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Exclusion Zone Formation in Fullerol-Deionized Water Interaction

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***Abstract*— Next to the hydrophilic surfaces, it is found existence of solute-free interfacial zones i.e. exclusion zones. It appears that their properties and structure are different than those in bulk water, and that their width can be up to several hundreds of micrometers. In this research is examined solution of hydrogenized carbon nanoparticles in which are added microspheres in order to observe if there will be exclusion of particles next to the nafion. It was shown in researches till now that exclusion zones develop near different physiological regions. The goal was to show that it will appear in presence of hydrogenized carbon nanoparticles.**

***Keywords* — deionized water, exclusion zone, fullerol, interaction.**

I. Introduction

Interactions of solutes in water solutions are in the range of nanometer scale. All interactions depend of particles itself, as also substances which are diluted, in what kind of solution are diluted, how they react between themselves, and regarding that, molecules exhibit or attractive, or repulsive forces [1]. Also, type of surface, next to which and with which interaction happen, play crucial role. Next to the hydrophilic surfaces is found existence of solute-free interfacial zones, i.e. exclusion zones, which are also known as „unstirred layers“. It appears that their properties and structure are different than those in bulk water, and that their width can be up to several hundreds of micrometers. Depending on laboratory conditions, zones may persist from short to relatively long times, even up to days [2]. Such exclusion zones, also named as EZ-water, can be seen by using the micron-sized microspheres, low-molecular weight dyes, proteins.

Smaller structured areas are seen with larger microspheres (>5 µm), but these particles are difficult to keep in suspension and they sediment due to gravity. Exclusion zones are not seen in presence of solely particles smaller than 200 nm, such as quantum dots, or buckyballs. However, visualization of these particles through light microscopy is difficult.

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Aside from micro particles, topsoil, claysoil, silt, bacteria and viruses have been excluded from the region [3]. Other biological interfaces, such as muscle myofibrils, tendon and seaweed have also shown clear exclusion zones [4, 5].

Independently of researches about „unstirred“ layers in clear water and interfacial zones, studies based on physical behavior of zones in water in vicinity of hydrophilic surfaces started several years ago [6]. These studies were not just about unstirred layers next to the cells, but also about nature of water parts next to the many hydrophilic surfaces.

Mollenhauer and More were the first to point out existence of zones where exclusion happens which can be seen on ultrastructure level by using the electronic microscopy in eukaryotic cells [7]. They described these areas as „differentiated areas of cytoplasm in which ribosomes, glycogen and organelles, as are mitochondria's, plastids or micro bodies, do not exit“. Also, they point out the other cell structures which are excluding. Conclusion can be made, from different researches, that clear zones can be found next to membranes or cytoskeleton structures, actin fibers [8] and microtubules [9], and that even can be found when most of water is osmotically removed [10].

Experiments which were done by Zheng and Pollack in 2006. were based on research of colloid particles behavior next to the hydrophilic surfaces. Energetically it is more favorable for colloid particles and dissolved substances to settle away from the structural parts so they can accept water easier (hydrate). Based on that, it was tried to examine separation of detached substances in the vicinity of surfaces. Hypothesis was that dissolved substances was excluded from the zones on bordering surfaces, and that zone without substances is structurally different than water in the rest of of examined sample.

In these experiments polyvinyl alcohol gel was placed in the chamber, and border of gel was observed with optical microscope [5]. Solution of latex microspheres was poured above gel into chamber. Right after pouring, microspheres started to exclude and move away from the surface of gel to the rest of the water. After a few minutes zone free of microspheres could be seen next to the borders of gel in the width of 50-100 µm (figure 1).

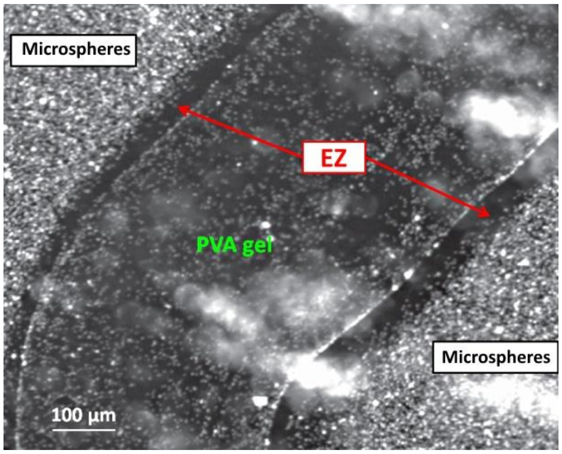

Figure 1. EZ (dark part) on both sides of polyvinil gel. Miicrosphere (up left and down right) areseparated from the part near gel. White foggy parts are reflection artefact [11].

