RHEOLOGICAL PROPERTIES AND SOLVENT STRUCTURE OF POLYSACCHARIDE HYDROGELS STUDIED BY MOLECULAR DYNAMICS SIMULATIONS

ANDREI NEAMTU[1*], ANA-MARIA OPREA[2], TUDOR PETREUS[3], LUCIAN GORGAN[4], CORNELIA VASILE[2]

Keywords: hydrogel, sulfated glycosaminoglycans, molecular dynamics simulations, rheology
Abstract: One important class of hydrogels based on natural polymers is the Glycosaminoglycan (GAG)-based hydrogels. In hydrogels biomaterial science, the mathematical modeling and computer simulation plays a complementary interpretative role in deciphering the complex physical/chemical and biological properties of this class of substances. **Aim:** the molecular modeling studies presented here aimed the information gathering regarding the particular molecular interactions responsible for rheological properties of this class of biomaterials. **Methods:** the methods included molecular dynamics simulations in the NPT ensemble for polysaccharidic matrices, radial function analysis for the solvent and viscosity calculations using periodic strain non-equilibrium molecular dynamics. All this methods were applied to models of 100%, 66% and 33% of maximum hydration compared to pure solvent simulations as control. **Results and conclusions:** decreasing the water content of the polymer matrix drastically affects the conformational flexibility of the polymer chains, the solvent percolation and viscosity coefficient of the biomaterials studied. The obtained viscosity coefficients were: $\eta_{H2O} = 0.982\times10^{-3}$ kg/(ms); $\eta_{100\%} = 1.520\times10^{-3}$ kg/(ms); $\eta_{66\%} = 1.862\times10^{-3}$ kg/(ms); $\eta_{33\%} = 2.602\times10^{-3}$ kg/(ms). The findings are useful for polysaccharidic hydrogel materials science as the rheological and solvent structuralisation can dramatically influence the physical stability of eventual macromolecular bioactive agents (e.g. therapeutic proteins) when they are loaded into such matrices for controlled delivery, especially during the storage period when the material is kept in lyophilised conditions.

INTRODUCTION

One of the most promissing class of polymers with adequate properties for drug or bioactive molecule/ macromolecule loading and release is represented by hydrogels. Hydrogels represent three-dimensional polymer networks that include chemically reticulated macromolecular chains (Hoffman 2002) embeeded in an aqueous environment. One important characteristic of hydrogels is that on the macroscopic scale they behave like solids while at molecular scale hydrogels have properties similar to solutions (Tanaka 2005). This is important especially for macromolecular active substances such as proteins because the mechanical properties of the reticulated polymer chains allow the immobilisation of the molecule inside the matrix while still keeping an aqueous medium similar to the natural one. Hydrogels are currently used in clinical practice and experimental medicine for: tissue engineering and regenerative medicine (Lee 2001); diagnosis (van der Linden 2003); controlled drug delivery (Lin 2006); surface cell immobilisations (Jen 1996); biomolecule and cell separations (electrophoresis) (Wang 1993); surface coatings for cell adhesion (Bennett 2003). Due to the increased water content and softness, similar to natural tissues, hydrogels may represent multicomponent systems that exhibit the characteristics of a natural material with an excellent biocompatibility (De Groot 2001). Regarding the chemical composition, the hydrogels are composed of 2%-80% polymer, 20%-98% water and 0.1%-5% additions. One important class of hydrogels based on natural polymers are the Glycosaminoglycan (GAG)-based hydrogels. Glycosaminoglycans are natural polymers made of specific repeating disaccharide units in which one sugar is uronic acid and the other is either N-acetylglucosamine or N-acetylgalactosamine. For obtaining succesfull hydrogel formulations GAGs have to be mixed with other polysaccharide types (ex. Cellulose or Xanthane) to increase the mechanical strength and to modulate the swelling degree (Oprea, 2010). In biomedical science these materials found their suitability in drug delivery (Soppimath 2002) (Byrne 2002), ophtalmology (Myers 1991; Compan 1998), tissue engineering (Darsov 1995; Draye 1998), urology (Di Tizio 1998), plastic and reconstructive surgery (San Roman 2001), orthopaedics (Broom 2000). Therewith, there are many important applications in pharmaceutics and biotechnologies. Also, GAG hydrogels (Hyaluronan, Chondroitin sulfate) and their derivatives were used for wound healing due to their potency of inducing re-epithelization (Luo 2000; Kirker 2004).

