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Theoretical study of hydrogen bond interactions of fluvastatin with ι-carrageenan and λ-carrageenan

Anastasios G. Papadopoulos · Michael P. Sigalas

Abstract The binding of the reductase inhibitor drug fluvastatin, hydroxy-3-methylglutaryl coenzyme A, with the hydrophilic ι- or λ-carrageenan polymers, serving as potential controllers of the drug's release rate, have been studied at the density functional level of theory with the B3LYP exchange correlation functional. Three low energy conformers of fluvastatin have been calculated. The vibrational spectroscopic properties calculated for the most stable conformer were in satisfactory agreement with the experimental data. A series of hydrogen bonded complexes of the most stable conformer of fluvastatin anion with low molecular weight models of the polymers have been fully optimized. In almost all, intermolecular H-bonds are formed between the sulfate groups of ι- or λ-carrageenan and fluvastatin's hydroxyls, resulting in a red shift of the fluvastatin's O−H stretching vibrations. Cooperative intramolecular H-bonds within fluvastatin or ι-, λ-carrageenan are also present. The BSSE and ZPE corrected interaction energies were estimated in the range 281–318 kJ mol^{-1} for ι-carrageenan - fluvastatin and 145–200 kJ mol^{-1} for λ-carrageenan - fluvastatin complexes. The electron density (ρ_{bcp}) and Laplacian ($\nabla^2\rho_{bcp}$) properties at critical points of the intermolecular hydrogen bonds, estimated by AIM (atoms in molecules) calculations, have a low and positive character ($\nabla^2\rho_{bcp}>0$), consistent with the electrostatic character of the hydrogen bonds. The structural and energetic data observed, as well as the extent of the red shift of the fluvastatin's O−H stretching vibrations upon complex formation and the

properties of electron density show a stronger binding of fluvastatin to ι- than to λ-carrageenan.

Keywords AIM · ι-Carrageenan · λ-Carrageenan · Density functional theory · Fluvastatin · Hydrogen bonding

Introduction

Hydrogen bonds (H-bonds) play a vital role in chemical, physical, biological and technological processes. While many experimental and theoretical studies have been directed toward understanding H-bonding, it remains an area of active research. An important application of hydrogen bonded complexes is the solid dispersion systems of drugs into inert polymer matrices, primarily aiming at the optimization of the dissolution rate of poorly soluble drugs [1, 2], as well as to control the drug's release rate [3]. As in such systems the drug molecules bind to the polymer with van der Waals interactions or H-bonds, the nature and strength of the interactions of a drug and the polymers are of particular importance [4–6]. Continuing our work on the theoretical study of H-bond interactions between drugs and hydrophilic polymers used as excipients in drug formulation, [7, 8] we present here a density functional study of the hydrogen bonding in solid dispersions of the reductase inhibitor drug fluvastatin sodium, hydroxy-3-methylglutaryl coenzyme A, with the hydrophilic ι-or λ-carrageenan polymers.

Fluvastatin sodium, a mevalonolactone derivative of a fluorophenyl substituted indole (Fig. 1), is a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor. It belongs to an inhibitor family of drugs, commonly called statins, which have been shown to slow the progression of coronary artery disease [9] and reduce mortality from cardiovascular disease [10]. They have been

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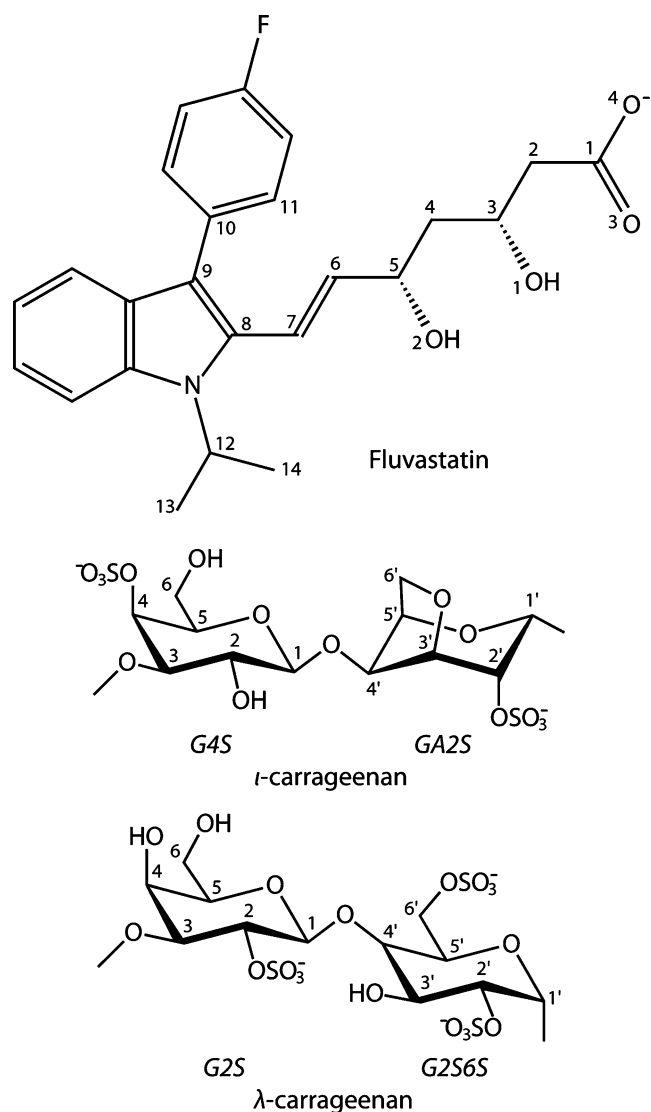e-mail: sigalas@chem.auth.gr

Fig. 1 Skeletal structure of the fluvastatin anion and ι-carageenan and λ-carageenan polymers studied in this work

suggested to have an anti-inflamatory and anti-cancer activity [11]. Some of them are also being tested against Alzheimer's disease and osteoporosis [12]. The crystal structure of fluvastatin has been solved by X-Ray crystallography [13], whereas its conformational space and hydroxy acid-lactone interconversion have been studied with DFT calculations [14].

