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Investigation of Influence of Nanophotonic Gas Permeable Contact Lenses on Saline By Aquaphotomics and OMI Spectroscopy

Contact lenses represent biomaterials whose main purpose is to correct the specific refractive anomaly of the eye. Since the visible light on its way to the perceptive part of the eye has to pass through the contact lens, the characteristics of the materials can significantly modify it. Biocompatibility of the lens surface is one of the most important issues in achieving contact lens wear without problems. We have developed new nanophotonic contact lens materials by adding nanoparticles of fullerene and their derivatives into standard PMMA RGP material. The aim of our investigation was to compare the influences of these materials on saline which is similar to tear film. We used NIR spectroscopy based on 12 vibration modes, called Aquaphotomics and Opto-magnetic imaging (OMI) spectroscopy as methods for characterizing the samples. The acquired spectrums were commented and compared with the standard contact lens material, which was analyzed by the same method.

Keywords: *contact lenses, NIR spectroscopy, fullerene, nanophotonic,OMI spectroscopy, biocompatibility, aquaphotomics.*

1. INTRODUCTION

Contact lenses represent a biomaterial that is widely used and relatively easy to study, due to its ease of removal from the ocular surface. Biocompatibility of the lens surface is one of the major unresolved issues in achieving contact lens wear without problems. A biocompatible or better to say ophthalmic compatible 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 the cornea doesn't get any oxygen from the blood vessels, like other tissues. 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.

The main purpose of the contact lens is to correct the specific refractive anomaly of the eye. Providing that the contact lens is produced with required optical power, the most often factors that influence the quality of sight while wearing RGP lenses are those related to the fact that visible light on its way to the perceptive part of the eye has to pass through the contact lens, thus the characteristics of the materials can significantly modify it. The imperfections of the material can cause changes in electromagnetic features of light waves.

The subject of this paper is new nanophotonic contact lens materials and their influence on saline (NaCl, 0.9%). The analysis of influence was made for standard RGP contact lens based on polymethyl methacrylate (PMMA) and the contact lenses doped with fullerene (C60), fullerol (C60(OH)24) and methformin hydroxylate fullerene (C60(OH)12(OC4N5H10)12). We used NIR spectroscopy based on 12 vibration modes, called Aquaphotomics and Opto-magnetic imaging spectroscopy as methods for characterizing the samples. Acquired results are practically applicable and according to them it is possible to develop a new generation of materials for rigid permeable and other types of contact lenses.

2. MATERIALS AND METHODS

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 (Fig. 1) [1].

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].

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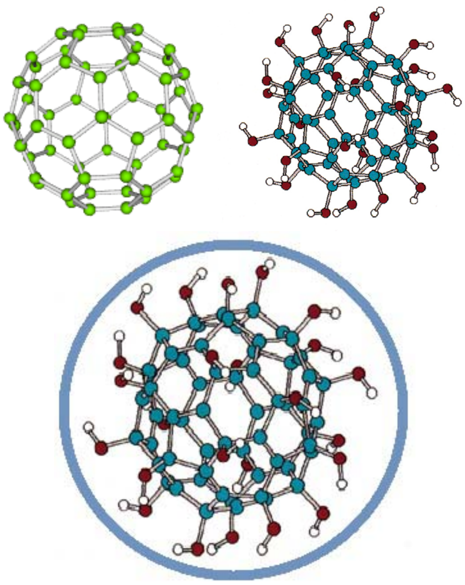

Figure 1. Fullerene C_{60} (left), fullerol $C_{60}(OH)_{24}$ (middle) and NHS-fullerol: $C_{60}(OH)_{24}$ @ $X(H_2O)$ or water shell fullerol (right) [3]

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 substance (NHS) (Fig. 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 (Fig. 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].

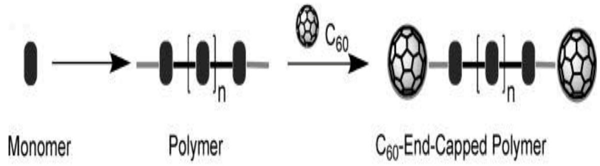

Figure 2. The synthesis of C_{60}-end-capped polymers [2].

