

## LIGHT SCATTERING IN RESEARCH AND QUALITY CONTROL OF DEUTERIUM DEPLETED WATER FOR PHARMACEUTICAL APPLICATION

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### ABSTRACT

**Objective:** Development of a methodology for measuring the deuterium content in water for pharmaceutical purposes by laser light scattering based on ideas about the cluster structure of water.

**Methods:** Samples of industrially manufactured drinking water from different manufacturers with varying deuterium content from 10 ppm to 115 ppm. For the titration of laboratory samples of deuterium depleted water in increments of 5 ppm the following reagents were used: Water, deuterium-depleted ( $\leq 1$  ppm ( $D_2O$ , Aldrich, USA); Deuterium oxide/Heavy water/Water- $d_2$  (99.9 atom % D, Aldrich, USA); water Milli-Q (specific resistance  $18.2 \mu S \cdot cm$  at  $25^\circ C$ ,  $TOC \leq 5$  ppb, Merck Millipore). The determination of deuterium content in samples of industrially manufactured water and water obtained in a laboratory manner was carried out by the method of low-angle laser light scattering (LALLS) at the Mastersizer (Malvern Instruments) analyzer and using a working measuring tool-laser dispersion meter/MDL («Cluster-1», Russia/Ukraine). The statistical methods-packages OriginPro®9.

**Results:** It was found that the content of isotopologies in water leads to physicochemical water's properties changes and morphology changes of giant heterogeneous clusters (GHC). The results of low-angle laser light scattering (LALLS) in the water samples under investigation showed the dependence of the water GHC "dispersibility" expressed in the differentiation of curves of the volume size distribution ("size spectra"), the volume concentration, w%, the laser obscuration values ( $I-I_0$ ) as the function of the water isotopic composition variations. The laser diffraction method results correlate with two-dimensional (2D) multi-descriptor mathematical analysis.

**Conclusion:** When identifying deuterium depleted water, it should be considered not only the indicators that determine its pharmacopoeial quality, but also the D/H ratio, because even small changes in the natural isotopic composition of water lead to significant biological effects. Our proposed approach using laser diffraction in combination with mathematical apparatus of (2D) multi-descriptor laser scattering analysis makes possible the exact calculation of individual signs of deuterium depleted water as the pharmaceutical object of study.

**Keywords:** Deuterium depleted water, Isotope control, D/H ratio in water, Mathematical descriptors, LALLS method

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### INTRODUCTION

Water quality control for pharmaceutical applications, according to the Pharmacopoeia requirements, includes routine tests of impurity content: nitrates, aluminum, heavy metals, control of bacterial endotoxins, number of viable aerobic microorganisms (Total Viable Aerobic Count), total organic carbon (total Organic Carbon in Water for Pharmaceutical Use) and specific electrical conductivity (not exceeding  $4.3 \mu S \cdot cm^{-1}$  at  $20^\circ C$  [1]. Potable Water is not covered by a pharmacopoeial monograph but must comply with the regulations on water intended for human consumption of a quality equivalent to that defined in Council Directive 98/83/EC or laid down by the competent authority [2]. However, the requirements for the isotopic composition of water currently remain beyond the scope of the pharmaceutical analysis. Differences in physical properties of water  $^1H_2O$  and  $^2H_2O$  cause abnormally high natural variations of the isotopic composition:  $^1H_2^{16}O$ ,  $^1H_2^{17}O$ ,  $^1H_2^{18}O$ ,  $^1HD^{16}O$ ,  $^1HD^{17}O$ ,  $^1HD^{18}O$ ,  $D_2^{16}O$ ,  $D_2^{17}O$ ,  $D_2^{18}O$  [3, 4]. The concentration of deuterium  $^2H(D)$  in natural water varies within the limits recorded in the standards of the isotopic composition of the hydrosphere SMOW-V, SLAP and GISP [5-7]. According to the SMOW-V standard, the content of stable isotopes in natural water is  $D/H_{SMOW} = (155.76 \pm 0.05) \cdot 10^{-6}$ . Therefore, water with a deuterium content below 155 ppm can be defined as deuterium depleted water (ddw). The modern equipment makes it possible to produce deuterium depleted water with deuterium content in the range from 140 to 1 ppm, for example, by rectification of water under vacuum using a heat pump [8]. At the same time, the physical and chemical properties of the samples of such water are not considered, and the subsequent induction biological effects are not recorded. It exacerbates the problem of objective ddw quality control in accordance with the basic principles