Carrageenans are linear, sulfated polysaccharides (galactans) extracted from various species of the red seaweed Rhodophyta [15, 16]. The carrageenan backbone is based on the repeating disaccharide sequence of D–galactopyranose residues linked alternately in 3-linked-β-D-galactopyranose and 4-linked-α-D-galactopyranose units. They are classified according to the degree of the substitution that occurs on their free hydroxyl groups, which are generally either the addition of ester sulfate or the presence of the 3,6-anhydride

on the 4-linked residue, and they are traditionally identified by a Greek prefix: ι- (iota) (mono-sulfate), κ- (kappa) (di-sulfate), and λ- (lambda) carrageenan (three-sulfate). Concerning the forms studied herein and shown in Fig. 1, ι-carrageenan is composed by 3-linked-β-D-galactopyranose-4-sulfate (G4S) and 4′-linked-3′,6′-anhydro-α-D-galactopyranose-2-sulfate (GA2S), whereas λ-carrageenan by 3-linked-β-D-galactopyranose-2-sulfate (G2S) and 4′-linked-3′,6′-anhydro-α-D-galactopyranose-2,6-disulfate (G2S6S).

Carrageenans have the ability to bind considerable quantities of materials. Besides their usage in food, ceramic, anti-ice fluids, paper and textile industries [17–20], complexes between carrageenan and drug molecules have been proved to control the drug's release rate and, thus, carrageenans, as well as carrageenan mixtures, have been used as drug carriers [3, 21].

In this article density functional theory (DFT) calculations are reported on the low energy conformation of fluvastatin and the structure of the hydrogen bonded complexes between fluvastatin and the ι- and λ-carrageenan polymers, in order to quantify the potential of the polymers to control the release rate of the drug. Because of the complexity of these interactions in the intact polymer our approach was to study the interaction of the drug with low molar mass molecules chosen to model each polymer. The key vibrational spectroscopy data of both fluvastatin and fluvastatin–polymer complexes indicative of the H-bond interactions responsible for the complexes' formation and the strength of the interactions calculated by means of the fluvastatin–polymer interaction energies are presented. Furthermore, the topological description of the electronic density concerning the observed hydrogen bonds has been studied by using the atoms in molecules theory (AIM).

Computational methods

Density functional theory (DFT) full geometry optimizations of the studied molecules and the H-bonded complexes have been carried out using the Gaussian 03 W suite of programs [22]. The hybrid method was applied (B3LYP) with Becke's three-parameter functional [23] and the nonlocal correlation is provided by the LYP expression [24]. The basis set used was 6–31 G [25, 26]. In order to achieve a better description of the H-bond the basis set for the atoms of the groups involved, that is hydroxyl group (OH) of fluvastatin, and the sulfate groups (OSO$_3$) of ι- and λ-carrageenan model, has been augmented to 6–31 G++(d, p). Harmonic frequency calculations were performed for all the optimized structures to establish that the stationary points found are minima. Vibrational frequency values in the harmonic approximation were scaled according to the prescriptions found in the literature [27].

In calculating the interaction energy in the hydrogen-bonded complexes studied, we accounted for the basis set superposition error (BSSE) by recalculating the monomer energies using the full dimer basis at the optimized geometry of the dimer using the counterpoise method. In this context, the BSSE corrected interaction energy of the A⋯B dimer is calculated as:

$$\Delta E(BSSE) = \Delta E + BSSE$$

$$\Delta E = E_{AB}^{\{AB\}}(AB) - E_A^{\{A\}}(A) - E_B^{\{B\}}(B)$$

$$BSSE = E_{AB}^{\{A\}}(A) + E_{AB}^{\{B\}}(B) - E_{AB}^{\{AB\}}(A) - E_{AB}^{\{AB\}}(B)$$

$$\Delta E(BSSE) = E_{AB}^{\{AB\}}(AB) - E_A^{\{A\}}(A) - E_B^{\{B\}}(B) + E_{AB}^{\{A\}}(A)$$
$$+ E_{AB}^{\{B\}}(B) - E_{AB}^{\{AB\}}(A) - E_{AB}^{\{AB\}}(B),$$

where, $E_{AB}^{\{AB\}}(AB)$ is the energy of the optimized dimer, $E_A^{\{A\}}(A)$ and $E_B^{\{B\}}(B)$ are the energies of the monomers A and B respectively at their equilibrium geometries calculated in their own bases $\{A\}$ and $\{B\}$, $E_{AB}^{\{A\}}(A)$ and $E_{AB}^{\{B\}}(B)$ are the energies of the monomers A and B respectively at geometries found in the dimer calculated in their own bases $\{A\}$ and $\{B\}$, and $E_{AB}^{\{AB\}}(A)$ and $E_{AB}^{\{AB\}}(B)$ are the energies of the monomers A and B respectively at geometries found in the dimer calculated with the full basis of the dimer $\{AB\}$ [28–32].

Analysis of the electronic charge density (ρ) and its Laplacian ($\nabla^2\rho$) was performed by means of the theory of atoms in molecules (AIM) proposed by Bader and coworkers [33–36]. According to this theory, when two neighboring atoms are chemically bonded, a bond critical point (bcp) appears between them, where the charge density is a minimum at r_c along the bond path ($\nabla\rho_{bcp}=0$) but a maximum along any orthogonal displacement. The sign of the Laplacian of the density at the bond critical point ($\nabla^2\rho_{bcp}$), reveals whether the charge is concentrated, as in covalent bond ($\nabla^2\rho_{bcp}<0$), or depleted, as in closed-shell (electrostatic) interactions ($\nabla^2\rho_{bcp}>0$). The AIM calculations were performed using the critical point option for the AIM keyword as implemented by Cioslowski [37–39] in Gaussian 03 W program [22].