More interesting than forming exclusion zones around polyvinyl alcohol gel were these zones around very charged polyacril-acid gel. In that case size of EZ was even till 250 μm (figure 2).

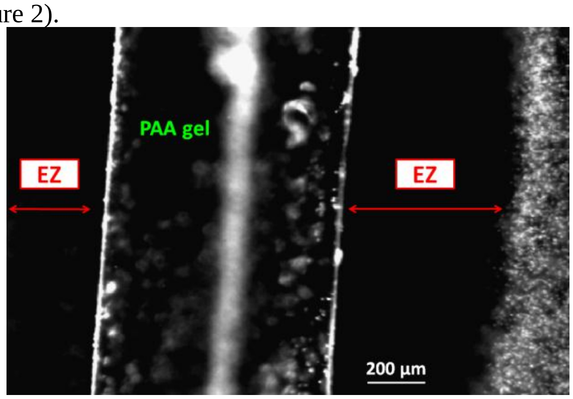

Figure 2. EZ (dar parts) n both sides of polyacril-acid gel. Microspheres which are separatedfrom the surface of gel are in the afar right part. Vertical white line in the middle of PPA gel represent optical artefact [11].

It was shown also that different dissolved substances are excluding, and that their sizes are very different, and depend on constitution itself: mud, sand, as also proteins, bacteria's, erythrocytes [12]. Materials whose structure influences exclusion are very different: polymer surfaces (nafion), hydrogels, biological surfaces (muscles), and live organisms (sea grass).

All these researches and results from different periods and laboratories, are showing that „unstirred" layers in classical physiology and clear exclusion zones in laboratories, are actually the same.

When nature of structure is observed – structure of water in unstirred layers and exclusion zones, there are 2 points of view. From one point it is thought that this water has the same characteristics as water in the rest of examined sample (bulk water), and that exclusion of particles happen only because of surfaces being near to each other. Because of entropic effects, dissolved substances should be in lower concentrations in areas near surfaces. It is considered that these effects influence that layers are wide even dozens of molecular layers. But, distances which are observed here are larger and reach even 250-300 μm, which is equivalent to quantity of around million molecules of water. On the other side, it is considered that water in this area is under influences of surface, i.e. that surface influences structure of water much more than expected. With first experiments, this is not confirmed, but recent results give base for statement that water in exclusion zone has different physical characteristics than the rest of examined water.

When all approaches are taken in account, it can be concluded that water in exclusion zone is physically and chemically different than the rest water in examined sample. Water in this area is under the significant influence of surface of material which surrounds it and that reaction which happens structurally tends to exclude particles.

In this paper was examined mechanism of formation of an exclusion zone (EZ), a region that becomes devoid of suspended hydrogenized nanoparticles and microspheres, near the surface of Nafion.

II. Materials

A. *Sample preparation. Chemicals*

Substances used for the experiments consisted of: hydrogenized nanomaterial fullerol (*MER Corporation, Tucson, Arizona, USA*), Nafion tubing (*Perma Pure, LLC*), Polybead carboxylate microspheres (*2.65% solid-latex, Polysciences Inc*.). Samples were made with deionized water, and all experiments were carried out at 22-23°C.

Deionized Water. For sample preparation for solutions is used deionized water obtained from the NANOpure Diamond ultrapure water system. The purity of this water is certified to have a resistivity value up to 18.2 MΩcm. For keeping the system to be clean, it is performed an automatic sanitization cycle. Additionally, a 0.2 μm hollow fiber filter is used for maintaining bacterial and particle-free water.

Fullerol. Fullerol solution was made of fullerol powder, purity 99,5 % dissolved in 50 ml of deionized water. In order to respect the protocol for preparation, and need that final solution of fullerol have pH = 4, in initial solution of fullerol and deionized water whose pH value was 10, it was added 120 μm of hydrochloric acid to get the pH = 3,85. Fullerol rapidly became suspension which could be seen by changing the color of solution from clear to brown. Final concentration of solution was 1mM.

Nafion. Nafion tubing TT-030 with 0.635 mm outside diameter, and 0.835 mm inside diameter was used in experiments. Before use, the tubing was cut on pieces 2mm high, and immersed in deionized water for 10 min.