of the pharmaceutical analysis, therefore, it requires a full description of the physical and chemical properties of ddw. The latter condition is necessary not only for the standardization and development of routine ddw control procedures but also for understanding the mechanisms of its biological action. Deuterium in human plasma is redundant with concentrations reaching 12 – 14 mmol/l in comparison with calcium (2.24–2.74 mmol/l), potassium (5.0–5.1 mmol/l), and glucose (3.3–6.1 mmol/l) circulating concentrations. Concentration of deuterium in the body water correlates well with the deuterium level in the environment [9].

Since the discovery of heavy water in 1932, the effects of high and low concentrations of  $D_2O$  on its biological objects have been studied. Replacing conventional hydrogen with heavy hydrogen leads to a decrease in the rate of chemical reactions (3 to 10 times), to a slowdown in many chemical reactions that determine complex biological processes in cells, tissues and the entire body [10–13].

The results of the research of using deuterium-depleted water that naturally occurring deuterium is essential for maintaining the normal cell growth rate study published in 1993 in the European Biochemical Societies journal, gave a new impetus to the development of scientific and practical interest in the biological effects of ddw [14]. It was shown [15] that long-term use of deuterium depleted water before irradiation (for 14 d) led to an increase in the sensitivity of the chromosomal apparatus of mice to  $\gamma$ -irradiation against the background of increased mitotic activity of cells. The numerous results reveal that the growth of different animal cell lines in tissue culture is inhibited in deuterium-depleted water. This striking observation suggests that the natural abundance of D is essential to trigger and maintain the normal growth rate of the cell. In the *in vivo* experiments

the longer survival and the fact that in 59% of the tumorous mice the tumor disappeared because of the treatment with deuterium depleted water also suggest that the presence of D in a certain concentration is necessary for normal cell [16-25].

In several publications, ddw is presented as a component for the preparation of combined pharmaceutical compositions, as well as a solvent for increasing the solubilization characteristics of API [26-30].

Drinking water with a low content of deuterium is widespread both in veterinary practice and for daily human consumption. Deuterium depleted water with the ratio of D/H =  $25 \pm 5$  ppm (Vetera-DDW-25 A. U. V., 13/99 FVM) induced complete or partial tumor regression in dogs and cats with spontaneous malignancies, it was registered as anticancer for veterinary use in 1999 [31]. The hypodermic preparation of the registered veterinary drug was successfully tested in clinical investigations.

Thus, there is every reason to believe that ddw is on the way from its consumption as drinking water to obtaining the status of "water for pharmaceutical purposes".

The existing methods of immediate off-line and on-line control of the isotopic composition of water in a wide range of concentrations densimetry, mass spectrometry, gravimetry, laser absorption spectroscopy, NMR, IR spectrometry are characterized by a significant error in the standard approach to the isotopic analysis of heavy water [32]. The proposed method of static low-angle laser light scattering with our technique of mathematical modeling of physical processes of agglomeration and formation of the cluster structure of water based on the calculation of physical and chemical descriptors is devoid of these shortcomings. Work [33] experimentally showed the influence of the isotopic composition of water samples with different D/H ratios on self-organization of water clusters

The isotopic analysis of drinking water is complicated by the influence of salt composition, taking into account the need for drinking water conditioning as a result of rectification and ion exchange [34].

Thus, this article refers to water systems with different variable ratios and macroscopic properties of D<sub>2</sub>O and H<sub>2</sub>O. Our work is aimed at studying the patterns of light scattering in samples of drinking and laboratory water with low deuterium content.

Thus, the aim of the present study was to develop a method for measuring the deuterium content in water for pharmaceutical purposes and further implement on-line control of its industrial production using laser light scattering based on ideas about the cluster structure of water.