Results and discussion

Molecular structure of fluvastatin

In order to study the H-bond complexes of fluvastatin with carrageenans we first located the global minimum in
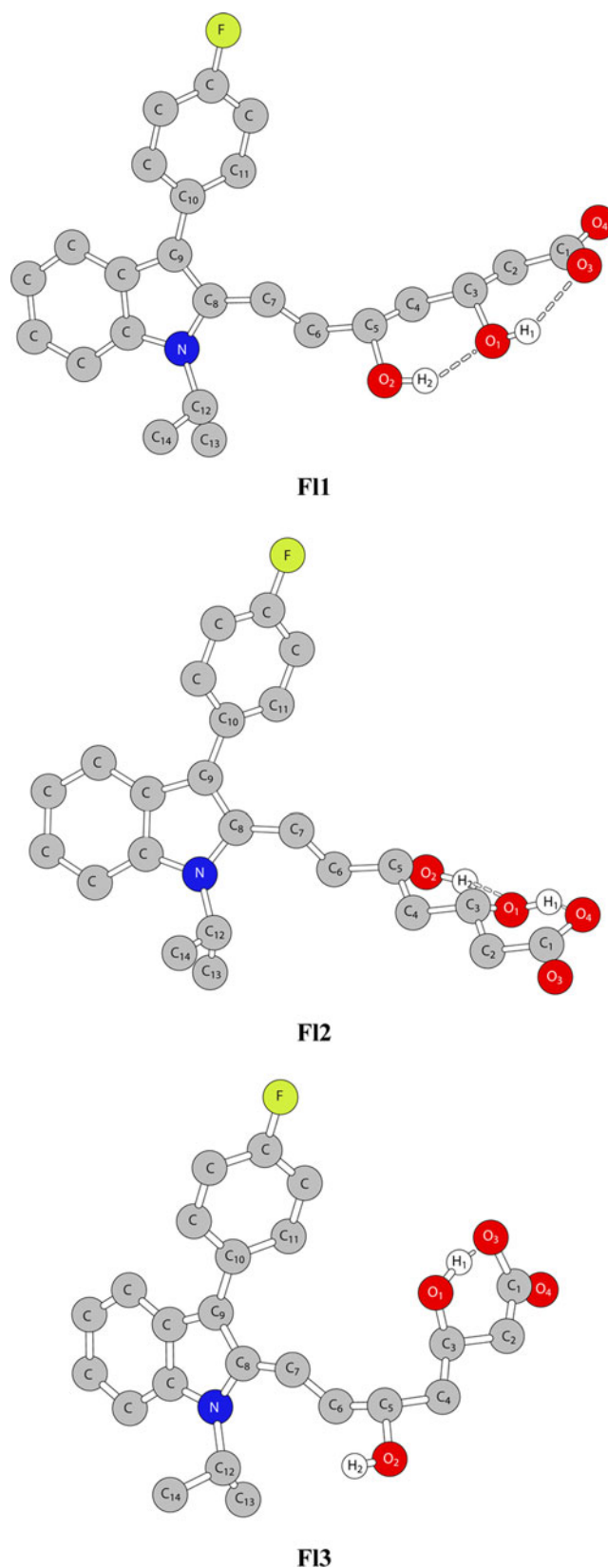

Fig. 2 Optimized structure of the fluvastatin conformers. For sake of clarity, only the hydrogen atoms eventually involved in hydrogen bond are shown

Table 1 Relative energies[a] and relevant optimized geometrical parameters[b,c] of fluvastatin conformers

	Fl1	Fl2	Fl3
ΔE	0.0	2	18
$O_3-C_1-C_2-C_3$	12.6	−12.3	44.5
$C_1-C_2-C_3-C_4$	179.2	−179.3	175.7
$C_2-C_3-C_4-C_5$	−177.7	177.3	176.8
$C_3-C_4-C_5-C_6$	171.2	−171.4	56.3
$C_4-C_5-C_6-C_7$	−116.2	109.4	−121.9
$C_6-C_7=C_8-C_9$	−145.5	−147.4	−148.0
$C_8-C_9-C_{10}-C_{11}$	45.0	45.1	52.5
$C_8-N-C_{12}-C_{13}$	−122.9	−120.7	−121.9
$C_8-N-C_{12}-C_{14}$	109.2	111.2	110.1
$H_1\cdots O_3$	2.122	2.122	2.111
$O_1-H_1\cdots O_3$	129.4	129.4	125.8
O_1-H_1	0.967	0.967	0.964
$H_2\cdots O_1$	1.932	1.932	
$O_2-H_2\cdots O_1$	142.8	142.7	
O_2-H_2	0.969	0.969	

[a] Energies in kJ mol^{-1}. [b] Bond distances in Å and angles in degrees. [c] Numbering scheme as in Figs. 1 and 2

the conformational space of the drug, which exhibits a tremendous conformational flexibility because of the many rotational degrees of freedom, associated with single bonds (scheme 2) and a very simple conformation analysis based on torsion angles for each bond, gives more than one million conformations. Hoffmann et al. has inspected the potential energy surface of fluvastatin

by means of DFT calculations and found small energetic differences between conformers [14]. Thus, in this study only the global minimum and some key conformers are presented.

The two low energy conformers of fluvastatin anion (**Fl1**, **Fl2**), shown in Fig. 2, have been located by a series of full optimizations at the B3LYP/6−31 G level with the basis set of the hydroxyl and carboxyl groups augmented to 6−311++G(d,p). Each initial geometry was this of the crystallographic observed structure [13] (pdb code: 1HWI) with crucial torsion angles perturbed. The most stable conformation found, **Fl1**, has two cooperative H-bonds between the two hydroxyl groups and a carboxyl group of fluvastatin. A similar structure has been found as the global minimum in the previous DFT study [14]. Conformation **Fl2**, having also two H-bonds, is less stable by 2 kJ mol^{-1}. A full optimization starting from the unperturbed X-ray structure gave structure, **Fl3** (Fig. 2), which is higher in energy by 18 kJ mol^{-1} and is very close to the experimental structure. The relative energies and relevant optimized geometrical parameters of the resulting conformers are given in Table 1. In all conformers, the $C_6-C_7=C_8-C_9$ group departs from planarity by 32–35° due to steric hindrance, whereas dihedral angles around C_2-C_3, C_3-C_4 and C_4-C_5 bonds show that they adopt the *gauche* orientations.