Microspheres. Polybead carboxylate microspheres of 1 μm diameter were used in experiments. All of the microspheres came suspended in water. According to the need of experiment, different concentrations of solutions were made.

III. Methods

All imaging of the interface was accomplished through bright field light microscopy using a 2.5x objective on an inverted microscope (*Axiovert 35, Zeiss, Inc.*). Video and images were recorded by a CCD camera system (*Color Digital Camera #CFW-1312C, Scion Corporation*) and analyzed through ImageJ (*v1.46f, NIH*). After calibrating the camera, measurements of the exclusion zone were made by taking 5-10 measurements of the microsphere-free region and averaging the results. Averaging these results over several images for a given experiment reduced error and increased statistical significance.

First experiment was based on exploring the behavior of solutes i.e. carboxylate microspheres in fulerol solution in the vicinity of nafion. Observations of the Exclusion zone were made with free-standing nafion tubes (*model #tt-030, outer diameter = 838.2 µm, wall thickness = 101.2 µm, height = 1-2 mm*) held in place with platinum wire whose outer diameter matched the inner diameter of the tube. Platinum was chosen to reduce the influence of corrosion and other electrochemical redox reactions with the ultrapure, deionized water.

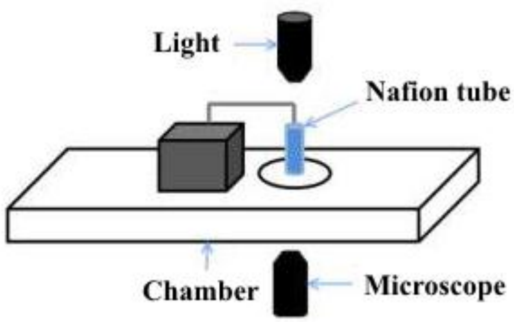

Figure 3. Experimental setup, view from above. Diameter of hole in chamber is 27mm [13].

Carboxylate (-) charged functionalized polystyrene microspheres (*model #8226-15, 18327-10, d = 1*) were suspended in fullerol solution at several dilutions (1:25, 1:100, 1:500 by volume) and were washed into the chamber containing the nafion tube to visualize the exclusion zone. Reaction between nafion and fullerol with microspheres leaded to exclusion of microspheres away from the nafion, and forming the exclusion zone. Images were taken in three intervals: 10, 15 and 20 minutes after pouring solutions. Full size exclusion zone reaches about 5 minutes after pouring solution into the chamber.

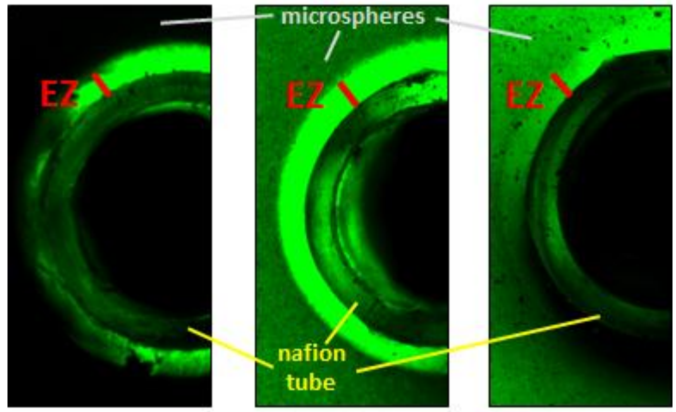

Figure 4. Exclusion zone in solutions of fullerol with microspheres in different dilutions 1:25 (left), 1:100 (middle) and 1:500 (right) [13].

IV. Results and Discussion

Carbon nanoparticles of fullerol are used because of their already known properties to accumulate [14]. Previous researches have shown that fullerol form large and flabbily associated aggregates in water [15]. Also, it was shown that even in low concentrations fullerol tends to aggregate in clusters.

The goal in this paper was to determine how and if fullerol particles will interact with particles of microspheres in deionized water solution, and if exclusion zone will appear. Hypothesis is that exclusion appears because of layers of water molecules which are ordered on different manner.

In both experiments microspheres distributed non-uniformly. They excluded almost completely from the area near the nafion tube. Away from the tube particles were uniformly distributed.

A. *Deionized water solution with fullerol and microspheres*

Zone begins to form immediately when nafion tube is immersed in solution with microspheres, and final biggest size develops around 7 minutes after pouring. Results are obtained at fixed times of 10, 15 and 20 minutes after poring solution into the chamber. Solution was added at the time t = 0 s. Table 1 shows averaged values of measured exclusion zones from images which are made. For purpose of measuring is used program ImageJ.

