INTRODUCTION

Pharmacopeias of different countries use the polarimetric method for identification and quantitative analysis of chiral active pharmaceutical ingredients (API) and excipients [1-3]. Polariometry does not lose its relevance and is technically improved in pharmaceutical research [4, 5], despite the long history of its existence [6] and the introduction of new optical methods [7,8]. Pharmacopoeia measurements of the optical activity of API are carried out in waters with different pH values depending on the nature of the substance. So solutions of ascorbic acid (H₂Z) are analyzed in aqueous solution at pH that corresponds to the predominance of the single-charged anion HZ⁻ (pKₐ1 < pH < pKₐ2) [9]. For the purpose of quality control of carbohydrates, ammonia is added into the aqueous solution. This is accompanied by a change in the hydration conditions of monosaccharide molecules [10], and the equilibrium between α- and β-anomers is achieved in non-hourly, but minute kinetics [11]. Polarimetric analysis of amino acids is usually carried out in 6-8 mol/l HCl, where the amino acid is fully protonated (HOOCH₂-NH⁺) and optical rotation is constant [12].

The choice of specific pH values for polarimetric measurements is not accidental. The interaction with giant heterogeneous clusters (GHC) of water depends on the chemical form of the optically active substance. Moreover, stereoisomers that interact with water clusters [10] are able to generate chirality of the latter [13-15]. For example, it has recently been experimentally proved for water structures adjacent to DNA macromolecules [7, 16].

In our previous studies, we demonstrated that the structure and size of water GHC depend both on pH and on the isotopic composition of water, primarily on the ratio of deuterium: protium (D/H) [17]. Now the contribution of chiral GHC optical rotation was studied using solutions of chiral substances with different D/H ratios at different pH values. The experimental results obtained from aqueous solutions of chiral compounds of various chemical classes confirm the chiral water clusters formation. It is confirmed by our observation of Biot’s law [6] infringement in low concentration ascorbic acid solutions, expressed in the absence of a constant value of the specific optical rotation [α]D at a concentration of below 0.1%, depends on the D/H ratio. The inequality was established in absolute values of optical rotation for L- and D-isomers of valine in solutions with different ratios of hydrogen isotopologues. The mutarotation of glucose confirmed the first-order kinetics, and the activation energies were statistically distinguishable for BD and DDW. The mutarotation of the natural galactose D-isomer proceeded with a lower energy consumption compared to the L-isomer. In heavy water, the mutarotation of monosaccharides had different kinetic mechanisms. Polarimetric results correlated with the number and size of GHC, which confirmed the possibility of chiral solvent structures induction by optically active pharmaceutical substances.

RESULTS:

The infringement of Biot’s Law was found for solutions of ascorbic acid, expressed in the absence of a constant value of the specific optical rotation [α]D at a concentration of below 0.1%, depends on the D/H ratio. The inequality was established in absolute values of optical rotation for L- and D-isomers of valine in solutions with different ratios of hydrogen isotopologues. The mutarotation of glucose confirmed the first-order kinetics, and the activation energies were statistically distinguishable for BD and DDW. The mutarotation of the natural galactose D-isomer proceeded with a lower energy consumption compared to the L-isomer. In heavy water, the mutarotation of monosaccharides had different kinetic mechanisms. Polarimetric results correlated with the number and size of GHC, which confirmed the possibility of chiral solvent structures induction by optically active pharmaceutical substances.

CONCLUSION:

In the optically active pharmaceutical substances quality control there should be considered the contribution of induced chiral GHC of water to the optical rotation value that depends on the isotopic D/H ratio, the substance nature and the form of its existence at a given pH.

Keywords: D/H ratio in water, Giant heterogeneous clusters of water, The influence of chiral compounds on water clusters chirality

ABSTRACT

Objective: Methodology development for quality control of optically active pharmaceutical substances based on water isotopologues.

