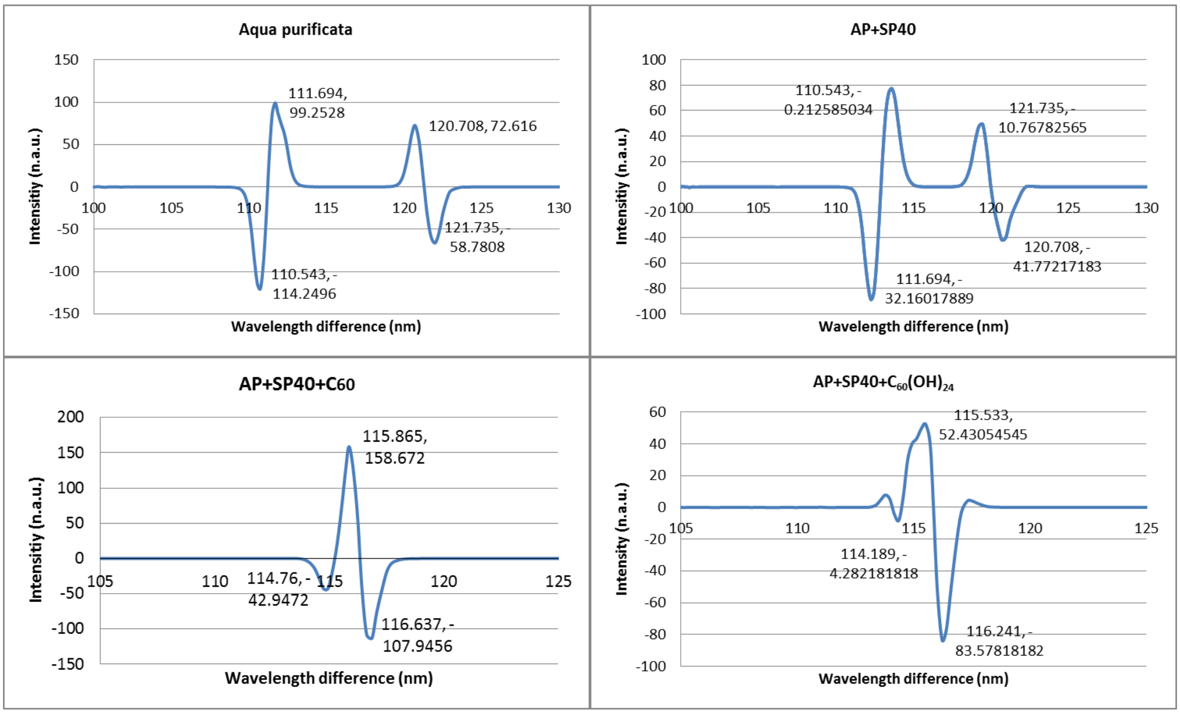

Figure 4. Comparison of wavelength difference (nm)-Intensity (n.a.u.) diagrams for aqua purificata, aqua purificata with SP40 contact lens (AP+SP40), aqua purificata with contact lens with C_{60} (AP+SP40+C_{60}) and aqua purificata with contact lens with $C_{60}(OH)_{24}$(AP+SP40+$C_{60}(OH)_{24}$).

For the class of saline the diagrams are slightly different, there are four peaks, two positive and two negative. Average wavelengths and intensities of the first and the second peak are 109.14/66.87 and 121.609/58.16, respectively, and the negative ones are 111.196/-37.53 and 119.51/-137.48. However, when this liquid was under the influence of the contact lenses the diagrams show two or three peaks. For the case of a standard contact lens SP40 and the one with incorporated C_{60} there are two peaks, one negative and one positive, at almost the same average wavelengths, and at slightly different amplitudes.