## MATERIALS AND METHODS

### Solutions under research

Samples of industrially manufactured drinking water from different manufacturers with varying deuterium content from 10 ppm to 115 ppm were investigated in the work: DDWater ("Super Light Water", Argentina, 115 ppm), DDW ("DDWater Bioengineering Co., Ltd.", Shanghai, China, 50 ppm), Helkare ("Henan Infinity Bioengineering Technology Co., Ltd.", Hebi, China (mainland), 70 ppm), DDW (Swiss AG, 50 ppm), Himali (Daudoug, 60 ppm), DDW Langvey (LLC MTK Iceberg, Moscow, Russia, 50 ppm), DDW VIVIDI (JSC "Almaz", Kotovsk, Russia, 10 ppm).

High-resistance waters with the [D/H] ratios of 45, 50, 55, 60, 70, 75, 80, 85, 90, 95, 100, 105, 115, 120, 125, 130, 140, 145, 150 (ppm) were investigated. For the titration of laboratory samples of deuterium depleted water in increments of 5 ppm the following reagents were used: Water, deuterium-depleted  $\leq 1$  ppm (D<sub>2</sub>O, Aldrich, USA); Deuterium oxide/Heavy water/Water-d<sub>2</sub> (99.9 atom % D, Aldrich, USA; water Mili-Q (specific resistance 18.2  $\mu\text{S}\cdot\text{cm}$  at 25 °C, TOC  $\leq 5$  ppb, Merck Millipore).

For conditioning of drinking water in terms of content of Cl<sup>-</sup>, HCO<sub>3</sub><sup>-</sup> and Na<sup>+</sup> ions to correct the hydrogen index (pH) of water, increase alkalinity and improve the taste of water, chemically pure substances were used as mineral additives. Aqueous solutions at NaCl and NaHCO<sub>3</sub> ionic strengths (pH= 7.0) of 1.2; 2.9; 4.6; 6.3; 8.0; 11.5 mmol with [D/H] ratios (ppm) of 45, 50, 55, 60, 70, 75, 80, 85, 90, 95, 100, 105, 115, 120, 125, 130, 140, 145, 150.

The pharmaceutical substance of sodium chloride NaCl and NaHCO<sub>3</sub> (manufactured by Sigma, Aldrich, USA).

### Methods of differentiation of samples for research

The solutions were filtered through a submicron inert membrane filter (Millex-GV Filter with a 0.22  $\mu\text{m}$  pore size hydrophilic PVDF membrane, Merck Millipore, UK).

### Laser diffraction method

The determination of deuterium content in water samples obtained in industrial and laboratory manners was performed by static low-angle laser light scattering (LALLS) using the Master Sizer 2000 instrument, Zeta Sizer Nano ZS instrument (MALVERN Instruments, UK) and "Cluster-1"/MDL-1, laser meter of dispersion (manufactured by the Institute of Colloid Chemistry and Chemistry of Water, Ukraine and RUDN University, Russia) [35]. Laser scattering occurs on optical inhomogeneities of the medium that appear when the refractive index changes [36]. To characterize the "dispersibility" of the samples, the grain-size distributions by volume and number fractions ("size spectra"), and the integral characteristics of "dispersibility" are subject to analysis: laser light scattering/laser obscuration value (1-T), volume concentration (%), specific surface area (sm<sup>3</sup>/g).

### 2D laser light scattering method

To study the kinetics of "flickering" of giant heterogeneous water clusters (GHC), we used our method of 2D laser light scattering [37]. The main stages of the algorithm for calculating interference patterns: image segmentation, representation, description and image analysis for identification purposes [38]. To describe the local vicinities of the IR characteristic points, special descriptors-analogs of topological indexes, which are characterized by invariance at different geometrical and photometric transformations of the received image, are constructed:  $SD_1 \div SD_3$ ,  $R_1 \div R_3$ , R, where  $(SD_1 \div SD_3)$  is the standard deviation from the mean;  $R_1 = \frac{d_i}{SD_i}$  is signal-to-noise ratio (Signal/Noise, SNR) [39].

### Statistics

The findings were processed by the statistical methods using software packages of Origin Pro 9.1. Each value on the fig. represents "mean $\pm$ SD", \*P<0.05.

