MOLECULAR DYNAMICS SIMULATIONS REGARDING THE PROPERTIES OF CELLULOSE/CHONDROITIN-SULPHATE BASED HYDROGELS AS DRUG DELIVERY MATRICES

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Abstract: Hydrogels represent three-dimensional polymer networks that exhibit the characters of a biomaterial with an excellent biocompatibility. 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 and indispensable role in deciphering the complex physical/chemical and biological properties of this class of substances. **Aim:** the molecular modeling studies depicted in the present paper aimed the information gathering at atomic-molecular level regarding the hydrophobic, hydrophilic, electrostatic and mechanical interactions that contributes to the internal organization of hydrogel matrices. **Material and methods:** the methods included quantum chemistry calculations for obtaining new parameters that were subsequently used in molecular dynamics simulations of polymer matrices with emphasis on their drug release properties. **Results and conclusions:** the parameters presented here for the sulfated form of GAGs are in good agreement with the more sophisticated models for the non-sulfated forms of GAGs, i.e. GLYCAM forcefield developed based on *ab initio* full quantum description, and can be regarded as a starting point for further development.

INTRODUCTION

Hydrogels represent three-dimensional polymer networks that include chemically reticulated macromolecular chains (Hoffman, 2002). Due to increased water content and softness, similar to natural tissues, hydrogels may represent multicomponent systems that exhibit the characters of a biomaterial with an excellent biocompatibility. 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. In biomedical science these materials found their suitability in drug delivery (Soppimath, et al. 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). Hydrogels based on GAGs are highly hydrated environments in which, when apposed peri-cellularly, a wide range of extracellular matrix components can readily assemble and even cell migration and proliferation can occur. The bioactive reparatory properties of synthetic materials based on GAGs could be explained at a certain extent by their ability of interacting with cell surface proteins involved in cell proliferation and differentiation, like fibroblast growth factor receptors, vascular endothelial growth factor/receptor (VEGF, VEGFR) and cell adhesion proteins (P-selectin, L-selectin, integrins). For example, mouse models treated either with Hyaluronan or Chondroitin sulfate displayed an increase of fibro-vascular reparatory tissue 10 days after a full thickness wound creation (Kirker, 2002).

The evaluation of the hydrogel-based materials is subject for a consistent number of research studies whose results can be found in different reviews (Dusek, 1993). Most of these researches have as purpose the synthesis of the hydrogel-based material followed by the experimental evaluation of the specific properties. On the other hand the extended applicability of this polymeric material category requires the design of more complex structures whose physical and chemical behavior are difficult to investigate experimentally. Thus, mathematical modeling and computer simulation plays a complementary but indispensable role in deciphering their complex properties (Wu, et al. 2004).

Molecular modeling studies depicted in the present paper had as purpose gathering information at atomo-molecular level regarding the hydrophobic, hydrophilic, electrostatic and mechanical interactions that contributes to the internal

organization of hydrogel matrices. In order to describe polysaccharide properties in the considered hydrogel structure we have used the molecular dynamics simulation technique **(MD – Molecular Dynamics)** (Alder, 1957; MacKerell, 1998).

MATERIALS AND METHODS

A. Obtaining parameters for disaccharides

Many references show a large variety of useful force fields in order to describe the molecular systems (Jensen, 2002). Literature database exploration regarding force fields developed mainly for polysaccharides revealed the presence of three variants frequently used for this purpose: CHARMM (Brooks, 1983); Kouwijzer (GROMOS) (van Gunsteren, 1996); GLYCAM (AMBER) (Cornell, 1995). Even if these force fields confer high accuracy characterized parameters regarding various oligo- and more complex polysaccharides (especially GLYCAM v. 06), the literature data shows no applicable parameters for the *sulfated* GAGs. The latter aspect led us to the development of special structures and parameters for these, using specific techniques of computational chemistry. For each of the two classes of substances we have built a disaccharide model (cellulose, chondroitin-sulfate), that represents the specific repetitive subunit in each polymeric chain. In **Figure 1** there are represented the molecular conformations and the compound atoms nomenclature. This step was performed with the GHEMICAL molecular editor. The models were then submitted to a standard procedure for geometrical optimization and partial electrostatic charges calculation for the compound atoms, using RESP method (Restrained Electrostaic Potential fit) (Bayly, 1993). In this method charges are derived from fitting a classical Coulomb model to quantum mechanical molecular electrostatic potential. For the quantum mechanics calculations we have used the MOPAC 6 software package, by choosing the AM1 semi-empirical method for molecular orbital (Austin Model-1) (Dewar, 1985). The AM1 method represents a molecular quantum description that supposes Schrödinger equation solving for the given molecule. The numeric Schrödinger equation result is based on LCAO method (Linear Combination of Atomic Orbitals) that consider the molecular orbital described as linear combinations of atomic orbitals. The AM1 method is characterized by the fact that for the analyzed molecule we have to consider only the valence electrons, those on the inner layers being described as a wave function, common with the nuclear one (Pople, 1965a,b).