The positive peak for the saline under influence of SP40 contact lens is at 115.51/78.03, and the negative one is 116.63/-72.89. When saline was under influence of C_{60} the peaks were at 115.73/86.62 and 116.92/-73.73, respectively. The differences in wavelengths are approximately 0.2 nm, and for intensities approximately 0.8 units for the negative peak and 8 units for the positive one. In the case of influence of the contact lens with incorporated $C_{60}(OH)_{24}$ on the saline there are three peaks, two positive and one negative (figure 5). The wavelengths of the peaks are approximately the same as in two previous cases. The first positive peak is at 114.91/99.74, and the negative one is at 116.19/-113.29.

In the case of eye drops the diagrams are similar (figure 6). For the eye drops only there are four peaks, two positive and two negative, as well as for the case of aqua purificata and saline. The first and the second positive peaks are at 111.94/91.855 and 121.28/69.611, respectively. The negative peaks are at 110.37/-99.73 and 122.34/-81.26. However, in this class we have four peaks in the case of eye drops under influence of contact lens with incorporated C_{60} as well.

Here the positive peaks are at 114.87/84.76 and 118.12/50.08, and the negative ones are at 115.372/-74.86 and 118.48/-104.73. When eye drops were under influence of contact lens SP40 and the one with incorporated $C_{60}(OH)_{24}$ there are three peaks. For the SP40 contact lens the peaks are at 114.39/105.89, 115.84/-112.02 and 118.25/22.18, and for the one with $C_{60}(OH)_{24}$the peaks are at 114.91/79.3, 116.09/-89.03 and 117.08/34.02.

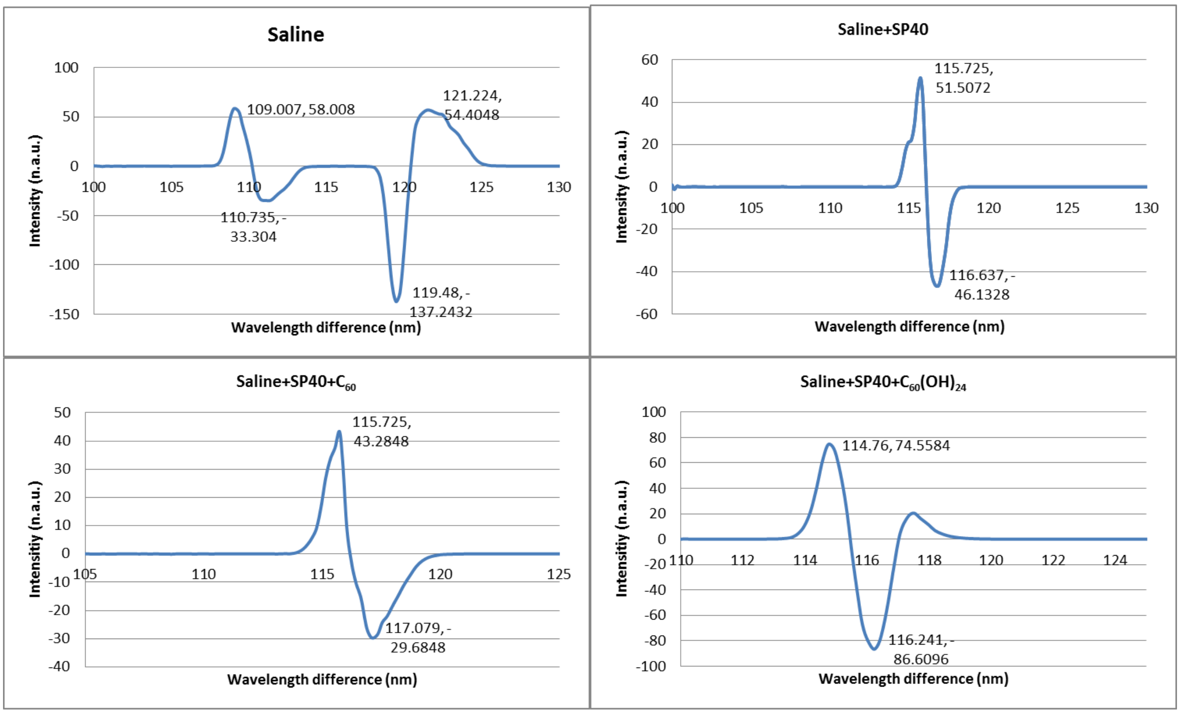

Figure 5. Comparison of wavelength difference (nm)-Intensity (n.a.u.) diagrams for saline, saline with SP40 contact lens (Saline+SP40), saline with contact lens with C_{60} (Saline+SP40+C_{60}) and saline with contact lens with $C_{60}(OH)_{24}$ (Saline+SP40+$C_{60}(OH)_{24}$).