The harmonic vibrational frequencies of all conformation have been calculated at the same level of theory and basis set and scaled with scaling factor 0.9614 [27]. The frequencies of O−H stretching vibrations for the most stable conformation **Fl1** have been calculated as ν_{O1-H1}= 3616 cm^{-1} and ν_{O2-H2}=3563 cm^{-1}.
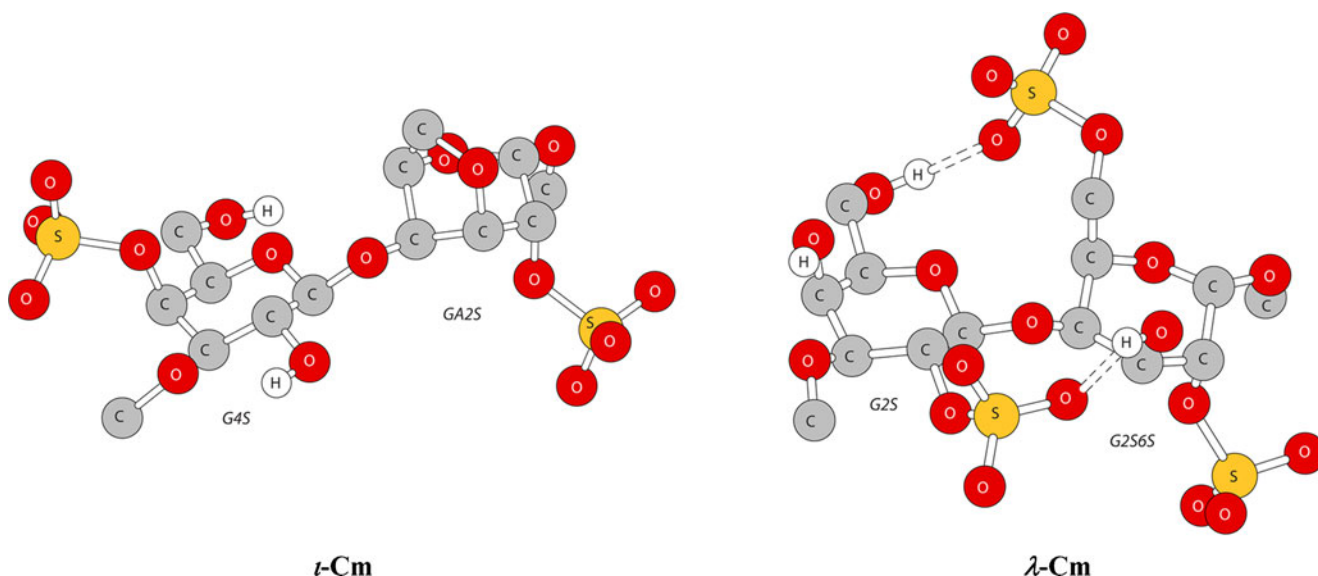
Fig. 3 Optimized structures of the models of ι-carageenan (ι-**Cm**) and λ-carageenan (λ-**Cm**). For sake of clarity, only the hydrogen atoms eventually involved in hydrogen bonds are shown

Fig. 4 Optimized structures of the H-bond complexes of *ι*-carrageenan model (**ι-Cm**) with fluvastatin anion (**Fl1**). For the sake of clarity, only the hydrogen atoms involved in the hydrogen bond are shown

ι-**Cm_Fl1_a**

ι-**Cm_Fl1_b**

ι-**Cm_Fl1_c**
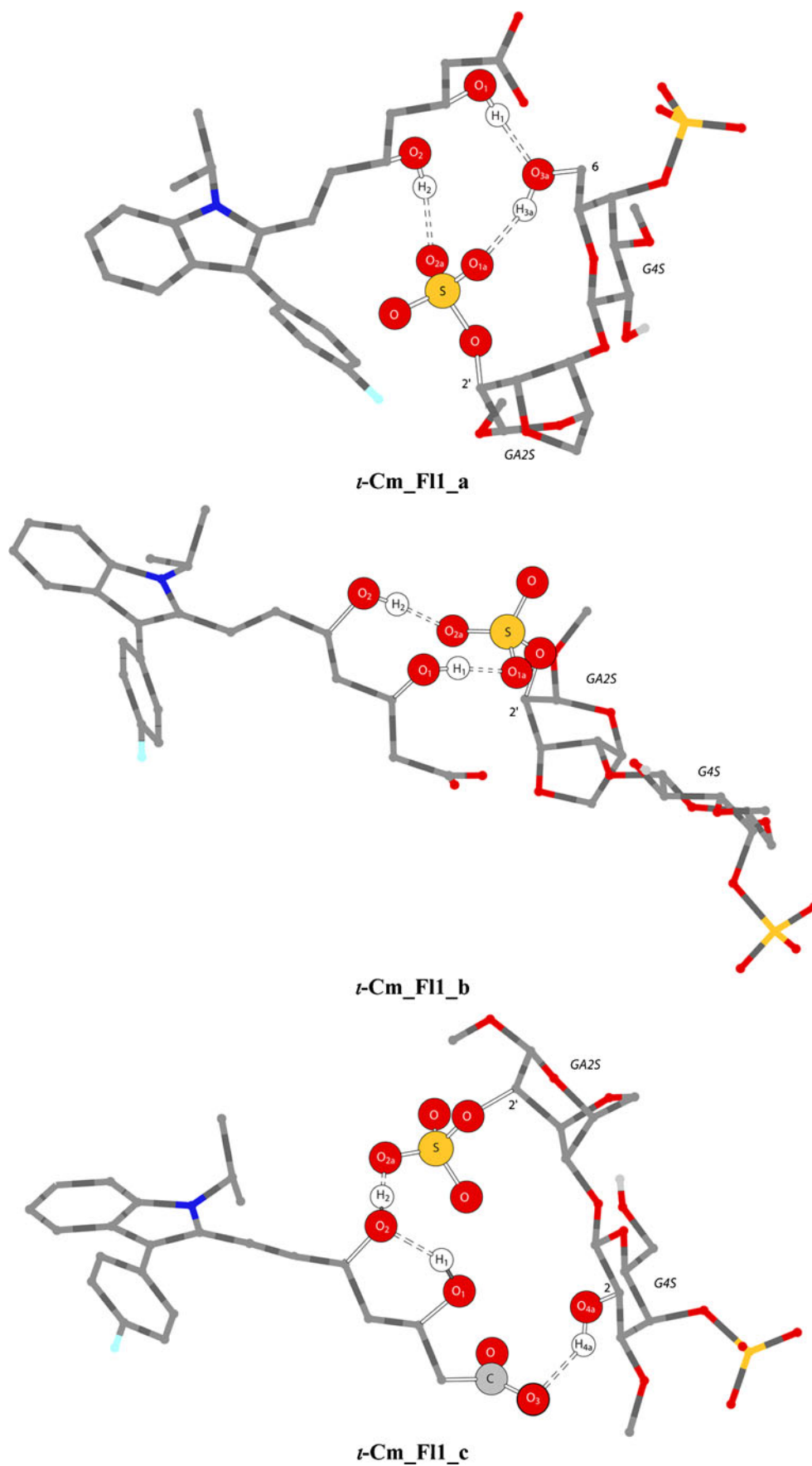
Although in 3,5 carboxyl diols the formation of lactone is very common at a rate which is usually pH dependent, at physiological pH and higher, the lactone form is unstable and the equilibrium favours hydrolysis to open the lactone and yield the hydroxyl acid form [40–43]. According to previous DFT calculations the lactone form is higher in energy by 25–79 kJ mol^{-1} [14]. Thus, in this study of hydrogen bonded complexes of fluvastatin the acidic form is used instead of the lactonic form.