Methods: Solutions of L-ascorbic acid, glucose, galactose and valine stereoisomers were prepared using deuterium depleted water (DDW-light-water, D/H=4 ppm), natural deionized high-ohmic water (BD, D/H=140 ppm), heavy water (99.9% D₂O). The optical rotation was observed using an automatic polarimeter Atago POL-1/2. The size distribution of giant heterogeneous clusters (GHC) of water was recorded by low angle laser light scattering (LALLS) method.

Results: The infringement of Biot’s Law was found for solutions of ascorbic acid, expressed in the absence of a constant value of the specific optical rotation [α]D at a concentration of below 0.1%, depends on the D/H ratio. The inequality was established in absolute values of optical rotation for L- and D-isomers of valine in solutions with different ratios of hydrogen isotopologues. The mutarotation of glucose confirmed the first-order kinetics, and the activation energies were statistically distinguishable for BD and DDW. The mutarotation of the natural galactose D-isomer proceeded with a lower energy consumption compared to the L-isomer. In heavy water, the mutarotation of monosaccharides had different kinetic mechanisms. Polarimetric results correlated with the number and size of GHC, which confirmed the possibility of chiral solvent structures induction by optically active pharmaceutical substances.

Conclusion: In the optically active pharmaceutical substances quality control there should be considered the contribution of induced chiral GHC of water to the optical rotation value that depends on the isotopic D/H ratio, the substance nature and the form of its existence at a given pH.

Acknowledgments: This study was financially supported by Russian Foundation for Basic Research, grant 17-03-00884-a.

© 2018 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/)
isotopology will improve pharmaceutical analysis and optimize their dosages, reducing the toxic load on the body.

MATERIALS AND METHODS

Water samples

Deionized high-ohmic water (specific electrical resistivity of 18.2 MΩ cm at 25 °C) was prepared by purifying pyrogenic distilled water (BD, D/H = 140 ppm) on a Milli-Q system (Millipore, Great Britain). Deuterium depleted water, «light» water (DDW, D/H=4 ppm) was purchased at the Research and Production Association (RPA) «Almaz» using the vacuum rectification technique. Deuterium oxide, heavy water—99.9% D₂O (Sigma Aldrich).

Definition of isotopic composition

The deuterium concentration was determined by using the mass-spectrometry method and multipass laser absorption spectrometry method (LWIA24d instrument, Los Gatos Research, USA).

Optically active pharmaceutical substances

D-valine (D-2-amino-3-methylbutanoic acid), L-valine (L-2-amino-3-methylbutanoic acid) and racemic valine (Sigma-Aldrich, USA), optical purity ≥ 99%; L-ascorbic acid (l-threo-ascorbic acid) (Sigma-Aldrich), content of API ≥99%; D-glucose, L-glucose, D-galactose, L-galactose (Sigma-Aldrich), content of API ≥99.5%.

Polarimetry

Optical activity was determined using the Atago POL-1/2 polarimeter (Japan), in a 100 mm cell, the measurement accuracy of±0.002 ° and the resolution of 0.0001 °. The electronic Peltier module was used for setting the required temperature (T=20 °C).

Determination of water clusters size distribution

Investigation of the water cluster size distribution was carried out by laser light diffraction spectroscopy and dynamic light scattering (DLS) methods on Master Sizer 2000 instrument and Zeta Sizer Nano ZS instrument (MALVERN Instruments, UK). Hexane was used as a background. Before the experiment, hexane, the water samples and solutions were filtered through 0.22 µm filters ("Millipore").

Statistics

The findings were processed by the statistical methods using software packages of Origin Pro 9.1. Each value on the fig. represents «mean±SD».