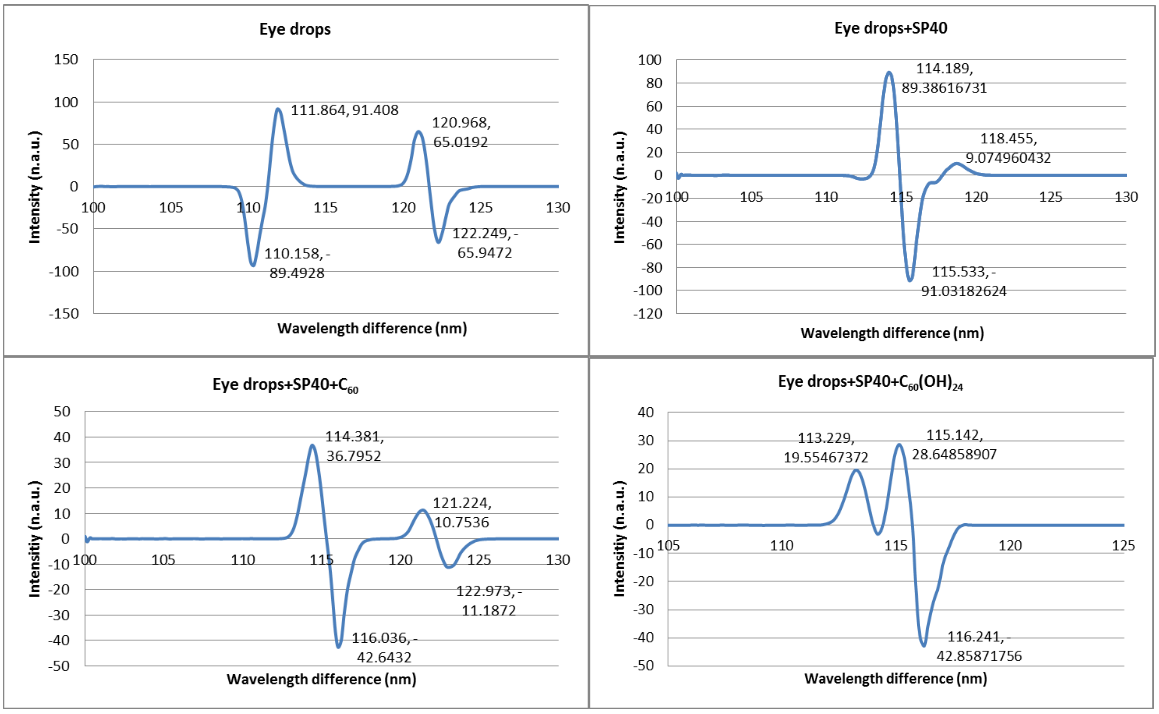

Figure 6. Comparison of wavelength difference (nm)-Intensity (n.a.u.) diagrams for eye drops, eye drops with SP40 contact lens (Eye drops+SP40) eye drops with contact lens with C_{60} (Eye drops+SP40+C_{60}) and eye drops with contact lens with $C_{60}(OH)_{24}$ (Eye drops+SP40+$C_{60}(OH)_{24}$).