Hydrogen bonded complexes of fluvastatin with ι- and λ-carrageenan polymers

The present reliability of high precision quantum mechanical calculations gives us the possibility to derive meaningful predictions about the strength of interactions between drugs and polymers. However, such calculations often require much long computational time and large sized memories and thus the actual analysis tend to be restricted to relatively small

Table 2 Relative energies^{a} and geometrical parameters^{b,c} for H-bonded complexes of fluvastatin anion with ι- and λ-carrageenan

ι-Cm_Fl1_a		ι-Cm_Fl1_b		ι-Cm_Fl1_c	
ΔE^{c}	0.0	ΔE	2	ΔE	35
Intermolecular hydrogen bonds					
O_1-H_1	0.981	O_1-H_1	0.980	O_2-H_2	0.994
$H_1 \cdots O_{3a}$	1.871	$H_1 \cdots O_{1a}$	1.862	$H_2 \cdots O_{2a}$	1.881
$O_1 \cdots O_{3a}$	2.788	$O_1 \cdots O_{1a}$	2.868	$O_2 \cdots O_{2a}$	2.858
$O_1-H_1 \cdots O_{3a}$	154.4	$O_1-H_1 \cdots O_{1a}$	169.6	$O_2-H_2 \cdots O_{2a}$	166.9
O_2-H_2	0.977	O_2-H_2	0.978	$O_{4a}-H_{4a}$	0.991
$H_2 \cdots O_{2a}$	1.887	$H_2 \cdots O_{2a}$	1.911	$H_{4a} \cdots O_3$	1.962
$O_2 \cdots O_{2a}$	2.847	$O_2 \cdots O_{2a}$	2.832	$O_{4a} \cdots O_3$	2.879
$O_2-H_2 \cdots O_{2a}$	166.8	$O_2-H_2 \cdots O_{2a}$	165.2	$O_{4a}-H_{4a} \cdots O_3$	152.7
Intramolecular hydrogen bonds					
$O_{3a}-H_{3a}$	0.976			O_1-H_1	0.980
$H_{3a} \cdots O_{1a}$	1.954			$H_1 \cdots O_2$	1.816
$O_{3a} \cdots O_{1a}$	2.874			$O_1 \cdots O_2$	2.704
$O_{3a}-H_{3a} \cdots O_{1a}$	156.0			$O_1-H_1 \cdots O_2$	148.9

λ-Cm_Fl1_a		λ-Cm_Fl1_b		λ-Cm_Fl1_c	
ΔE	0.0	ΔE	72	ΔE	79
Intermolecular hydrogen bonds					
O_1-H_1	0.986	O_1-H_1	0.985	O_1-H_1	0.990
$H_1 \cdots O_{1a}$	1.881	$H_1 \cdots O_{8a}$	1.876	$H_1 \cdots O_{5a}$	1.903
$O_1 \cdots O_{1a}$	2.822	$O_1 \cdots O_{8a}$	2.834	$O_1 \cdots O_{5a}$	2.885
$O_1-H_1 \cdots O_{1a}$	158.6	$O_1-H_1 \cdots O_{8a}$	163.4	$O_1-H_1 \cdots O_{5a}$	171.0
O_2-H_2	0.986	O_2-H_2	0.988		
$H_2 \cdots O_{2a}$	1.897	$H_2 \cdots O_{9a}$	1.880		
$O_2 \cdots O_{2a}$	2.847	$O_2 \cdots O_{9a}$	2.822		
$O_2-H_2 \cdots O_{2a}$	160.9	$O_2-H_2 \cdots O_{9a}$	158.4		
Intramolecular hydrogen bonds					
				O_2-H_2	0.981
				$H_2 \cdots O_1$	1.833
				$O_2 \cdots O_1$	2.706
				$O_2-H_2 \cdots O_1$	146.5
$O_{6a}-H_{6a}$	0.986	$O_{6a}-H_{6a}$	0.995	$O_{6a}-H_{6a}$	0.993
$H_{6a} \cdots O_{3a}$	1.916	$H_{6a} \cdots O_{3a}$	1.733	$H_{6a} \cdots O_{3a}$	1.756
$O_{6a} \cdots O_{3a}$	2.901	$O_{6a} \cdots O_{3a}$	2.722	$O_{6a} \cdots O_{3a}$	2.745
$O_{6a}-H_{6a} \cdots O_{3a}$	174.6	$O_{6a}-H_{6a} \cdots O_{3a}$	172.7	$O_{6a}-H_{6a} \cdots O_{3a}$	173.7
$O_{7a}-H_{7a}$	0.999	$O_{7a}-H_{7a}$	0.993	$O_{7a}-H_{7a}$	0.997
$H_{7a} \cdots O_{4a}$	1.831	$H_{7a} \cdots O_{4a}$	1.866	$H_{7a} \cdots O_{4a}$	1.921
$O_{7a} \cdots O_{4a}$	2.778	$O_{7a} \cdots O_{4a}$	2.820	$O_{7a} \cdots O_{4a}$	2.885
$O_{7a}-H_{7a} \cdots O_{4a}$	157.4	$O_{7a}-H_{7a} \cdots O_{4a}$	160.2	$O_{7a}-H_{7a} \cdots O_{4a}$	161.9