For obtaining an optimum hydrogel material with superior biomedical characteristics for a particular application the research has to be focused not only on the synthesis of the material but also on the physical and chemical characterzation of their properties which finally dictates the quality of the obtained therapeutic system. Along with the experimental techniques routinely used in the area of the biomaterials, in the recent years the computer simulation and modelling is playing an incresing role due to their ability to microscopically describe processes inaccessile or very difficult to asses by experimental means (Lam 2003, 2004). The tremendous progress in computing power and algorithmics makes now

possible to simulate systems at the mesoscale level (10-100nm and 10ns-10μs) (Rex 1998; Papisov 1998; Marrink 2003, 2004; Lee 2009a,b).

Although the Molecular Dynamics (MD) technique for simulating molecular systems is largely used in the material science there is still a lack of such studies for hydrogel based systems (Tamai 1996a,b; Oldiges 2002a,b,c; Jiang 2007; Lee 2009a,b).

The aim of the current study is to implement a methodological framework for glycosaminoglycan hydrogel simulations and to study the rheological properties and solvent organization in relation to the degree of hydration.

METHODS

Molecular dynamics technique was used to simulate pure cellulose (Figure 1) matrices in aqueous environment in order to evaluate the phenomena that occur on the microscopic scale in this class of materials. The GLYCAM06 forcefield (Kirschner 2008) was used for the description of the polysaccharidic chains while for the solvent the TIP3P model of Jorgensen et al. (1983) was selected. The initial structure of the hydrogel was constructed by generating with the aid of the xLeap program (AMBER Tools 10 suite, Cornell 1995) of a polymeric chain of 32 repeating units of β-D-Glucose linked in 1 – 4 positions.

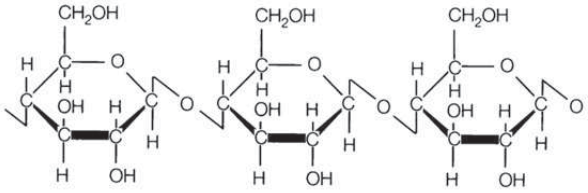

Figure 1. Cellulose structure

The resultant topology and coordinate files were then transformed to GROMACS (Berendsen, 1995) compatible input files with the aid of amb2gmx.pl script of Mobley (2006) corrected for negative dihedral potential barriers (GLYCAM forcefield in contrast with AMBER does not use phase shift for proper torsions and consequently it contains also negative values for some of the terms of the torsional energy barriers). In total there have been constructed 12 chains by replicating the original one and subsequently they were aligned to be perpendicular on the simulation cell faces as such there are 4 chains along each direction of the coordinate system (Figure 2). Due to the difficulties associated with the simulation of an infinite network (hydrogel) which can not make use of usual periodic boundary conditions, for each chain present in the system one of its was chemically connected to the other end of its corresponding periodic image using the „periodic molecules" option in GROMACS. Due to periodic boundary conditions the simulated system thus consists of infinite molecules arranged in a three dimensional network which is a good model for a hydrogel.