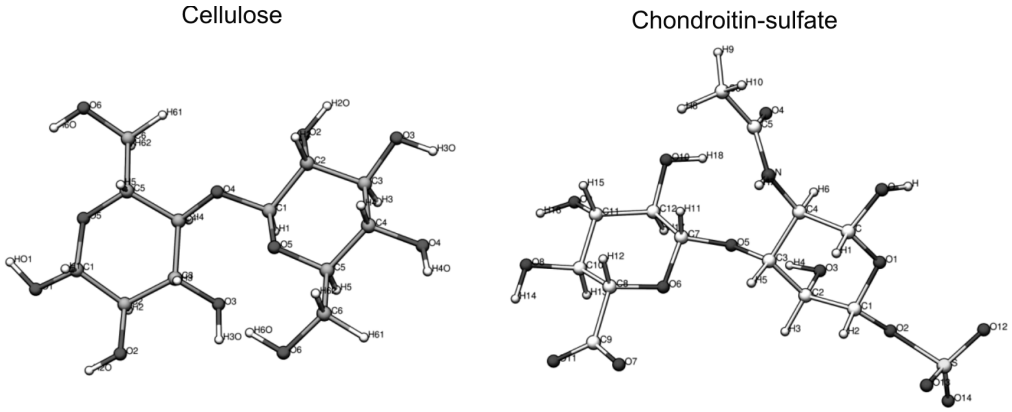

Figure 1. The conformations and atoms nomenclature used for cellulose and chondroitin sulfate

In order to describe covalent intermolecular interactions (chemical bonds, valence angles, dihedral angles) we have used GAFF force field (Generalized Amber Force Field) (Cornell, 1995). To validate the proposed model we have performed two molecular dynamics simulations in explicit solvent either for the cellulosic disaccharide described by GLYCAM force field and our own force field, and also for the chondroitin-sulphate. We have investigated series of intramolecular structure parameters (e.g. dihedral angle variations that defines the specific geometry for the pyranosic cycle or the glucosidic bridge) and solvent distribution around the saccharide molecules, described by radial distribution functions. Electrostatic interactions were evaluated using the PME method (Particle Mesh Ewald) while the thermodynamic conditions of temperature and pressure were constant (T = 27 °C, P = 1 atm). Each simulation lasted for 20ns.

B. Hydrogel matrices simulation

Based on the parameters obtained in the previous step we have built models for cellulose/chondroitin sulphate mixture hydrogels in various ratios. We have performed simulations for hydrogels with 90/10 and 50/50 cellulose/chondroitin-sulphate ratios. We have added Na^+ ions to the modeled systems up to neutralization. Electrostatic

interactions were evaluated by using the PME method while the thermodynamic conditions of temperature and pressure were constant (T = 27 °C, P = 1 atm). Each simulation lasted for 20ns. All simulations were performed with AMBER 10 (Cornell, 1995) and GROMACS 4.0.5 (Berendsen, 1995) software packages on a HPC server cluster HPC (High Performance Computing) with 64 Intel Xeon QuadCore 2,66GHz cores (Dell PowerEdge 1950), 1GB RAM/and high speed Infiniband SDR connection.

RESULTS AND DISCUSSIONS

Partial electrostatic charges obtained for the cellulosic disaccharide and the chondroitin-sulphate are shown in **Table I**, together with cellulose charges in the GLYCAM 06 force field, for comparison.