^{a} Corrected for zero point energy in kJ mol^{-1}. ^{b} Bond distances in Å and angles in degrees.
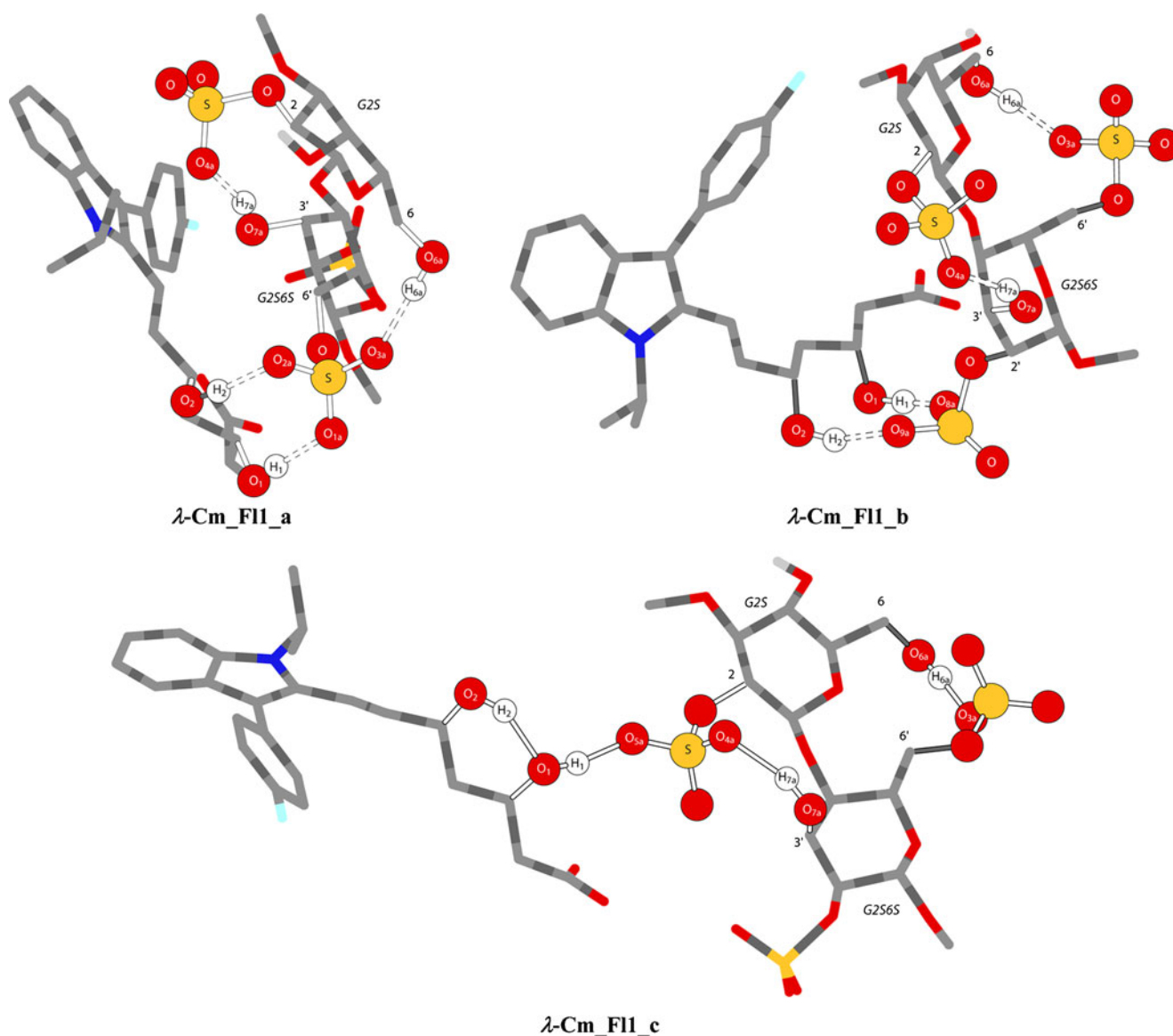^{c} Numbering scheme as in Figs. 4 and 5

Fig. 5 Optimized structures of the H-bond complexes of λ-carrageenan model (λ-**Cm**) with fluvastatin anion (**Fl1**). For the sake of clarity, only the hydrogen atoms involved in the hydrogen bond are shown

system representative of the whole polymer in vacuo [6–8]. In our study we used two low molecular weight models, ι-**Cm** and λ-**Cm**, of ι- and λ-carrageenan polymers respectively. Their calculated as global minimum structures are shown in Fig. 3. In the model of λ-carrageenan, λ-**Cm**, there are two strong H-bonds between the hydroxyl group in the 6-position of the galactopyranose-4-sulfate ring and the sulfate group in the 6′-position of the galactopyranose-2,6-disulfate ring (H···O = 1.742 Å) and between the hydroxyl group in the 3′-position of the galactopyranose-2,6-disulfate ring and the sulfate group in the 2-position of the galactopyranose-4-sulfate ring (H···O = 2.132 Å).

Initial structures of the complexes formed from the most stable conformer of fluvastatin anion (conformer **Fl1**) and the polymer models of ι- and λ-carrageenan (ι-**Cm** and λ-

Table 3 Interaction energy (kJ mol^{-1}) of H-bonded complexes of fluvastatin anion with ι-Carrageenan and λ-Carrageenan

	ΔE	ΔE+BSSE	ΔE+BSSE+ΔZPE [a]
ι-Cm_Fl1_a	−383	−318	−318
ι-Cm_Fl1_b	−381	−316	−314
ι-Cm_Fl1_c	−345	−282	−281
λ-Cm_Fl1_a	−284	−201	−200
λ-Cm_Fl1_b	−199	−154	−152
λ-Cm_Fl1_c	−189	−146	−145

[a] ZPE calculated as 0.9614$\Sigma h\nu_i/2$.

Cm) have been modeled, where fluvastatin acts as a hydrogen donor through hydroxyl groups or as an acceptor through hydroxylic oxygens, whereas ι-carrageenan and λ-carrageenan acts as acceptors through oxygen atoms of the sulfate groups or donors through hydroxylic hydrogens. All trial structures have been fully optimized at the B3LYP/6-31 G level by adding polarization and diffuse functions, 6–31++G(d,p), on the atoms involved in the H-bonds.