The materials used in this investigation are new contact lens materials based on polymethylmethacrylate (PMMA) and three different nanomaterials: fullerene C_{60}, fullerol ($C_{60}(OH)_{24}$) and methformin hydroxylate fullerene ($C_{60}(OH)_{12}(OC_4N_5H_{10})_{12}$). These materials were designated as material A, B and C, respectively. The percentage of nanoparticles in PMMA was 0.33%, but they were not completely dissolved in methylmethacrylate. Polymerization was homogenous in all samples, so the new nanophotonic Soleko SP40™ material for rigid gas permeable lenses was made. The fourth polymerization was done without nanomaterials therefore this material was used as a reference sample.

The purpose of our new materials is to increase the sensibility of the eye to contrast and color perception, to decrease permeability of UV and near UV part of the spectrum, as well as to decrease the effect of light aberrations of low and high order. The results of the characterization of these materials and contact lenses by Opto-magnetic imaging spectroscopy and UV/Vis spectroscopy methods have shown that optical and mechanical characteristics such as refractive index, oxygen permeability and hardness are satisfactory. Characteristics such as transmissivity of the wavelengths from the visible part of the spectrum, protection from UV radiation, wettability and the quality of surfaces are significantly improved as well [4].

Since contact lenses are devices used directly in contact with the eye tissue, their biocompatibility is extremely important. Therefore the study of cytotoxicity according to the ISO 10993 standard: Biological Evaluation of Medical Devices was done. This study showed that contact lenses of standard material with incorporated C60 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 [5].

2.1 Set of 12 infrared spectroscopic modes – Aquaphotomics

Distinct water configurations, dimers, trimers, solvation shells, are known to contribute very specifically to the NIR water spectrum. As these configurations are very sensitive to the configuration and charges of the solvated molecules or clusters the, NIR spectrum of the solvent has been found to contain significant information about the solutes. This information could be concentration of solutes, concentrations of nano particles of various sizes, concentrations of molecules which don't absorb light in the vis-NIR range, temperature, etc.

For the characterization of the solution we used NIR spectroscopy based on 12 vibration modes, called 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 [6].

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 four 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.

2.2 Opto-magnetic imaging spectroscopy method (OMIS)

When the incident white light is diffuse, the reflected white light is composed of electrical and magnetic components, whereas diffuse incident light inclined under certain angle will produce reflected light which contains only electrical component of light. 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 [7].

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). In addition to customized camera, a software solution is used for analyzing the obtained images, yielding a characteristic diagram 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. 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 [7].

The pictures of the samples were taken one day and 6 days after leaving the contact lenses in saline solution. The solutions were poured into Petri dishes and the pictures were acquired. Every sample was shot 10 times, with white and reflected polarized light. The pictures of clear saline solution, without influence of contact lenses, were taken as well, for comparison.

The results of influence of nanophotonic contact lenses on three different aqueous solutions were previously published. The results were acquired by the same method but the conditions differed. First of all the solutions were poured into small white plastic cups instead of Petri dishes. Second, the contact lenses were taken out of the solutions after 72 hours [8].

3. RESULTS AND DISCUSSION

This paper presents the comparison of the influence of the standard RGP material and two nanophotonic materials, material A and material C. The aqaugrams and the opto-magnetic imaging results for material B, the standard RGP material with incorporated fullerol, are presented in the paper previously published [5].

3.1 Aquaphotomics results

The first results were acquired after 1h and 40 minutes (Fig. 3). 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. Material C (SP40+$C_{60}(OH)_{12}(OC_4N_5H_{10})_{12}$) and material A (SP40+$C_{60}$) show the decrease of free OH bonds and free water molecules.