RESULTS AND DISCUSSION

The infringement of the Biot’s Law in ascorbic acid solutions with different D/H ratio

According to Biot’s law ([J. B. Biot] α = [α]) • [T] • [C], the specific rotation of optically active pharmaceutical substances’ solutions in selected temperature (T) and wavelength (λ) does not depend on the concentration (C): [α] = 1/[T] • [C] [6]. However, in quantum-mechanical calculations of the last, it was shown, that in some cases such relation may be infringed [25]. In the non-linear function of specific optical rotation for 0.03-4 mol/l solutions of D-levoglucosan, the authors explain the presence of heterogeneous water structures, which sizes enlarge in case of dilution. The mutual effect of optically active substances and solvent associates was called «solvent imprinting», i.e. the, contribution of supramolecular structures to the value of specific optical rotation [26, 27].

In our experiments, the fact of «solvent imprinting» presence is confirmed, which is displayed in an increase of special optical rotation in the case of L(+)-ascorbic acid concentration decline. We investigated ascorbic acid solutions with a different D/H ratio and concentrations of 1% to 0.025% (pH=3.3–3.6). Simultaneously, we controlled the presence of water GHC by LALLS method considering that their size spectrum changes in dependence of the D/H ratio [28, 29]. The function of «α-C» in the observed concentrations interval is depicted in ascending straight-lines, which did not allow us to identify any solvent isotopic composition features in the selected scale (fig. 1). At the same time, we noticed an increase of specific optical rotation in case of solutions dilution below 0.5%. Taking into account numerous information about the impact of the solvent nature on the optical rotation values of chiral compounds [6] and the chirality induction in water supramolecular structures of [7, 29], we can assume, that observable the Biot’s law infringement is manifested in optical active water GHC contribution to the [α] value. Undoubtedly, the structure and quantity of water supramolecular associates play a significant role in this process. Since the heterogeneity of water is constituted in a row where BD>DDW>D₂O, the strongest contribution to the specific optical rotation value was awaited for the ascorbic acid solutions with natural isotopic composition (D/H=140 ppm). Indeed, our assumption was confirmed, and in the case of dilution [α] was maximal for BD. It declined according to demonstrated row and practically disappeared for D₂O.

The fact concerning the specific optical rotation dependence on the concentration of the chiral compound has been discussed for half a century [6, 30]. There were indications that this dependence corresponds to a parabolic function and the necessary extrapolation conditions [α] to the zero point at C→ 0 [30]. But specific rotation did not have to get a zero value in case of the zero solution concentration, as soon as it depended on the nature of the solvent [6]. In specific cases, where the limitless dilution of the solution leads to the extrapolation of the specific optical rotation to zero, it is worth speaking about “intrinsic” specific optical rotation, which is displayed not in square brackets, but in braces [α]. This contributes to the «substance-solvent» systems, in which a relation does not exist. The experimental search for these systems is complicated by the absence of the mentioned relation is accompanied by a decrease in substance solubility.
Thus, the Biot’s law infringement is revealed as a specific optical rotation in the case of L-ascorbic acid solution dilution, which depends on the D/H ratio and is correlated with a water heterogeneity degree. Obtained results show the chirality induction in supramolecular complexes in water.

**Polarimetric analysis of valine stereoisomers in waters with different D/H ratio**

Pharmacopoeia quality control assay of L-valine substance [1, 3] is held in 6-7 mol/l HCl solution, which ensures the relative stability of the optical rotation in accordance with the shift rule (Lutz’ and Jirgensons’ shift-rule) [12]. In these conditions of pH values which are considerably lower than valine $pK_{a1}$, cationic, fully protonated form of the amino acid only be present (fig. 2), which presumes the monotype mechanism of action between water GHC and valine that results in the constant value of optical rotation. Nevertheless, even in these tough conditions of acidity, world pharmacopeias mention an ambiguity in specific rotation values in 2-3 units interval for aqueous solutions of amino-acids (not only for valine). For instance, for 8% aqueous solution of L-valine this interval is $+26.5 ÷+29.0$ which reflects the contribution of other chiral structures to the optical rotation value. The possible contribution of chiral supramolecular water structures on the optical rotation value was indicated by different authors [7, 14, 29, 31].

\[
pH = pK_{a1} = 2.3 \quad \quad \quad \quad pH = pI = 5.9 \quad \quad \quad \quad pH = pK_{a2} = 9.6
\]