4.2. Aquaphotomics results

The first results were acquired after 1h and 40 minutes (figure 7). Comparing to the pure water, the aquagram shows the decrease in strongly bounded water (the left part of the aquagram, 1493-1517 nm), an increase in free water molecules (S0) as well as the existence of water molecules in fist water shell (1360 nm). The presence of SP40 contact lens in saline induced a slight increase of water molecules in the first water shell, free water molecules and water molecules with free OH group which is different from the case of pure saline. In the presence of doped contact lenses there is an increase on free water molecules and molecules with free OH group which implies on breaking strongly bounded water. The highest absorption could be seen in the case of a material doped with fullerol (SP40+$C_{60}(OH)_{24}$).

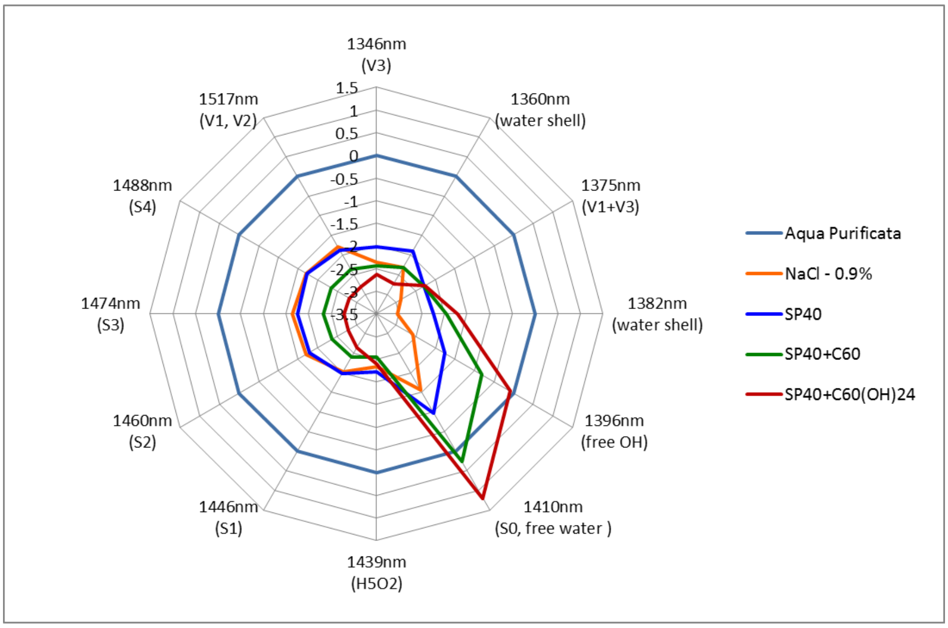

Figure 7. The aquagram: nanophotonic contact lenses in saline after 1h 40 min.

The second spectrum acquisition (figure 8) was made after two days and the aquagram shows the reorganization of water in NaCl 0.9%. This is most likely the consequence of evaporation i.e. the breaking of hydrogen bonds between the water molecules. Therefore there is an increase of free water molecules and molecules with free OH groups (1360 nm, 1382 nm – hydration shells).

Comparing to the basic material (SP40) the presence of the material doped with fullerene (SP40+ C_{60}) induces more water molecules responsible for hydration and even more for the material doped with fullerol (SP40+$C_{60}(OH)_{24}$). The reason for this is that free water molecules and molecules with free OH groups form stable structures and take part in hydration of NaCl molecules and of nanoparticles that are probably released from the material. The proof for this is the highest absorption in the presence of SP40+$C_{60}(OH)_{24}$ because it has the most OH groups, it is very hydrophilic and it easily forms bonds with available water molecules.

After five days (figure 9) NaCl 0.9% showed reorganization of water with slightly decreased number of free water molecules and molecules with free OH groups, but there is a significant amount of hydration shells at 1360 nm and 1382 nm. There is also strongly bounded water (left part of the aquagram at 1460 nm and 1488 nm), which is not the case for saline and for the solution in presence of all types of contact lenses.