## RESULTS

### The influence of isotopy on the physicochemical water's properties

The substitution of the hydrogen atom with deuterium leads to the fact that many physical, chemical and biological properties of H<sub>2</sub>O and D<sub>2</sub>O differ from each other. Compared with H<sub>2</sub>O<sup>16</sup>, the heavy water is characterized by higher values of density, boiling point, and melting point. The heat capacity of the heavy water at 20 °C is 10% higher than that of the "light" one, the mobility of D<sup>+</sup> and OD<sup>-</sup> ions is almost 50% lower than that of H<sup>+</sup> and OH<sup>-</sup> ions. Earlier, we published the results of a study of the properties of water with different contents of heavy isotopes of hydrogen by NMR spin echo, quasi-elastic scattering of slow neutron QSSN (13,289 MeV), viscometry, and others [40, 41]. The experimental data provide direct evidence that the density, surface tension, and water viscosity anomalies caused by variable deuterium concentration lead to the formation of clusters (density inhomogeneities) of water of different sizes, hence the realization of the isotope effect through the cluster organization of the associated liquid (fig. 1) [42].

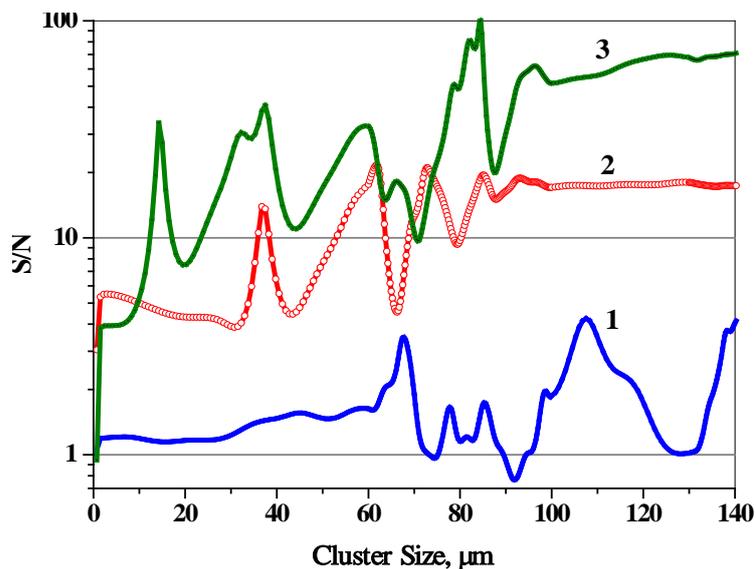


Fig. 1: Signal-to-noise ratio (SNR) when registering the size of water clusters by LALLS-method in water samples with the different content of heavy isotope of  $^2\text{H}(\text{D})$ : 1-140 ppm; 2-99.9%  $\text{D}_2\text{O}$ ; 3-5 ppm (deuterium depleted water);  $n=15$ ;  $*P<0.05$ ,  $\text{SNR} = \frac{\bar{V}}{SD}$ ,  $\bar{V}$ -average volume fraction (%) of water clusters, SD-standard deviation

The graph shows a downward trend in the level of "noise" in the water in case of the replacement of protium by deuterium. Consequently, the D/H ratio can act as the "control" parameter of water GHC size distribution.

#### The influence of the water isotopologies content on the morphology of water GHC

We used the method of low-angle light scattering to study the structure of deuterium-stabilized water giant heterogeneous

clusters (GHC) formed in solutions. The feature of the problem to solve is the fact that in the study of aqueous solutions with different D/H ratios, there is no possibility of analyzing them using well-known analytical methods. Using the physical principle of electromagnetic wave scattering on density inhomogeneities deuterium-stabilized water giant heterogeneous clusters, the angular distribution of the intensity of scattered light was measured as the laser beam was passing through a cell consecutively filled with laboratory samples of water with different D/H ratios (fig. 2).