In the present work, we performed the analysis of GHC structures in water and valine stereoisomers aqueous solutions as a function of the D/H ratio in different pH conditions. For example, in DDW, at pH=pK$_{a1}$=2.3 the GHC dimensional spectra of both stereoisomers are identical in their forms, whereas for L-isomer those spectra are larger and they are shifted to the right (fig. 3). At pH values corresponding to the zwitterion presence (isoelectric point), an increase in GHC heterogeneity for D-isomer is observed. On the other hand, for the L-isomer, small clusters disappear, and a band appears with a maximum at $r = 100 \mu m$ on the dimensional spectra.

![Fig. 2: Protolytic balance of valine in aqueous solutions](image)

![Fig. 3: Dimensional spectra of water GHC in solutions of D-valine: pH=2,3 (a), pH=6,3 (b) and L-valine: pH=2,3 (c), pH=6,6 (d). (DDW, D/H=4 ppm). n=5, mean±SD](image)
The difference in water clusters dispersion is characteristic for valine stereoisomers not only in aqueous solutions with an isotopic composition of D/H= 4 ppm and D/H= 140 ppm but also in heavy water. The obtained results for GHC in valine solutions allowed us to assume the absence of mirror identity in optical properties of L- and D-valine solutions in different pH conditions. Earlier, the computer simulation showed [31] that in accordance with the size of water clusters, valine stereoisomers induce both right and left rotating water clusters. L-valine induces the equal amount of water clusters stereoisomers, whereas D-valine significantly induces left rotating structures. Polarimetric analysis of valine solutions with different isotopic compositions in pH from 1 to 12 illustrates the conformity of theoretical calculations (fig. 4). In deionized water with the natural isotopic composition, the sum of specific rotation values of valine stereoisomers solutions is always<0, and this is particularly expressed in acidic solutions.

The solvent nature influence on the stereoisomers optical activities attracted the attention of many researchers. For instance, the specific rotation of L-valine increases more than three times when passing from aqueous solution to the hydrochloric acid solution (+22.9) then to the acetic acid solution (+7.26) [33]. The change in the solvent can not only increase or decrease the angle of optical rotation of the chiral compounds but also lead to a change in the sign of rotation. This is clearly demonstrated by \([\alpha]_D^{20}\) of tartaric acid solutions, which is a configuration standard in different solvents: +21.3 (H2O); +6.6 (C2H5OH); +0.3 (N, N-dimethyl formamide);−12.9 (dioxane);−14 (diethyl ether) [6]. These and other examples illustrate the fact, that optical rotation is a function of the interaction between a chiral compound and solvent molecules. Discovered in the 30's of XX century, the connection between the amino acids and peptides optical activity and pH of the water solutions [12] became a basis for the implementation of pharmacopoeia assays for polarimetric analysis of these compounds which should hold only in a strongly acidic environment [1]. The uniqueness of an amino acid structure in a fully protonated stable form (for example, valine in the form of HOOC-Val-NH3+) results in the formation of chiral water clusters in a manner analogous to that, which was experimentally proved for a DNA fragment [7].

It is known that the transition from one living matter hierarchic organization level to another, the optically active isomer is replaced by its antipode [34]. For instance, natural proteins consist of only L-amino acids, while the composition of cell membranes includes D-amino acids. This kind of selectivity can be associated with the isotopic composition of water and the change in a number of its physicochemical properties that affect the formation of bio systems.