Table I. The charges obtained from quantum mechanical computations and RESP fitting for cellulose and chondroitin sulfate (for cellulose also the GLYCAM 06 charges are given for a direct comparison)

Cellulose atomic partial charges											
No.	*Atom*	*Q(e) Glycam06*	*Q(e) AM1*	*No.*	*Atom*	*Q(e) Glycam06*	*Q(e) AM1*	*No.*	*Atom*	*Q(e) Glycam06*	*Q(e) AM1*
1	HO	0.4450	0.4313	16	H1	0.0000	0.0716	31	H1	0.0000	0.0376
2	OH	-0.6390	-0.6030	17	OH	-0.7090	-0.6042	32	OH	-0.6880	-0.5942
3	CG	0.3840	0.3118	18	HO	0.4320	0.4257	33	HO	0.4240	0.4162
4	H2	0.0000	0.0542	19	CG	0.3100	0.0863	34	CG	0.2760	0.0792
5	OS	-0.4710	-0.4603	20	H1	0.0000	0.0782	35	H1	0.0000	0.0649
6	CG	0.2250	0.1123	21	OH	-0.7180	-0.6020	36	OH	-0.7140	-0.6003
7	H1	0.0000	0.0732	22	HO	0.4370	0.4247	37	HO	0.4400	0.4114
8	CG	0.2820	0.1375	23	OS	-0.4680	-0.3960	38	CG	0.2840	0.1102
9	H1	0.0000	0.0951	24	CG	0.3840	0.3473	39	H1	0.0000	0.0659
10	H1	0.0000	0.0398	25	H2	0.0000	0.0856	40	OH	-0.7090	-0.6052
11	OH	-0.6880	-0.5929	26	OS	-0.4710	-0.4425	41	HO	0.4320	0.4260
12	HO	0.4240	0.4073	27	CG	0.2250	0.0739	42	CG	0.3100	0.1240
13	CG	0.2760	0.1252	28	H1	0.0000	0.0724	43	H1	0.0000	0.0779
14	H1	0.0000	0.0770	29	CG	0.2820	0.1372	44	OH	-0.7180	-0.5920
15	CG	0.2840	0.1207	30	H1	0.0000	0.0662	45	HO	0.4370	0.4246
Chondroitin-sulfate atomic partial charges											
No.	*Atom*	*Q(e) Glycam06*	*Q(e) AM1*	*No.*	*Atom*	*Q(e) Glycam06*	*Q(e) AM1*	*No.*	*Atom*	*Q(e) Glycam06*	*Q(e) AM1*
1	H	-	0.4332	18	H7	-	0.3055	35	O8	-	-0.6613
2	O	-	-0.6218	19	C5	-	0.6646	36	H14	-	0.4601
3	C	-	0.3474	20	O4	-	-0.6296	37	C11	-	0.1413
4	H1	-	0.0510	21	C6	-	-0.1748	38	H15	-	0.0461
5	O1	-	-0.4690	22	H8	-	0.0625	39	O9	-	-0.6087
6	C1	-	0.4414	23	H9	-	0.0634	40	H16	-	0.4078
7	H2	-	0.0455	24	H10	-	0.0457	41	C12	-	0.1256
8	O2	-	-0.5715	25	O5	-	-0.4298	42	H17	-	0.0676
9	C2	-	0.1149	26	C7	-	0.2995	43	O10	-	-0.6106
10	H3	-	0.0645	27	H11	-	0.0322	44	H18	-	0.4246
11	O3	-	-0.5654	28	O6	-	-0.3966	45	O11	-	-0.8610
12	H4	-	0.3961	29	C8	-	-0.0109	46	S	-	1.7099
13	C3	-	0.0970	30	H12	-	0.0272	47	O12	-	-0.7417
14	H5	-	0.0814	31	C9	-	0.9346	48	O13	-	-0.7653
15	C4	-	0.0147	32	O7	-	-0.7847	49	O14	-	-0.7378
16	H6	-	0.1172	33	C10	-	0.1058			-	
17	N	-	-0.5493	34	H13	-	0.0612			-	