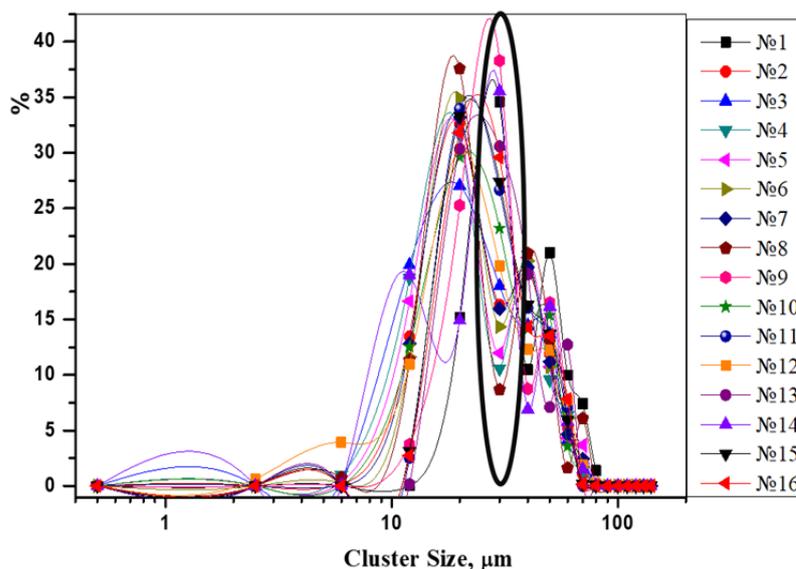
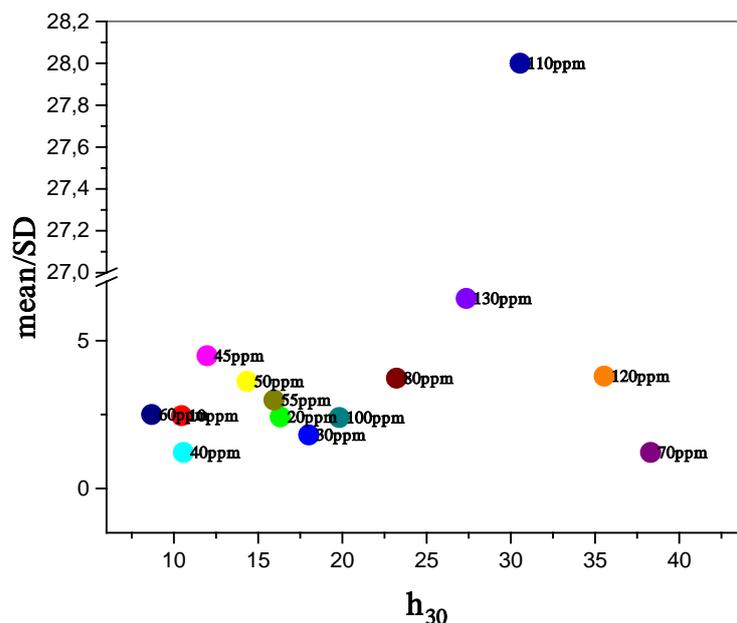


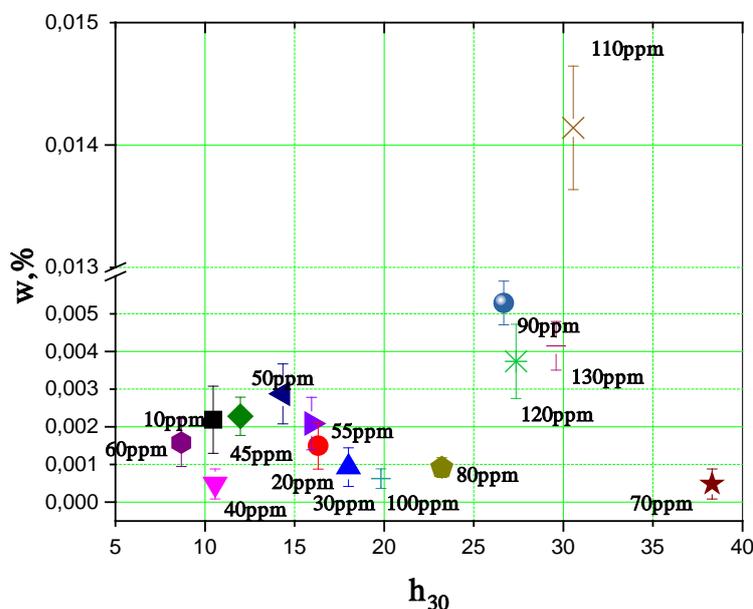
Fig. 2: The grain-size distributions by volume fractions ("size spectra") in laboratory samples of deuterium depleted water with different D/H ratios: № 1-10 ppm; № 2-20 ppm; № 3-30 ppm; № 4-40 ppm; № 5-45 ppm; № 6-50 ppm; № 7-55 ppm; № 8-60 ppm; № 9-70 ppm; № 10-80 ppm; № 11-90 ppm; № 12-100 ppm; № 13-110 ppm; № 14-120 ppm; № 15-130 ppm; № 16-140 ppm;  $n \geq 5$ ,  $*P<0.05$