The greatest difference in the total specific rotation of enantiomers equimolecular solutions in deionized high-ohmic water of natural isotopic composition (D/H = 140 ppm) is observed at pH = 2.3. To confirm the sign of the optical rotation, a control experiment was conducted with an aqueous solution of the valine racemic substance in BD, where amino acid was present as a zwitterion -OOC-Val-NH3+. During two hours of measurements, the rotation angle values had a certain drift, the range of which can be estimated by the standard deviation (N = 9):

\[
[\alpha] = \pi \pm SD = -0.75 \pm 0.132
\]

Thus, the solution of the valine racemic substance in BD has a negative optical rotation, the value of which depends on pH and reaches a maximum in an acidic environment where the amino acid is in the cationic form of HOOC-Val-NH3+. For solutions in DDW, the total value of specific rotation of the enantiomers, on the contrary, has a positive sign and reaches a maximum value in an alkaline environment where the anionic forms of the amino acid-OOC-Val-NH4+ predominate. Unlike natural and "light" water, in heavy water (99.9% D2O), the total value of specific rotation is practically independent of pH in solutions of valine enantiomers equimolecular mixture. This result is extremely important, as in heavy water the number of GHC is minimal [17, 28].

Thus, the differences in the specific rotation values of valine stereoisomers and its racemate can be explained by the mutual effect between the solvent and optical active substance. The obtained experimental results show the important role of the solvent isotopic effect in the manifestation of the chiral pharmaceutical substances aqueous solutions optical activity and the induction of optically active GHC. The presence of stable sub millimeter in homogeneities in valine aqueous solutions, proved by the LALLS method, depends on the chemical form of the stereoisomer at a given pH and the isotopic D/H ratio. Long-lasting water clusters possess chirality and contribute to the optical activity of the pharmaceutical substances.

Monosaccharides mutarotation kinetics in waters with different D/H ratio

It is known that mutarotation of freshly prepared monosaccharides aqueous solutions lead to the establishment of the dynamic balance between acyclic form and cyclic anomers with different polyacetal hydroxyl group position. Anomers are considered diastereomers and, thus, they differ with their physicochemical and biological
properties, which can be reflected in their *in vivo* activity. Mutarotation of glucose and galactose in the liquid biological environment can alter the biological processes which require their participation. It is worth to mention that, according to pharmacopeial methods [1], to increase the mutarotation rate of D-isomers of galactose and glucose, a change in pH with the addition of ammonia leads to a certain reduction in the time to reach the equilibrium state, which possibly results in GHC structure changes when the latter participate in the hydration of monosaccharides [35].

In the present study, we investigate the influence of the D/H ratio on glucose mutarotation rate. In the aqueous solution of different D/H ratio, initial kinetic curves \( \alpha_D T^{-t} \) and the semi-logarithmic anamorphosis \( \ln(\alpha_D T^{-t}) \) demonstrate the acceleration of the mutarotation process with a temperature increase to 22-28 °C. Mutarotation rate values in deuterium depleted and natural waters in different temperatures allowed us to calculate the activation energy of the mutarotation process using Arrhenius coordinates (fig. 5). It turned out, the activation energy values are statistically reliably distinguishable: BD–(33.8±3.8) kJ/mol, DDW–(54.5±6.3) kJ/mol.

Calculations of the results obtained in our previous study [36] evidence the differences in \( \alpha \) values of galactose enantiomers mutarotation. Native galactose mutarotation occurs in low energy expenditure. For example, in deuterium depleted water (DDW) activation energy of D-isomer is \( \alpha_d=(85 \pm 8.7) \) kJ/mol, whereas for L-isomer its value increases to \( (130\pm9.7) \) kJ/mol. Considering the influence of water isotopic composition on optical properties of galactose isomers, it is worth paying attention to the geometric positions complementation between OH-groups and the structural matrix of the solvent [37].

Since the structure of water GHC depends on deuterium content, the difference between obtaining values of galactose enantiomers activation energies in their mutarotation are is obvious [37]. In heavy water, the specific rotation of both monosaccharides do not follow the rules of first-order reaction kinetics. This phenomenon may be due to the fact that mutarotation proceeds with the formation of acyclic D-containing intermediates and kinetic limitation of C1D bond breaking arises. Besides, the specific rotation alteration in D2O can be influenced by the novel chiral centre of C6 in case of protium replacement with deuterium. Despite this, it is worth mentioning, that monosaccharides hydration character should visibly alter from natural and "light" waters depending on their homogeneity.