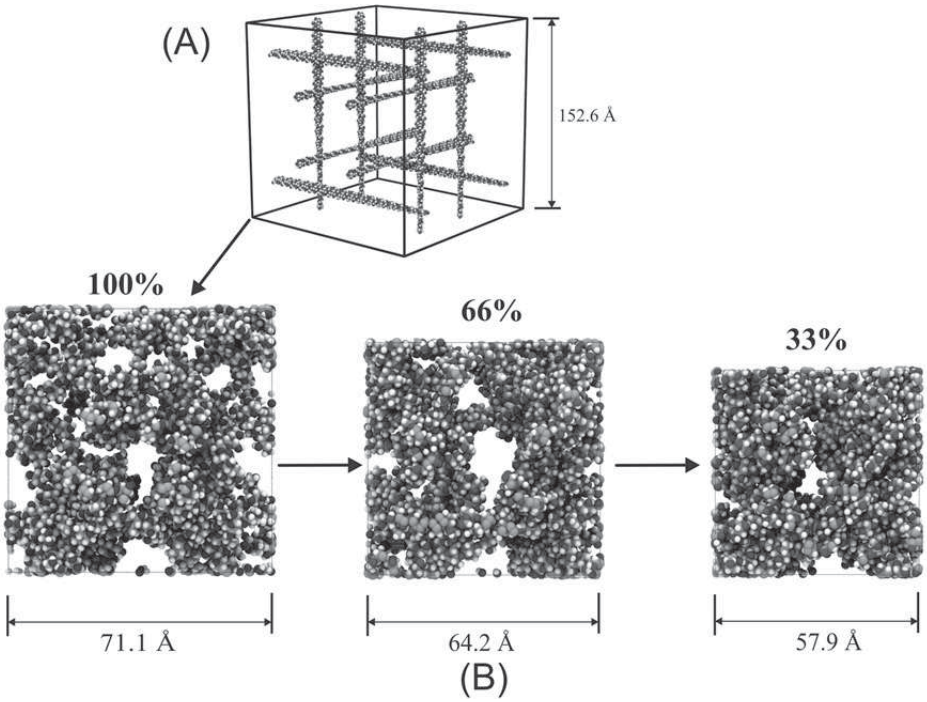

Figure 2. Depiction of the initial simulation cell constructed using the periodic molecules approach (A) and the compression of the cell to reach the necessary volume for 100%, 66% and 33% hydration (B).

The system constructed in this way has all the chains fully extended which is far from equilibrium even for 100% hydration. To obtain the desired density at 100% hydration the initial expanded simulation cell was gradually compressed in an iterative manner until its volume reached the correct value. For each step the simulation cell was scaled down a geometry optimization simulation was performed to relax the compressed polymer chains. The 66% and 33% box geometries were obtained in a similar manner by further scale down the simulation boxes. The polymeric chains in each simulation box were hydrated with TIP3P water molecules as follows: 9458 molecules for 100% hydration, 6170 molecules for 66% hydration and 3498 molecules for 33% hydration. The simulations were performed in NPT ensemble (number, pressure and temperature constant) at 1 atm and 300K. Integration step was 0.001 ps, each simulations being 100ps long.

For the viscosity calculations the Hess (2002) nonequilibrium method was applied. This method apply an external acceleration cosine profile in one direction of the simulation box and it is based on the fact that the energy, which is fed into system by external forces, is dissipated through viscous friction. The generated heat is removed by coupling to a heat bath. The viscosity simulations used the same parameters as the equilibrium ones but they were two times longer (200ps). All the simulations were performed in parallel on a Dell Cluster with 64 computing cores (Dell PowerEdge 1950) with Infiniband interconnect in the Molecular Modeling Laboratory of the Center for The study and Therapy of Pain, UMF “Gr. T. Popa” Iasi.

RESULTS AND DISCUSSIONS

The radial distribution functions (or equivalently “pair correlation functions”) describes how the number of a certain species of atoms varies with the distance from one particular atom. They are very useful in determine which is the particular structural microenvironment in which certain type of atoms are located. The data resulted from the simulations were analysed in order to

compute the radial distribution functions between water oxygens and between water oxygen and the O5 atom of the pyranose ring. Some of the results are depicted in the Figure 3.