The grain-size distribution curves show the area of differentiation of all laboratory samples of deuterium depleted water at water GHC diameter  $d = 30 \mu\text{m}$ . This difference allowed

us to determine the characteristic areas occupied by deuterium depleted water samples when measuring calibration diagrams (fig. 3).



A



B

Fig. 3: The one-to-one correspondence between the SNR (A), the volume concentration, w% (B) and maxima of the 30 μm water GHC size group; n=5, \*P<0.05

**Measurement the content deuterium in drinking water based on mathematical models of diffraction method**

The technique is based on the calculations of the interference pattern formed by light beams after laser light scattering in the test sample on the acoustic wave (fig. 4).

**Mathematical descriptors**

The interference patterns recorded on the diode matrix undergo changes in time due to the kinetics of the formation and decay of water GHC and they were subjected to the mathematical analysis using descriptors  $d_1$ ,  $d_2$  and  $d_3$ , which are characterized by low

variability at various geometric and photometric transformations of the image obtained in time (table 1).

**2D Laser light scattering mathematical model**

The dependencies of the analytical signal and the standard deviation of the results on the D/H ratio in the studied water samples were investigated. The results of the correlation method are presented in the form of two-dimensional (2D) diagrams of multi-descriptor analysis [43] (similar to the "fingerprint" in molecular biology) and make it possible to visually illustrate the topology of 2D laser scattering for a series of images of deuterium depleted water obtained by laboratory dilution and in industrial manner (fig. 5).

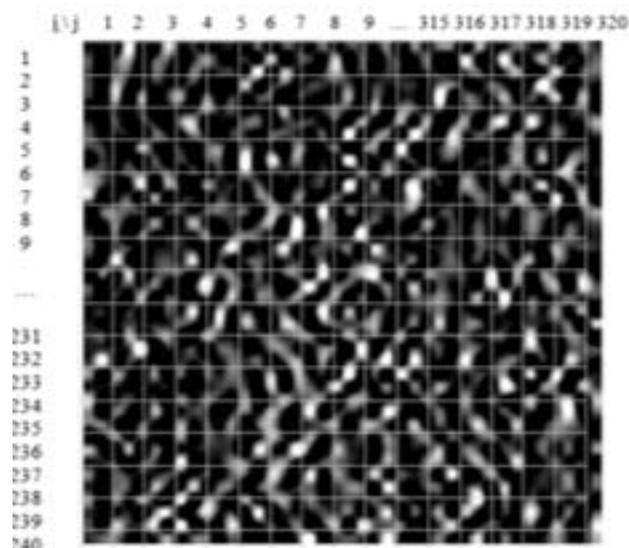


Fig. 4: Discrete representation of the interference pattern

Table 1: Mathematical representation of descriptors

Descriptor	Mathematical representation	Description
$d_1$	$d_1 = \frac{i_{\Delta S_i > S_b}}{i_t} \cdot 100\%$ Where $i_t$ is the total number of elements; $\Delta S_i$ is the value of differences in the signal level of the elements of two interference patterns; $S_b$ is the threshold level of the signal.	– the number of different elements, regardless of the degree of difference
$d_2$	$d_2 = \frac{\sum_{\Delta S_i > S_b} \Delta S_i}{i_t \cdot \bar{S}} \cdot 100\%$ where $\sum_{\Delta S_i > S_b} \Delta S_i$ is the average value of the signal level of all the elements of the original interference pattern	– the degree of difference for each discrete element based on the original interference pattern and the total intensity of the level of its signal
$d_3$	$d_3 = \frac{\sum_{\Delta S_i > S_b} \Delta S_i}{i_t \cdot \Delta S_{max}} \cdot 100\%$ where $\Delta S_{max}$ is the sum of max possible differences in terms of the signal level of all the relevant elements of the interference patterns of absolute black and absolute white	– the max value of possible differences between the interference patterns of absolute black and absolute white