Monosaccharides are of interest as a model for studying solvation interactions. However, the comprehensive understanding of hydration nature and solutions structures of carbohydrates is not achieved yet. At the same time, it is obvious [35], that hydration of carbohydrates occurs via hydrogen bonds formed between OH-groups and water molecules. The varying orientation of OH-groups in «glucose and galactose», «L-and D-isomers», «α-and β-anomers» pairs influences hydration energy [38] and induces alterations in the optical rotation.

Thereby, the observed water isotopic composition effect on the conformational equilibrium of monosaccharides allows one to assume the participation of chiral GHC in the physiological functions of carbohydrates as active pharmaceutical ingredients.

The results obtained show that the control of the deuterium content in water for pharmaceutical use and of deuterated pharmaceutical substances is no less important than the determination of the elemental profile of heavy and essential elements in medicinal plants and other biological materials [39-41]. In the long term, the methods for estimating the D/H ratio in pharmaceutical objects should be standardized and introduced into pharmacopeias.

**CONCLUSION**

In the optically active pharmaceutical substances quality control there should be considered the contribution of induced chiral GHC of water to the optical rotation value that depends on the isotopic D/H ratio, the substance nature and the form of its existence at a given pH.

**ACKNOWLEDGMENT**

The publication has been prepared with the support of the «RUDN University Program 5-100».

**AUTHORS CONTRIBUTIONS**

All the author has contributed equally

**CONFLICT OF INTERESTS**

Declared none

**REFERENCES**

1. European Pharmacopoeia 8.0 V.1-2. Strasbourg: Council of Europe; 2014.
2. U. S. Pharmacopoeia 40-National Formulary 35. USA; 2017.
3. Japanese Pharmacopoeia. 17th ed. Japon; 2016.
4. Calisto S, Martínez Ponce G, Ganica G, Figueroa Gerstenmaier S. A wavefront division polarimeter for the measurements of solute concentrations in solutions. Sensors 2017;17:2844.

5. Ajithkumar KC, Pramod K. Development and validation of a modified polarimetric assay method for small volume samples. Int J Appl Pharm 2018;5:17.89.

6. Ellul EL, Samuel H, Wilen SH, Doyle MP. Basic organic stereochemistry. New York: Wiley-Interscience; 2001.

7. McDermott ML, Vanselow H, Corcelli SA, Petersen PB. DNA’s chiral spine of hydration. ACS Cent Sci 2017;3:708-14.

8. Sharma BK, Singh J, Raj P. Spectrophotometric determination of propranolol hydrochloride and metoprolol tartrate in pharmaceutical dosage forms, spiked water and biological fluids. Int J Pharm Pharm Sci 2018;10;107-15.

9. Linthorst JA, van der Wal-Neugter J. Polariometry and stereochemistry: the optical rotation of Vitamin C as a function of pH. Educ Quim 2014:25:135-8.

10. Shiraga K, Suzuki T, Kondo N, De Baerdemaeker J, Ogawa Y. Quantitative characterization of hydration state and destructuring effect of monosaccharides and disaccharides on water hydrogen bond network. Carbohydr Res 2015;406:46-54.

11. Velsik J. The chemistry of food. New York: John Wiley and Sons, Inc; 2013.

12. Jirgensons B. Optical Activity of Proteins and Other Macromolecules. Berlin: Springer-Verlag; 1973.

13. Scherrer A, Vuilleumier R, Sebastiani D. Vibrational circular dichroism from ab initio molecular dynamics and nuclear velocity perturbation theory in the liquid phase. J Chem Phys 2016;145:84-101.

14. Khakhlin AV, Gradoboeva ON. A method to determine and classify the chirality of the water medium. J Struct Chem 2016;57:934–9.