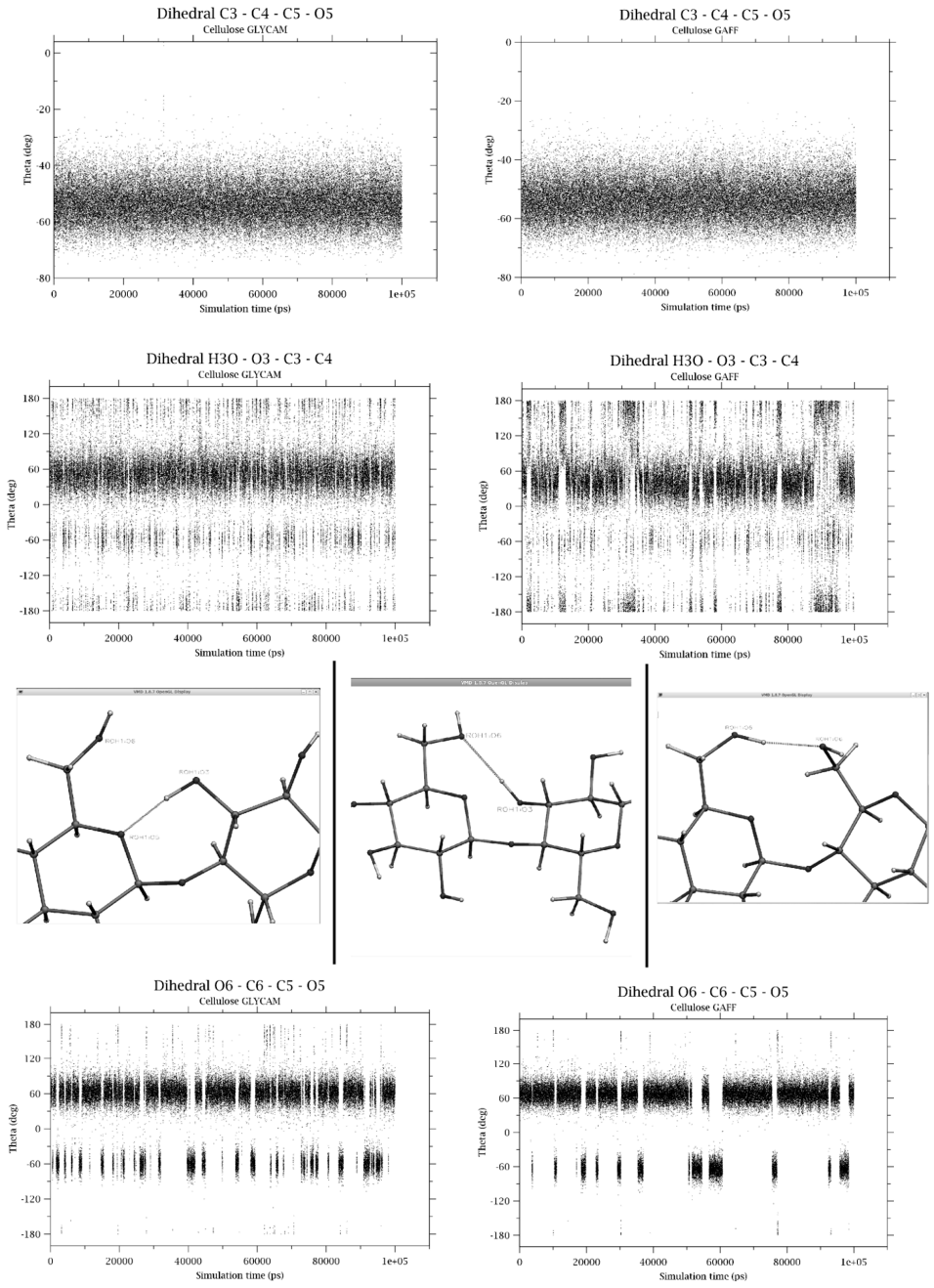

Figure 2. Comparative representation (GLYCAM vs. GAFF) of some dihedral angle variation during simulation. These angles characterize the considered polysaccharides in the present study.

Comparative Molecular dynamics simulation results for cellulosic disaccharide are shown in **Figure 2**. We can observe that the dihedral angle C3 - C4 - C5 - O5 is around 55° in both cases that demonstrates the dominance of chair-pyranosic form, that is conserved even when using GAFF force-field and the special charges computed by the above described procedure.

Therewith, the dihedral angle H3O – O3 – C3 – C4 shows a preferential conformation for a 60° value, that corresponds to an intermolecular stable H-bridge formed between the –OH equatorial group in the 3 position of one pyranosic cycle and the cyclic oxygen of the second. This behavior is shown both in the GLYCAM force-field and also for the parameters depicted in the present study. The dihedral angle O6 – C6 – C5 – O5 favors the 60° value that corresponds to an intermolecular stable H-bridge formed between the –OH in 6 position of a monosaccharide and the equatorial -OH group in the 3 position of the neighbor residue. The forming of the two intramolecular H-bridges is competitive while they uses the same -OH group in the 3 position.