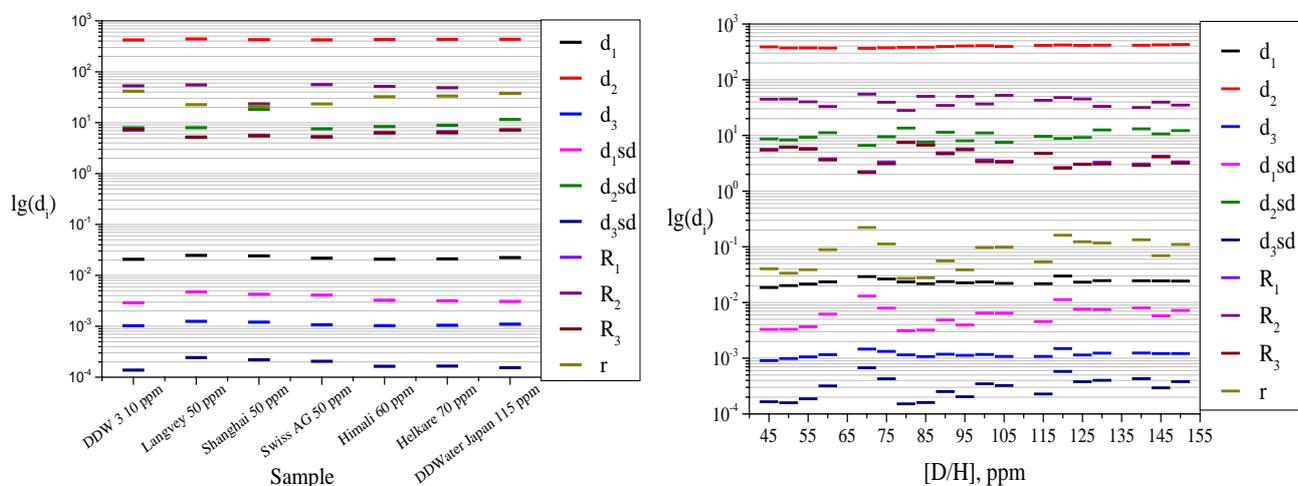


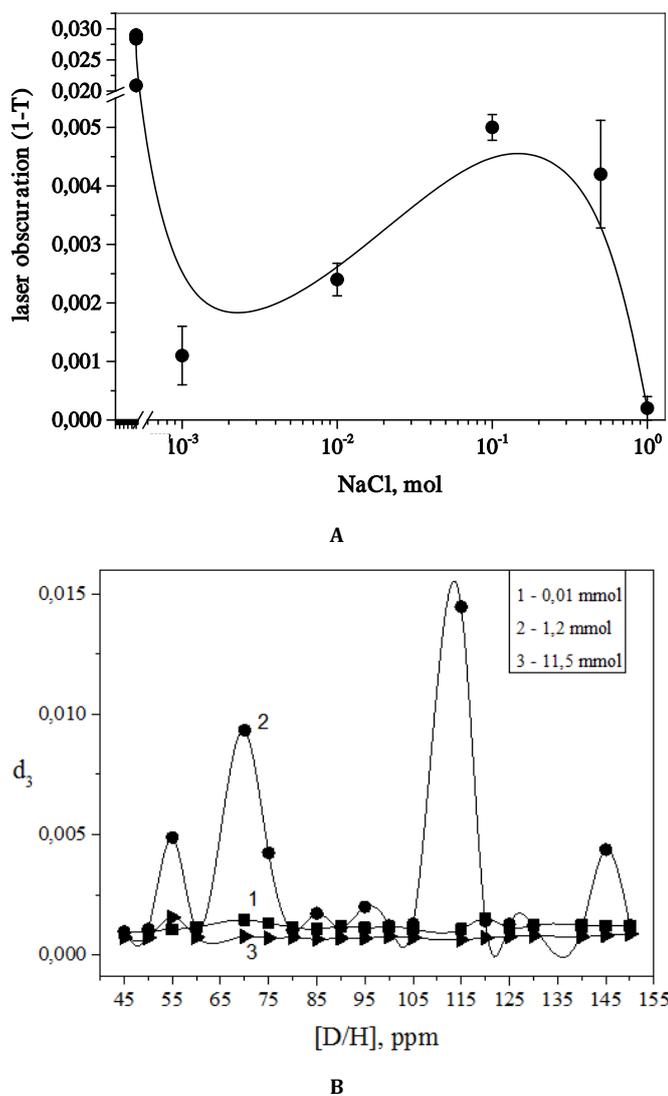
Fig. 5: Two-dimensional (2D) diagrams (“fingerprint”) of the industrial water samples for ten descriptors for each sample (A) and the laboratory samples with different ratio [D/H] (B);  $n \geq 100$ ,  $*P < 0.05$