The three minima found for ι-carrageenan - fluvastatin complex (ι-**Cm_Fl1_a**, ι-**Cm_Fl1_b**, ι-**Cm_Fl1_c**) are shown in Fig. 4 and their relative energies and geometrical parameters of the H-bonds are depicted in Table 2. In all minima the groups of ι-carrageenan involved in intermolecular H-bonds are the sulfate group in the 2′-position of the *GA2S* galactopyranose unit or the hydroxyl groups in the 2- or 6-position of the G4S galactopyranose unit. Optimization of initial complex structures with the sulfate group in the 4-position of the *G4S* galactopyranose unit acting as hydrogen acceptor led to the same minima. Complex ι-**Cm_Fl1_a** with two intermolecular H-bonds is the most stable. The first H-bond is this between fluvastatin's O_1-H_1 hydroxyl and the *GA2S*-2′-sulfate group, whereas the second is this between fluvastatin's O_2-H_2 hydroxyl and the *G4S*-6-hydroxyl group of ι-carrageenan with the latter acting as a hydrogen acceptor. The complex is further stabilized by the intramolecular hydrogen between *G4S*-6-hydroxyl group and the *GA2S*-2′-sulfate group of ι-carrageenan. The calculated H···O distances are 1.871 Å, 1.917 Å and 1.954 Å respectively. The involvement of the hydroxyl groups of fluvastatin in H-bonds results in an elongation of O−H bonds and a red shift of the calculated frequencies of the O−H stretching vibrations relative to free fluvastatin (ν_{O1-H1}=3395 cm^{-1} and ν_{O2-H2}= 3487 cm^{-1}). In the ι-**Cm_Fl1_b** complex, which is less stable by 2 kJ mol^{-1}, only the *GA2S*-2′-sulfate group of ι-carrageenan is involved in two H-bonds with both

hydroxyls of fluvastatin, with the H···O distances being 1.862 Å, 1.911 Å. Finally, in the less stable by 35 kJ mol^{-1} complex, ι-**Cm_Fl1_c**, intramolecular H-bonds are formed between the *GA2S*-2′-sulfate group and fluvastatin's O_2-H_2 and between *G4S*-2-hydroxyl group and fluvastatin's carboxylate, whereas the intramolecular H_1···O_2 H-bond found in free fluvastatin (**Fl1**) is also present.

In the case of the interactions between λ-carrageenan and fluvastatin three complexes were also located, namely λ-**Cm_Fl1_a**, λ-**Cm_Fl1_b** and λ-**Cm_Fl1_c**, whose optimized structures are shown in Fig. 5 and their relative energies and geometrical parameters of the H-bonds are depicted in Table 2. In all minima the intramolecular H-bond between *G2S*-2-sulfate and *G2S6S*-3′-hydroxyl group, as well as this between *G2S6S*-6′-sulfate and *G2S*-2-hydroxyl group, both found in the free λ-carrageenan model, λ-**Cm**, are retained. In the complex λ-**Cm_Fl1_a**, being the most stable, two intermolecular H-bonds are formed between the *G2S6S*-6′-sulfate group of λ-carrageenan and fluvastatin's hydroxyls with H···O distances 1.881 Å and 1.897 Å. The red shift of the calculated frequencies of O−H stretching vibrations of the hydroxyl groups of fluvastatin involved in H-bonds (ν_{O1-H1}=3424 cm^{-1} and ν_{O2-H2}=3501 cm^{-1}) relative to free fluvastatin for this complex is smaller than this for ι-**Cm_Fl1_a**, a fact that serves as an indication of a stronger binding of fluvastatin to ι-carrageenan than to λ-carrageenan. In the complex λ-**Cm_Fl1_b**, which is less stable by 72 kJ mol^{-1}, the two intermolecular H-bonds are those between fluvastatin's hydroxyls and the *G2S6S*-2′-sulfate group of λ-carrageenan. The H···O distances are equal to 1.876 Å and 1.880 Å. Finally, the complex with the higher energy (79 kJ mol^{-1}), λ-**Cm_Fl1_c**, has only one intermolecular H-bond between the *G2S*-2-sulfate group of λ-carrageenan and fluvastatin's O_1-H_1 hydroxyl with H···O distance are equal to 1.903 Å. Furthermore, besides the aforementioned intramolecular bonds within λ-carrageenan

Table 4 Properties of the electron density, ρ_{bcp}, at H-bond critical points, its Laplacian, $\nabla^2\rho_{bcp}$, and ellipticity, ε, for H-bonded complexes of fluvastatin anion with ι- and λ-carrageenan. [a,b]

ι-Cm_Fl1_a				ι-Cm_Fl1_b				ι-Cm_Fl1_c			
	ρ_{bcp}	$\nabla^2\rho_{bcp}$	ε		ρ_{bcp}	$\nabla^2\rho_{bcp}$	ε		ρ_{bcp}	$\nabla^2\rho_{bcp}$	ε
H_1···O_{3a}	0.0319	0.0992	0.0821	H_1···O_{1a}	0.0385	0.0955	0.0329	H_2···O_{2a}	0.0472	0.1517	0.0316
H_2···O_{2a}	0.0288	0.0983	0.0400	H_2···O_{2a}	0.0291	0.0872	0.0379	H_{4a}···O_3	0.0283	0.0780	0.0963
H_{3a}···O_{1a}	0.0252	0.0800	0.0452					H_1···O_2	0.0385	0.1189	0.0321
λ-Cm_Fl1_a				λ-Cm_Fl1_b				λ-Cm_Fl1_c			
	ρ_{bcp}	$\nabla^2\rho_{bcp}$	ε		ρ_{bcp}	$\nabla^2\rho_{bcp}$	ε		ρ_{bcp}	$\nabla^2\rho_{bcp}$	ε
H_1···O_{1a}	0.0305	0.0931	0.0299	H_1···O_{8a}	0.0369	0.1211	0.0299	H_1···O_{5a}	0.0331	0.1099	0.0089
H_2···O_{2a}	0.0256	0.0947	0.0351	H_2···O_{9a}	0.0311	0.1040	0.0351	H_2···O_1	0.0375	0.1047	0.0333
H_{6a}···O_{3a}	0.0425	0.1297	0.0328	H_{6a}···O_{3a}	0.0452	0.1307	0.0309	H_{6a}···O_{3a}	0.0394	0.1225	0.0333
H_{7a}···O_{4a}	0.0288	0.0953	0.0300	H_{7a}···O_{4a}	0.0297	0.0968	0.0278	H_{7a}···O_{4a}	0.0278	0.0835	0.0395

[a] Values of ρ_{bcp} and $\nabla^2\rho_{bcp}$ in atomic units. [b] Numbering scheme as in Figs. 4 and 5

the $H_2 \cdots O_1$ H-bond found in free fulvastatin (**Fl1**) is also present.

The calculated H-bond distances ($O_{Fl1} \cdots O\iota, \lambda_{-Cm}$) for the intermolecular H-bonds between ι- and λ-carrageenan and fluvastatin, being in the range of 2.788–2.879 Å and 2.822–2.885 Å respectively, are indicative of a stronger binding of fluvastastin to ι-carrageenan. This is further confirmed by the higher interaction energy values in fluvastatin–ι-carrageenan complexes (mean 304 kJ mol^{-1}) compared to those of fluvastatin–λ-carrageenan complexes (mean 166 kJ mol^{-1}), listed before and after inclusion of the BSSE correction calculated by the counterpoise method, as well as the ZPE correction, in Table 3.