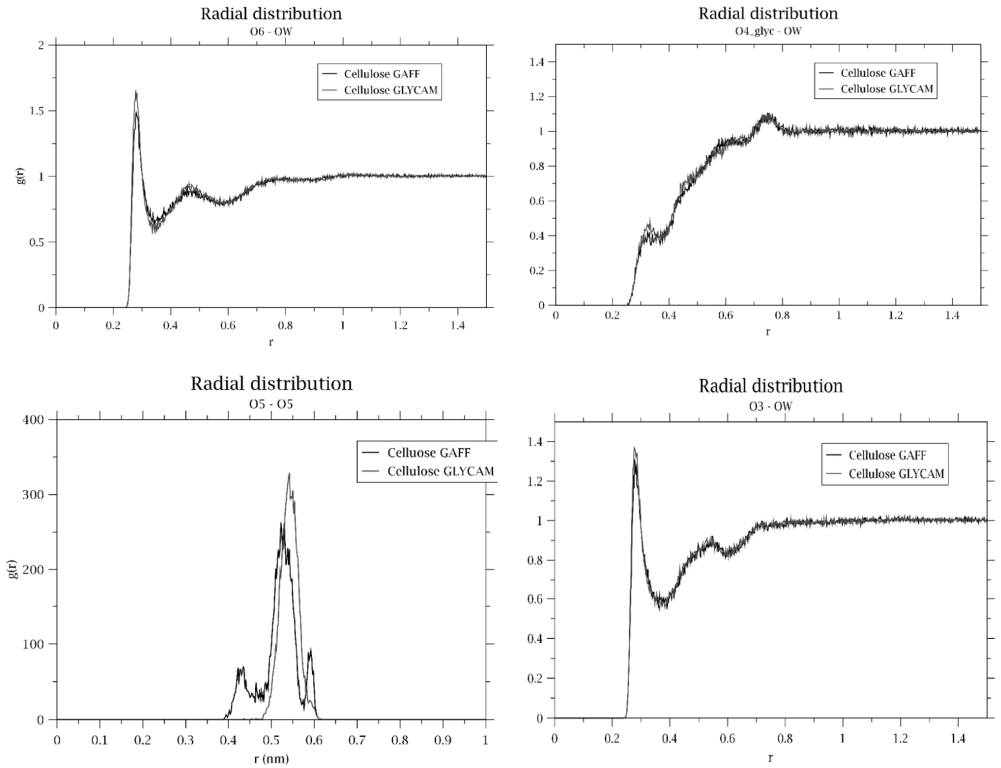

Figure 3 Comparative representations (GLYCAM vs. GAFF) of structural organization for solvent molecules (water), expressed by radial distribution functions, around selected atoms of investigated polisaccharides.

Radial function calculation results for oxygen atoms O3, O4, O5, O6 according to the water molecules oxygen are shown in **Figure 3**. We can observe a very good correspondence for water distribution despite the chosen force field, thus validating the usage of the GAFF force field with

partial electric charges determined by AM1 method, for using them on polysaccharides as cellulose and chondroitin-sulfate.