The comparison of different D<sub>2</sub>O and H<sub>2</sub>O dilutions both in laboratory dilutions and in samples of drinking water for pharmaceutical application shows that the multi-descriptor set is individual for each sample of dilution in a given interval. Therefore,

comparing the value of each descriptor of an unknown dilution with the values of the corresponding descriptors of each of the dilutions from the calibration interval from 45 to 155 ppm, it is possible to determine the exact deuterium content in the water.

Since drinking water and water for pharmaceutical recognition are subject to mandatory conditioning procedure for correcting the acidity (pH) of water and enriching it with salts, we found it interesting to measure the calibration diagrams of deuterium

depleted water samples saturated with sodium salts (fig. 6). From the literature, the effect of concentration and ionic strength of electrolyte solutions of the water GHC structure is well known [44].



**Fig. 6: The influence of concentration (ionic strength) of electrolyte NaCl solutions on the water GHC structure and the laser obscuration values (A);  $n=15$ ,  $*P<0.05$ , Diagram of  $sd_3$  variability dependence on the value of  $d_3$  descriptor for the laboratory samples with different ratio [D/H] and concentration (ionic strength) of electrolyte NaCl+NaHCO<sub>3</sub> solutions (B);  $n\geq 100$ ,  $*P<0.05$**

In NaCl solutions with ionic strength  $I = 0.001$  mol, the light scattering ability of the solution drops sharply and becomes close to that of deionized water. A 100-time increase in the ionic strength of the solution contributes to the formation of water GHC, the light scattering ability of the solution increases dramatically.

## DISCUSSION

It was found that the content of isotopologies in water leads to changes in the morphology of water giant heterogeneous clusters (GHC) and physicochemical water's properties, similarly with [45]. The results of low-angle laser light scattering in the water samples under investigation showed the dependence of the GHC "dispersibility" expressed in the differentiation of the laser obscuration values ( $I_{I_0}$ ), the volume concentration, % and the curves of the volume size distribution function of the variations of the isotopic composition of water. The analysis of the signal-to-noise diagrams in the size group of  $30 \mu\text{m}$  revealed a differentiated location of the maxima in the histogram of the volume distribution.

The results of the LALLS-method correlate with the calculations made by us using a computer model based on the multi-descriptor analysis [46] and reliably describe the analytical characteristics of the samples for the comparative research using mathematical descriptors. It was shown [47] that the molecular topological indices are numerical invariants of a molecular graph and are useful to predict their physicochemical and biological properties. The study of laboratory dilutions of D<sub>2</sub>O and H<sub>2</sub>O and samples of industrially manufactured drinking water in the range of the D/H ratio from 45 to 155 ppm by the method of laser 2D light scattering [48] allowed the accurate calculation of the individual characteristics of deuterium depleted water due to the existence of "flickering" deuterium-stabilized water clusters.

## CONCLUSION

There has been developed a method for measuring the deuterium content in water for pharmaceutical purposes and created a database that includes characteristics of industrially manufactured

water samples and samples obtained in the laboratory. Laser diffraction in combination with mathematical apparatus of two-dimensional laser scattering analysis (2D) using variables of ten descriptors for each sample could to determine the D/H ratio from 45 to 155 ppm in water exactly and relate a test sample with a sample from the database. The results obtained reflect the distribution of density inhomogeneities in aqueous solutions (LALLS method) and mathematical description using descriptors. The developed method can be used during the water quality control in the production for pharmaceutical purposes, given that even small changes in the natural isotopic composition of water lead to significant biological effects.

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#### AUTHORS CONTRIBUTIONS

All the authors have contributed equally

#### CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest

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