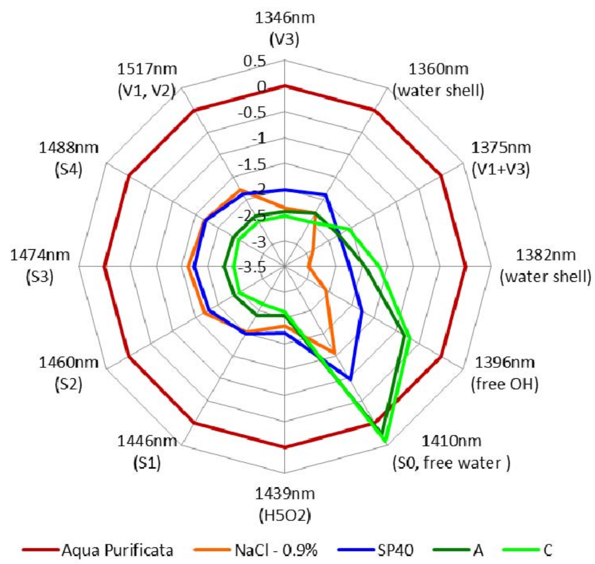

Figure 3. Aquagram: nanophotonic contact lenses in saline after 1h 40 min

The second spectrum acquisition (Fig. 4) 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 A (SP40+C_{60}) induces more water molecules responsible for hydration, which is the case for material C (SP40+ $C_{60}(OH)_{12}(OC_4N_5H_{10})_{12}$). 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.

After five days (Fig. 5) 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 except material C. 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).

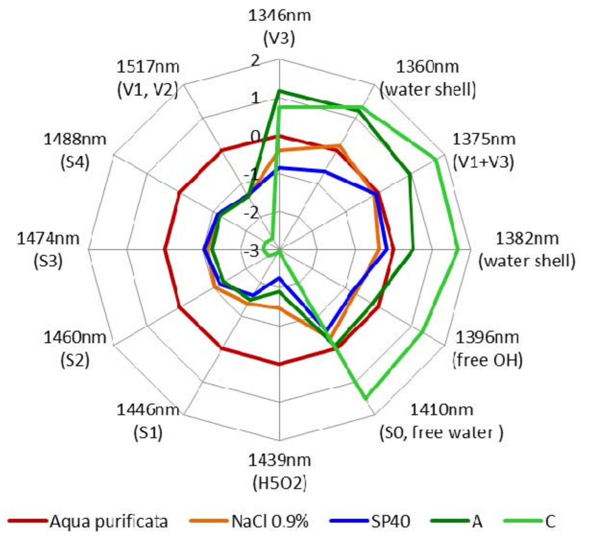

Figure 4. Aquagram: nanophotonic contact lenses in saline after 2 days

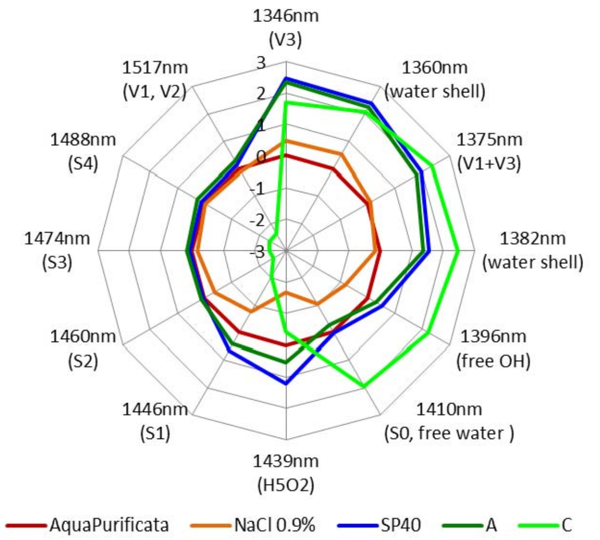

Figure 5. Aquagram: nanophotonic contact lenses in saline after five days

3.2 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. From the average spectrum diagram for saline and for saline under influence of SP40 contact lens after one day (Fig. 6) we can see there are four peaks, two positive and two negative ones. For the class of saline the average wavelengths and intensities of the first and the second positive peak are 101.139/67.57 and 115.865/58.61, respectively, and the negative ones are 104.209/-101.91 and 113.038/-47.04. However, when this liquid was under the influence of the SP40 contact lens there is a slight shift to the right that is the wavelength differences are slightly higher. The first and the second positive peaks are 101.522/63.89 and 118.712/87.47, respectively, and the negative ones are 105.354/-91.93 and 115.725/-37.87.