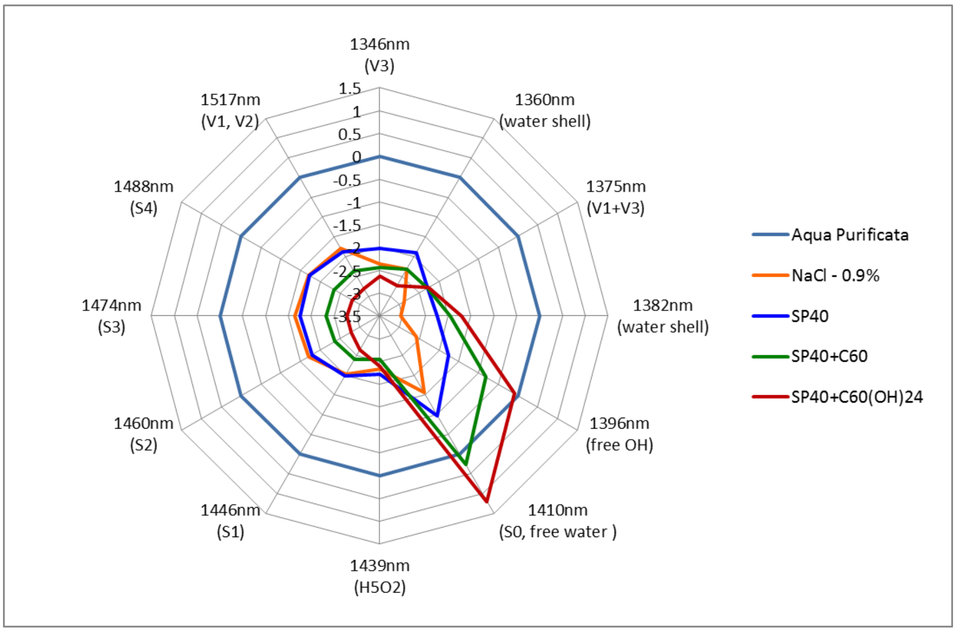

Figure 8. The aquagram: nanophotonic contact lenses in saline after 2 days.

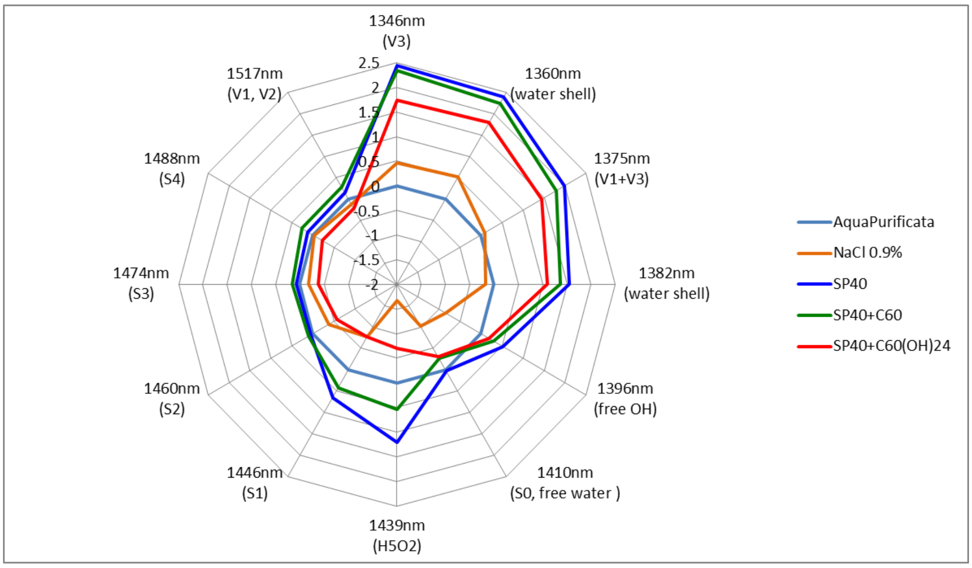

Figure 9. The aquagram: nanophotonic contact lenses in saline after five days.