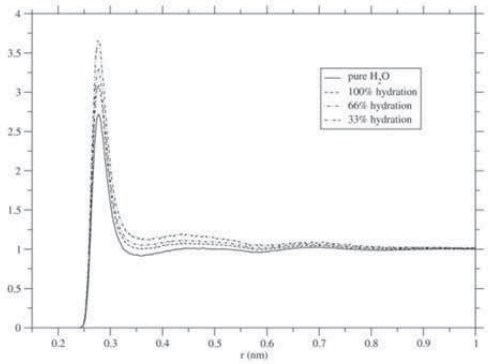

Figure 3. The radial distribution functions of OW (water) oxygen atoms for cellulose hydrogels at 100%, 66% and 33% hydration compared to the pure water .

It can be seen from the plots that the radial distribution function between water oxygens (OW) presents three peaks corresponding to the first three layers of hydration in all the three polymer matrices discussed here. This is comparable with the structuralization into a pure volume of water. The difference is that the peaks are increasing in amplitude when the water content of the hydrogel matrix is decreasing. In analysing these results we must state that there are two water populations in a hydrogel matrix: the bounded water compartment in which water molecules have strong interactions with the polymer chains and the bulk water compartment in which water molecules are located far from the chains and behave like in a pure volume of water. As the measurements were done on all the water molecules present in the system the increase in height of the first and subsequent peaks reflects that the bounded water compartment became more representative as the water content of the hydrogel lowers. This has an impact on the solvent mobility inside the polymer matrix as can be further analysed by computing the Mean Square Displacement (MSD) of water molecules.

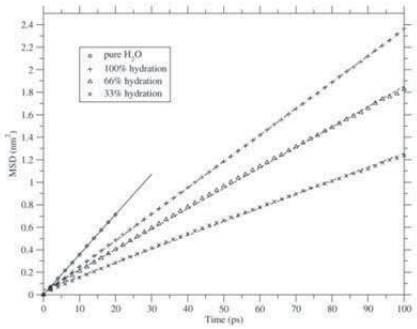

Figure 4. The Mean Square Displacement (MSD) of water molecules for cellulose hydrogels at 100%, 66% and 33% hydration compared to the pure water.

The MSD was computed from all the equilibrium simulations by following the motions of atoms from their initial positions. The results are presented in the Figure 4. The MSD, which is related to the diffusion coefficient of a molecule by the Einstein equation, is decreasing as the water content is decreasing. Again, as the MSD calculations were performed on the whole water population, and taking into account the results on pair correlation functions above, this decrease in the overall water mobility demonstrates that the bounded water compartment, beside being more ordered, is also far less mobile than the bulk water. These results sustain the experimental findings which correlates the degree of internal diffusion of different embeeded substances to the hydration level of hydrogels.

The solvent structure, along with the polymer chain packing, also influences the rheological properties of hydrogels. The percolation (filtration) of the solvent through the polymet network was evaluated using a non-equilibrium molecular dynamics technique as described in the *„Methods”* section.

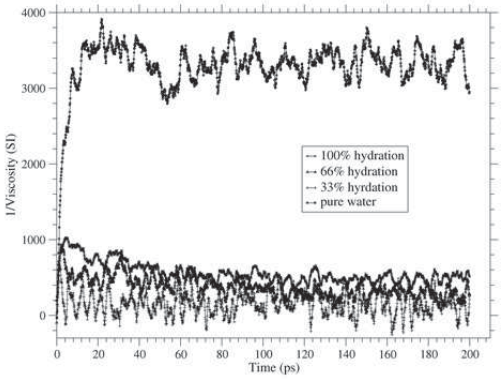

Figure 5. The viscosity of water flowing through the polymer network of cellulose hydrogels at 100%, 66% and 33% hydration compared to the pure water.