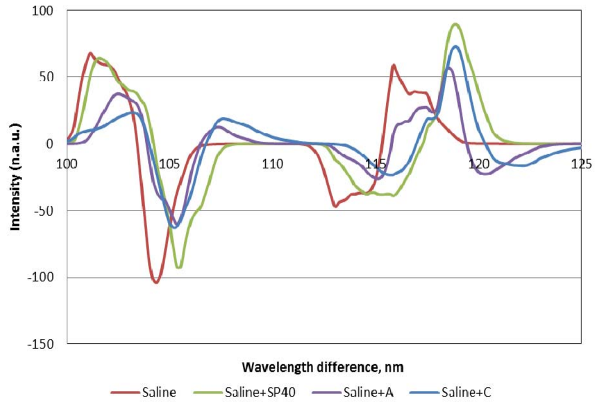

Figure 6. Comparison of Wavelength difference (nm)-Intensity (n.a.u.) diagrams for saline, saline with SP40 contact lens, saline with contact lens with C_{60} (material A) and saline with contact lens with $C_{60}(OH)_{12}(OC_4N_5H_{10})_{12}$ (material C) after one day in the solution.

For the cases of a contact lens with incorporated C_{60} (material A) and incorporated $C_{60}(OH)_{12}(OC_4N_5H_{10})_{12}$ (material C) there are also four major peaks, but the difference is the appearance of two peaks small peaks, one positive and one negative. For the material A the first and the second major peaks are 102.477/37.33 and 118.455/56.09, respectively, and the two negative ones are 105.354/-60.15 and 115.142/-26.19. The two peaks with low amplitudes are at 107.275/12.38 and 115.142/-26.19. For the material C the first and the second major peaks are 103.054/23.07 and 118.967/72.51, respectively, and the two negative ones are 105.165/-62.59 and 115.725/-23.44. The two peaks with low amplitudes are at 107.472/18.29 and 121.992/-16.31.

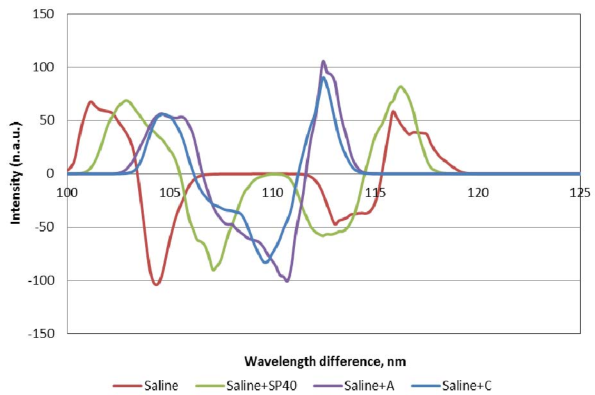

Figure 7. Comparison of wavelength difference (nm)-Intensity (n.a.u.) diagrams for saline, saline with SP40 contact lens, saline with contact lens with C_{60} (material A) and saline with contact lens with $C_{60}(OH)_{12}(OC_4N_5H_{10})_{12}$ (material C) after six days in the solution.

From the average spectrum diagram for saline and for saline under influence of SP40 contact lens after six days (Fig. 7) we can see there are four peaks, two positive and two negative ones, the same as it was after one day. Under the influence of the SP40 contact lens there is also a slight shift to the right that is the wavelength differences are slightly higher. The first and the second positive peaks are 102.865/68.97 and 116.423/78.09, respectively, and the negative ones are 107.086/-89.92 and 112.461/-58.13.

For the cases of a contact lens with incorporated C_{60} (material A) and incorporated $C_{60}(OH)_{12}(OC_4N_5H_{10})_{12}$ (material C) the difference is obvious since there are three peaks. For the material A the first and the second positive peaks are 104.593/56.73 and 112.461/105.74, respectively, and the negative one is 110.543/-96.47. For the material C the first and the second positive peaks are 104.593/55.75 and 112.461/90.19, respectively, and the negative one is 109.583/-82.73. It is obvious that the influences of material A and C after six days are almost the same; the differences are only in the amplitudes which can imply on the bonding of free electrons in the saline solution.

4. CONCLUSION

Biocompatibility of fullerene materials is still insufficiently investigated and according to the existing results precaution is necessary. 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. Near 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 $C_{60}(OH)_{24}$. The difference after five days is that now there is strongly bounded water in all cases except for material C. Same as for the previous cases after five days it can be concluded that higher absorption in the cases for doped contact lenses implicates their influence on the solution. After five days it is obvious that the material C had the highest influence on the solution while material B had the lowest influence.