In the presence of the basic material and the material with fullerene C_{60}, the organization of water is almost the same, in both cases there is large amount of hydration shells and there is absorption at 1439 nm (H5O2). In the presence of the material with $C_{60}(OH)_{24}$ the difference is significant due to the presence of strongly bounded water in a left part of the aquagram (1446 nm, 1460nm, 1474 nm and 1488 nm) which implies the presence of water molecules forming 1, 2, 3 and 4 hydrogen bonds.

4.3. Cytotoxicity study

For the cytotoxicity study we have chosen PMMA with C_{60} incorporated, as the "worst case". A cytotoxicity test on extract of nanophotonic contact lenses with incorporated C_{60} was conducted to evaluate the potential for cytotoxicity. This study was conducted according to the requirements of the United States Pharmacopeia and ISO 10993 standard: Biological Evaluation of Medical Devices, Part 5 (2009): Tests for in vitro cytotoxicity. Under the conditions of this study, the extract of the test article showed no evidence of causing cell lysis or toxicity greater than a grade 2 (mild reactivity), grade 0 for the test article (table 1). The extract of the test article met the requirements of the USP and ISO 10993 standard.

Table 1. Cytotoxicity test results.

	GRADE	REACTIVITY
	0	None
Test article	0	None
	0	None
	4	Severe
Positive control	4	Severe
	4	Severe
	0	None
Negative control	0	None
	0	None

5. Conclusion

Biocompatibility of fullerene materials is still insufficiently investigated and according to the existing results precaution is necessary. According to the study of cytotoxicity, contact lenses of standard material with incorporated C_{60} have no toxic effect on mouse fibroblast cells, therefore it can be concluded that other types of materials should not have cytotoxic effect since they contain functionalized fullerenes.

By the method of opto-magnetic imaging spectroscopy we have detected the differences in influences, but the meaning of those differences is not known until comparative toxicological investigations are done. The acquired Wavelength difference (nm) - Intensity (n.a.u.) diagrams show that all three sorts of contact lenses have almost the same influence on all three used liquids. Therefore, for aqua purificata before adding the contact lenses there are four peaks, two positive and two negative. The diagrams are the same for saline and eye drops as well. Also, it is obvious that the SP40 contact lens didn't make significant changes for aqua purificata, and for saline and eye drops there is a change, there are three peaks. For the case of contact lenses with incorporated C_{60} and $C_{60}(OH)_{24}$ in aqua purificata the peaks are at approximately the same wavelengths, only different intensities, which can imply to the same influence of both sorts of contact lenses.

In the case of saline under influence of contact lenses with incorporated C_{60} and $C_{60}(OH)_{24}$ the diagrams are slightly different. It is obvious that those sorts of contact lenses have different influences, because there are two and three peaks, at different wavelengths. It is the same for the eye drops that were under influence of contact lenses with incorporated C_{60} and $C_{60}(OH)_{24}$, but here we have four and three peaks. Also it is marked that the peaks appear at wavelengths in range of 108 nm to 122 nm, while intensities have bigger variations.

According to the aquagrams it can be concluded that there is a certain influence of the materials on saline. The water is organized around some molecules, but it is not certain whether these molecules are nanoparticles from the material or some other molecules. Infrared spectroscopy (aquaphotomics) method showed specific influences of nanophotonic materials on saline solution.

After acquiring first aquagrams it could be concluded that doped materials induce restructuring of saline meaning the number of strongly bounded water molecules decreases and the number of free water molecules and molecules with free OH groups increases. After two days the water restructured even more and the absorbance was higher for the saline with doped materials, especially in the case of a material doped with $C_{60}(OH)_{24}$. After five days it is obvious that the material doped with fullerol (SP40+$C_{60}(OH)_{24}$ has the lowest influence.

The future investigation should be focused on eye irritation and sensitivity testing, acute systemic and sub chronic toxicity and on *in vitro* genotoxicity testing according to the appropriate ISO standards.

Acknowledgments
This research has been partially funded by Ministry of Science and Technological Development of Republic of Serbia, through Project III 45009.

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