TABLE I. AVERAGE VALUES OF EXCLUSION ZONES IN FULLEROL-DEIONIZED WATER SOLUTION WITH DIFFERENT DILUTIONS OF MICROSPHERES.

Solution of fullerol-deionized water with microspheres		Concentration		
		1:25	1:50	1:100
Time of taking images	10 min	66	82	82
	15 min	58	77	75
	20 min	48	67	70

Results, i.e. sizes of exclusion zones are observed through dependence of time when image was taken and three dilutions of fullerol-deionized water solution.

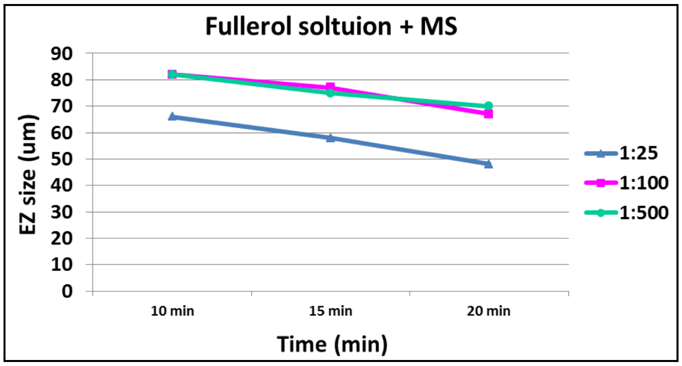

Figure 5. Diagram shows dependence of size of exclusion zone regarding to the time and dilutions.

Size of the exclusion zone through time, in all chambers and all dilutions, decreases. Exclusion zone forms rapidly and maintains sharp shape through the time.

In order to compare sizes of exclusion zones which appeared, series of other experiments were carried out. In those experiments, instead of fullerol with deionized water, is used just deionized water with microspheres. Size of chamber was the same 27 mm, as also dilutions 1:25, 1:100 and 1:500. Results are compared, and there is evidence that differences between sizes of exclusion zones exist.

TABLE II. AVERAGE VALUES OF EXCLUSION ZONES IN DEIONIZED WATER SOLUTION WITH DIFFERENT DILUTIONS OF MICROSPHERES.

Solution of deionized water with microspheres		Concentration		
		1:25	1:50	1:100
Time of taking images	10 min	93	101	98
	15 min	85	95	87
	20 min	76	87	79

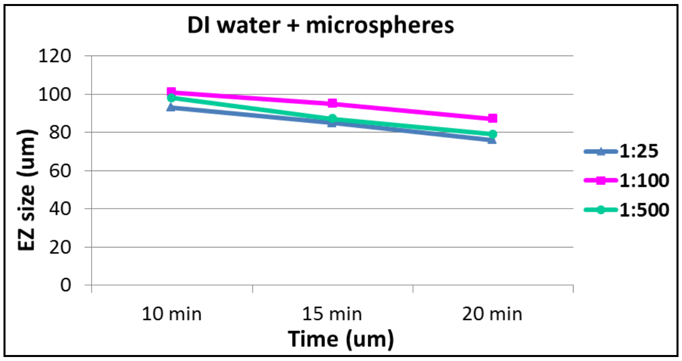

Figure 6. Diagram shows dependence of size of exclusion zone regarding to the time and dilutions.

Size of the exclusion zone is significantly smaller in fullerol-deionized water solution with microspheres, comparing to the sizes in simple deionized water solutions. Smallest sizes are notices in highest concentrations, for both types of solutions, but there is the difference in sizes between dilutions. During time, sizes are decreasing in all chambers, no matter which solution.

V. Conclusion

Identification and examining of the exclusion zones provide unique opportunities in understanding the basic mechanisms of different biomolecular interactions within water structures, as also exploring the importance of water organization in life [16].There are a numerous results which are describing the phenomenon of exclusion zone in aqueous solutions with large variety of substances and surfaces which are interacting. But, there are no exact results of examining the exclusion zone with nanoparticles. It was found that growth and size of fullerol aggregations in aqueous solutions is dynamical and sensitive on changes in pH, temperature and other environmental conditions [14].

In this research, is proved existence of differently ordered areas in water solution of nanoparticles, and it's length is dependent of concentration of solution which is used. Although the EZ zone has been demonstrated as a general phenomenon next to hydrophilic surfaces, as also phenomena which generates *in vitro* with hydrogenized nanomaterial fullerol, exact role of exclusion zone in this setup remains to be elucidated.

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