15. Green MM, Reidy MP. Macromolecular stereochemistry: the out-of-proportion influence of optically active comonomers on the conformational characteristics of polysaccharides. The sergeants and soldiers experiment. J Am Chem Soc 1989;11:6451-4.

16. Kopka ML, Frattini AV, Drew HR, Dickerson RE. Ordered water structure around a B-DNA dodecamer: a quantitative study. J Mol Biol 1983;163:1-29-49.

17. Goncharuk VV, Syroeshkin AV, Pleteneva TV, Uspenskaya EV, Levitskas VV, Tverdiislov VA. On the possibility of chiral structure-density submillimeter inhomogeneities existing in water. J Water Chem Technol 2017;39:319-24.

18. Timmins GS. Deuterated drugs: where are we now? Expert Opin Ther Pat 2014;24:1067-75.

19. Somlyai G. Defeating cancer! The biological effects of deuterium depletion. Bockington: Author House; 2002.

20. Somlyai G, Kovacs A, Goller I. Deuterium has a key role in tumour development--new target in anticancer drug development. Eur J Cancer 2010;36 Suppl 1:155-225.

21. Shao L, Hewitt MC. The kinetic isotope effect in the search for deuterated drugs. Drug News Perspect 2010;23:398-404.

22. Guengerich FP. Kinetic deuterium isotope effects in cytochrome P450 reactions. Methods Enzymol 2017;596:217-38.

23. Xie X, Zubarev RA. On the effect of planetary stable isotope compositions on growth and survival of the terrestrial organism. PLOS One 2017;12:1.9.

24. Atzrodt J, Derduv V, Kerr WJ, Reid M. Deuterium-and tritium-labeled compounds: applications in the life sciences. Chem Int Ed 2018;57:17.91.

25. Covington CL, Polavarapu PL. Concentration dependent specific rotations of chiral surfactants: experimental and computational studies. J Phys Chem A 2016;120:5715-25.

26. Orkova AV, Andrade RR, da Silva OD, Zinin AI, Kononov L.O. The chemistry of food. Part II Fourth Edition. London: A and C Black; 1988.

27. Kolomiet S, Berl V, Lenn JM. Chirality induction and protonation-induced molecular motions in helical molecular strands. Chem Eur J 2007;13:5466-79.

28. Khakhlin AV, Gradoboeva ON. Aqueous shell chirality research of varying thicknesses. Proceeding CBRA; 2017, p. 934–9.

29. O’Neill MJ, editor. The Merck Index An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, New York: Merck and Co. Inc; 2006.

30. Tverdislov VA, Sidórova AE, Yakovenko LV. From symmetries to the laws of evolution. I. Chirality as a means of active media stratification. Biofizika 2012;57:120-6.

31. Mauger I, Busch S, McLaren SE, Pardo LC, Bruni F, Ricci MA. Structure-activity relationships in carbohydrates revealed by their hydration. Biochim Bioph Phys Acta Gen Subj 2017;186:1486-93.

32. Zrelov OYu, Syroeshkin AV, Uspenskaya EV, Titorovich OV, Pleteneva TV. Effect of water isotope composition on galactose mutarotation kinetics. Pharm Chem J 2015;49;413-6.

33. Kholmanskj A. Chirality anomalies of water solutions of saccharides. J Mol Liq 2016;216:683-7.

34. Miljovic M. Carbohydrates: synthesis, mechanisms, and stereoelectronic effects. New York: Springer; 2009.

35. Kripa KG, Sangeetha R, Chamundeeswari D. Pharmacognostical studies. J Phys Chem A 2016;120:5715-25.

36. Mulik A, Bhadekar R. Extracellular polymeric substance (EPS) from Kocuria sp. BRI 36: a key component in heavy metal resistance. Int J Pharm Pharm Sci 2018;10:50-4.

37. Clowes BJ, Divya B, Suman M, Venkatasswamy K. Thiyagaraju A. Study on phytochemicals, functional groups and mineral composition of Allium sativum (Garlic). Int J Curr Pharm Res 2017;9:42-5.