The results are expressed as an „average” viscosity coefficient which must not be identified with the usual shear viscosity coefficient of the entire hydrogel as the calculations performed here are subjected only to water molecules (which themselves further can be dinstinguished into two compartments as explained above). The „viscosity” coefficients computed qualitatively express the degree of water percolation through hydrogel matrices as a result of an applied external stress. The values obtained for the viscosity coefficients are: $\eta_{H2O} = 0.982\times10^{-3}$ kg/(ms); $\eta_{100\%} = 1.520\times10^{-3}$ kg/(ms); $\eta_{66\%} = 1.862\times10^{-3}$ kg/(ms); $\eta_{33\%} = 2.602\times10^{-3}$ kg/(ms). The results, presented in the Figure 5, demonstrate that the rheological properties of the solvent depend to a large extent on the hydration degree. This is due to either an increase of the bounded solvent population and also to a decrease of the pore dimensions of the polymer network as they become more tightly packed.

CONCLUSIONS

Molecular dynamics simulations were performed on cellulose hydrogel networks in order to establish a methodology suitable for the simulation of reticulated polysaccharidic biopolymers. The study also included the evaluation of the relationship between solvent organization,

rheological properties and the degree of hydration of the hydrogel matrices. While the first aspect is more general in its nature, providing methods of simulation not only for polysaccharidic polymers but for other reticulated structures too, the second one gives us more specific information about how water structure inside the hydrogel molecular microenvironment can influence its macroscopic properties. During the initial construction of the network model it clearly appeared that the usual periodic boundary conditions are not suitable for hydrogel simulations and further analysis proved that the 'periodic molecule' algorithm is the one to be chosen when three dimensional polymer networks are to be modeled. Regarding the solvent organization and dynamics the radial distribution functions and MSD suggest that as the water content decreases there is an increase of the bounded water fraction over the 'free' bulky water. The bounded water is more organized in the succesive layers around polymer chains than free water and also far less mobile which impose a limited diffusion of solvent (and of the dissolved substances) in matrices with low hydration. The degree of hydration in polysaccharidic hydrogels can be fine-tuned by inclusion in a mixed network of glycosaminoglycans (polyelectrolytes) on one hand and of cellulose on the other. Also, the reticulation density is the second major factor that can affect the degree of swelling of a hydrogel. The data presented here is important for the intimate understanding of the hydrogels behavior on molecular scale and to ease the correlations which can be made between the chemical structure of the reticulated polymers and the macroscopic properties (which can easily be measured experimentally).

REFERENCES

Bayly CI, Cieplak P, Cornell WD, Kollman PA *J. Phys. Chem.* 97:10269-10280, 1993

Cornell WD, Cieplak P, Bayly CI, Gould IR, Merz KM Jr, Ferguson DM, Spellmeyer DC, Fox T, Caldwell JW, Kollman PA (1995) A Second Generation Force Field for the Simulation of Proteins, Nucleic Acids, and Organic Molecules. *J. Am. Chem. Soc.* 117: 5179–5197

D. L. Mobley, J. D. Chodera, K. A. Dill. (2006) On the use of orientational restraints and symmetry number corrections in alchemical free energy calculations, *Journal of Chemical Physics,* 125:084902.

Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. (1983) Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys*, 79: 926-935

Soppimath, K. S., T. M. Aminabhavi, A. M. Dave, S. G. Kumbar, and W. E. Rudzinsky. *Drug Dev. Ind. Pharm.*, 28:957., 2002

Tanaka Y. et al. (2005), *Prog. Polym. Sci.,* 30:1-9

[1]Center for the Study and Therapy of Pain (CSTD) Iaşi, „Gr. T. Popa" University of Medicine and Pharmacy of Iaşi
[2]Laboratory for Polymer Physics and Chemistry, "P. Poni" Institute of Macromolecular Chemistry
[3]Department of Cell and Molecular Biology, „Gr. T. Popa" University of Medicine and Pharmacy of Iaşi
[4]Laboratory of Molecular Genetics, Faculty of Biology„Al. I. Cuza" University of Iaşi
* neamtuandrei@gmail.com

Acknowledgements: This paper was supported by funding from research Grant no. 2561/2008 CNCSIS