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 saline. Therefore, for saline before adding the contact lenses as well as after adding the contact lenses there are four peaks, two positive and two negative. The exception is in the case of material A and C after six days in saline solution. It is obvious that the SP40 contact lens does not change the structure of saline significantly after one day or after six days, since the average diagrams have the same form and the differences in wavelength difference and intensities are not significant.

After one day in saline the contact lens with incorporated C_{60} (material A) and $C_{60}(OH)_{12}(OC_4N_5H_{10})_{12}$ (material C) have almost the same influence on saline since the form of the average diagrams are the same and the wavelength differences are right shifted. The intensities are slightly smaller than in the case of saline, which implies that the present C_{60} bonds free electrons from the saline.

After six days in saline the contact lens with incorporated C_{60} (material A) and $C_{60}(OH)_{12}(OC_4N_5H_{10})_{12}$ (material C) have significantly changed the structure of saline since the diagrams show three peaks, two positive and one negative. However, it is obvious that the there is almost no difference between the influences of these two materials. Also it is marked that the peaks appear at wavelength differences in range of 101 nm to 121 nm, while intensities have bigger variations.

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.

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REFERENCES

[1] Giacalone, F. and Martín, N.: Fullerene polymers: synthesis and properties, Chemical Reviews, Vol. 106, pp. 5136-5190, 2006.

[2] Giacalone, F. and Martín, N.: *Fullerene Polymers: Synthesis, Properties and Applications*, Wiley-VCH VerlagGmbH & Co. Weinheim, 2009

[3] Koruga, Dj.: Composition of Matter Containing Harmonized Hydroxyl Modified Fullerene Substance, US Patent 8,058,483 B2, November 15, 2011.

[4] Stamenković, D., Tomić, M., Debeljković, A., Munćan, J. and Matija, L.: How incorporated nanomaterials in contact lenses affect their mechanical and optical properties, in: *Proceedings of the 29th Danubia-Adria-Symposium*, 26-29.09.2012, Belgrade, pp. 158-161

[5] Tomić, M., Munćan, J., Stamenković, D., Jokanović, M. and Matija, L., Biocompatibility and cytotoxicity study of nanophotonic rigid gas permeable contact lens material, Journal of Physics: Conference Series, 429 012016, pp. 1-10, 2013.

[6] Tsenkova, R.: Aquaphotomics: dynamic spectroscopy of aqueous and biological systems describes peculiarities of water, Journal of Near Infrared Spectroscopy, Vol. 17, pp. 303-314, 2009.

[7] Koruga, Đ. and Tomić, A., System and Method for Analysis of Light-matter Interaction Based on Spectral Convolution, US Patent Pub. No.: 2009/0245603, 2009.

[8] Tomić, M.: *Investigation of Classical and Fullerene Contact Lenses Influence on Biological Liquids by Opto-magnetic Spectroscopy Method*, MSc Thesis, Faculty of Mechanical Engineering University of Belgrade, 2011. (in serbian)

ИСПИТИВАЊЕ УТИЦАЈА НАНОФОТОНИЧНИХ ТВРДИХ ГАСПРОПУСНИХ КОНТАКТНИХ СОЧИВА НА ФИЗИОЛОШКИ РАСТВОР АКВАФОТОМИКОМ И ОПТО-МАГНЕТНОМ ИМИЏИНГ СПЕКТРОСКОПИЈОМ

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Контактна сочива представљају биоматеријале чија је главна сврха исправљање специфичних рефрактивних аномалија ока. С обзиром да видљива светлост на свом путу до перцептивног дела ока мора да прође кроз контактно сочиво, карактеристике материјала могу значајно да је измене. Биокомпатибилност површине сочива је једно од најважнијих питања у постизању ношења контактних сочива без проблема. Развијени су нови нанофотонични материјали за контактна сочива додавањем наночестица фулерена и њихових деривата у стандардни PMMA материјал за тврда гас пропусна контактна сочива. Циљ нашег истраживања је било поређење утицаја ових материјала на физиолошки раствор који је сличан сузном филму. За карактеризацију узорака користили смо блиску инфрацрвену спектроскопију засновану на 12 вибрационих модова, познатију као Аквафотомика. Добијени спектри су коментарисани и упоређени са стандардним материјалом за контактна сочива, који је анализиран истом методом.