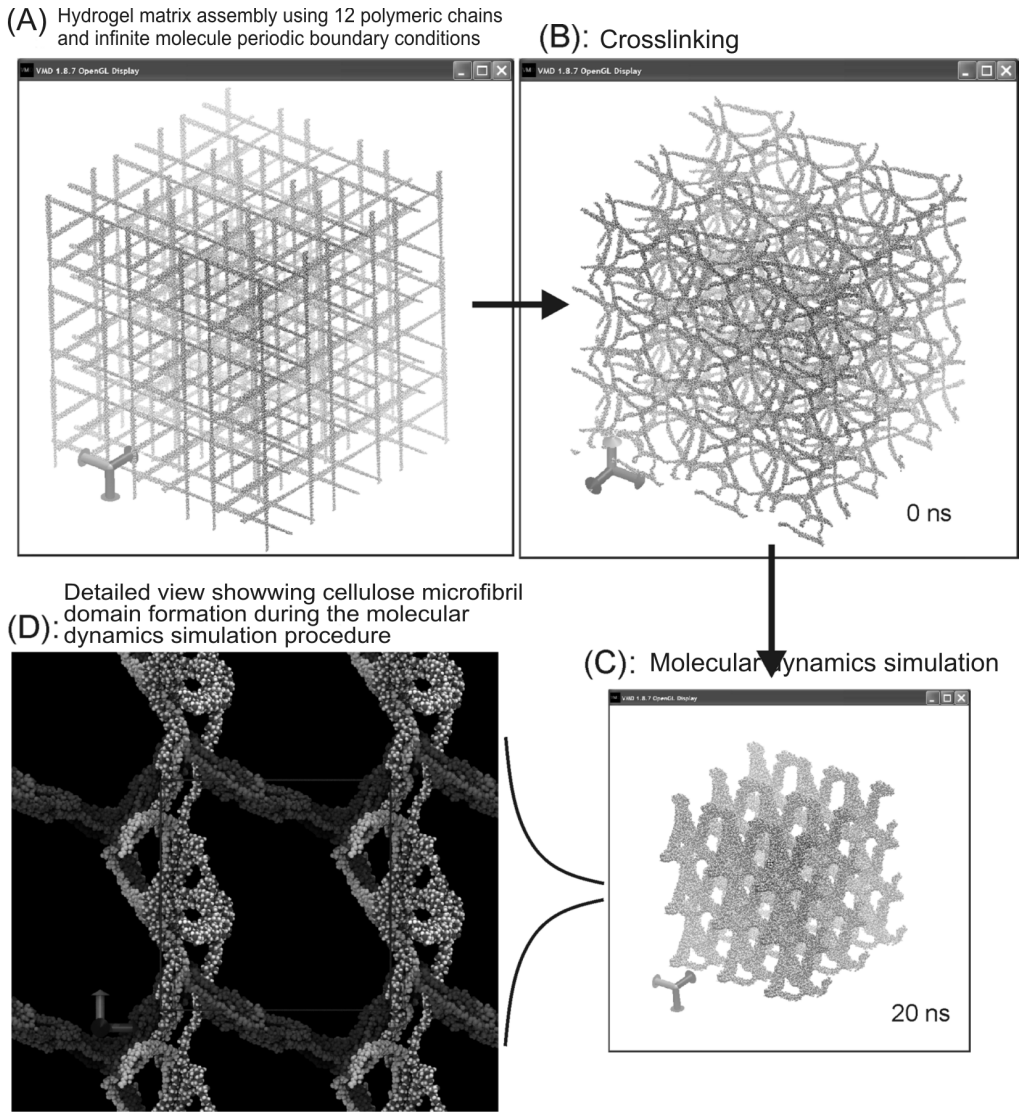

Figure 4. Model building and chondroitin-sulphate and cellulose/xantan based hydrogels (A,B,C); fibrillar cellulose organization (color chains) obtained following the 50/50 cellulose/chondroitin-sulphate hydrogel simulation (D).

The charges obtained in the previous step were used for the simulation of a defined small domain of a hydrogel three dimensional matrix in explicit solvent and ionic environment. Some of the preliminary results are presented below. In **figure 4** we have represented the cellulose

chain representation obtained during simulation for 50/50 cellulose/chondroitin-sulphate hydrogel for 20 ns interval.

We can observe the formation of cellulose microfibrils (color chains in figure 4D), an expected result regarding the pure cellulose insolubility into water. This fibril formation limits the hydrogel water swelling, increasing the mechanical stiffness, which relates to experimental rheological measurements performed on this mixture (Oprea, 2009).

CONCLUSIONS

Quantum mechanics, molecular mechanics and molecular dynamics simulations were performed on disaccharidic molecules (cellulose and chondroitin sulfate dimers) in order to derive partial atomic charge sets that can further be used in molecular dynamics simulations of hydrogel matrices. These last simulations are able to provide detailed information on solvent and ions distribution and polymer chains arrangement inside the complex structure of such biomaterials. Moreover the succesful modelling of the hydrogel matrices loaded with a certain drug molecule could give us the rate of diffusion and the correlation between the water content/swelling and the controlled release properties of the material. For such a simulation to be feasible, high quality charge models must be devised, especially when dealing with poly-electrolyte systems such as glycosaminoglycans. The charges presented here for the sulfated form of GAGs are in good agreement with the more sophisticated models for the non-sulfated forms of GAGs, i.e. GLYCAM forcefield developed based on *ab initio* full quantum description, and can be regarded as a starting point for further development.

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