The use of the Laplacian of the electron density for the determination of regions of concentration and depletion of the electron density has been applied to the classification of the atomic interactions [36]. The two general classes of atomic interactions are shared interactions and closed-shell interactions. In the shared interactions (covalent and polar bonds) the charge density is contracted toward the line of interaction linking the nuclei and the Laplacian has negative values ($\nabla^2 \rho_{bcp} < 0$). In the closed-shell interactions (hydrogen-bonds, ionic bonds, and van der Waal's interactions) the charge density is contracted toward each of the interacting nuclei, the electronic charge is depleted in the interatomic surface and the Laplacian has positive values ($\nabla^2 \rho_{bcp} > 0$). The calculated electron density (ρ_{bpc}) and its Laplacian ($\nabla^2 \rho_b$) bcpat bond critical points of the H-bonds found in the optimized complexes of fluvastatin and ι- and λ-carrageenan are given in Table 4. As the magnitude of both the electron density and its Laplacian within complexes of ι- or λ-carrageenan group are almost the same, the discussion is restricted in the representative cases of the most stable complexes ι-**Cm_Fl1_a** and λ-**Cm_Fl1_a**.

The calculated electron density properties for the H-bonds of all complexes show that the intermolecular O$\cdots$H bonds have low ρ_{bcp} and positive $\nabla^2 \rho_{bcp}$ values, typical for closed-shell interactions as H-bonds and indicate an electrostatic character of the O$\cdots$H bondings [36]. Moreover, the low values of ellipticity, e, at BCPs indicate stability of the H-bonds. The ι-**Cm_Fl1_a** shows higher ρ_{bcp} and $\nabla^2 \rho_{bcp}$ values than λ-**Cm_Fl1_a**, indicating a more electrostatic O$\cdots$H bonding and increased bond strength in the fluvastatin–ι-carrageenan complex. This correlation of ρ_b and $\nabla^2 \rho_b$ was first reported by Carroll and Bader [44] and confirmed later by other authors [45, 46].

All theoretical data reveal that ι- and λ-carrageenan polymers form hydrogen bonded complexes with fluvastatin. Although the present study is based on low molecular weight models of the polymers, one expects analogous binding in long polymer chains. Thus, both polymers could serve as a depot for the drug, leading to a stabilization of the drug during storage as well as a prolonged release profile. As ι-

carageenan shows a stronger binding with the drug, it is expected to be a better controller of the drug's release rate. A related experimental study is in progress in our Department.

Conclusions

The molecular structure of fluvastatin was studied at B3LYP level. Three low energy conformers have been located. The vibrational properties calculated for the most stable conformer were in satisfactory agreement with the experimental data. The hydrogen bonded complexes of the most stable conformer of fluvastatin anion with low molecular weight models of ι-carrageenan or λ-carrageenan polymers have been fully optimized. In almost all cases intermolecular H-bonds are formed between the sulfate groups of ι- or λ-carrageenan and fluvastatin's hydroxyls, along with cooperative intramolecular H-bonds within fluvastatin or ι-, λ-carrageenan. The BSSE and ZPE corrected interaction energies were estimated in the range of 281–318 kJ mol^{-1} for ι-carageenan and 145–200 kJ mol^{-1} for λ-carageenan complexes. The low values of electron density ρ_{bcp} and positive values of the Laplacian $\nabla^2 \rho_{bcp}$ at the intermolecular H-bonds' critical points, estimated by AIM calculations, are consistent with electrostatic character of the H-bond. The stronger binding of fluvastatin to ι-carageenan than to λ-carageenan, according to the structural and energetic data observed as well as to the extent of the red shift of the fluvastatin's O−H stretching vibrations upon complex formation and the properties of electron density, indicates that ι-carageenan would be a better controller of the drug's release rate.

References

1. Serajuddin ATM (1999) J Pharm Sci 88:1058–1066
2. Craig DQM (2002) Int J Pharm 231:131–144
3. Gupta VK, Hariharan M, Wheatley TA, Price JC (2001) Eur J Pharm Biopharm 51:241–248
4. Bogdanova S, Pajeva I, Nikolova P, Tsakovska I, Muller B (2005) Pharm Res 22:806–815
5. Gupta R, Thilagavathi R, Chakraborti AK, Bansal AK (2005) Mol Pharm 2:384–391
6. Oliveira BG, Lima MCA, Pitta IR, Galdino SL, Hernades MZ (2010) J Mol Model 16:119–127
7. Teberekidis VI, Sigalas MP (2007) J Mol Struct THEOCHEM 803:29–38
8. Karavas E, Georgarakis E, Sigalas MP, Avgoustakis K, Bikiaris D (2007) Eur J Pharm Biopharm 66:334–347
9. La Rosa JC, He J, Vupputuri S (1999) J Am Med Assoc 282:2340–2346
10. Almuti K, Rimawi R, Spevack D, Ostfeld RJ (2006) Int J Cardiol 109:7–15

11. Yoshida M (2003) J Atheroscler Thromb 10:140–144
12. Miida T, Hirayanna S, Nakamura Y (2004) J Atheroscler Thromb 11:253–264
13. Istvan ES, Deisenhofer J (2001) Science 292:1160–1164
14. Grabarkiewicz T, Grobelny P, Hoffmann M, Mielcarek J (2006) Org Biomol Chem 4:4299–4306
15. Piculell L (2006) Gelling carrageenans. In: Stephen AM, Phillips GO, Williams PA (eds) Food polysaccharides and their applications, 2nd edn. Taylor and Francis Group, London, New York, pp 239–276
16. Falshaw R, Fourneaux RH, Stevenson DE (2005) Carbohyd Res 340:1149–1158
17. De Ruiter GA, Rudolph B (1997) Trends Food Sci Technol 8:389–395
18. Tye RJ (1989) Carbohyd Polym 10:259–280
19. Shchipunov YA (2003) J Colloid Interf Sci 268:68–76
20. Langendorff V, Guveliev G, Michon C, Launay B, Parker A, De Kruif CG (2000) Food Hydrocolloid 14:273–280
21. Nanaki S, Karavas E, Kalantzi L, Bikiaris D (2010) Carbohyd Res 79:1157–1167, and references therein
22. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Montgomery JA, Vreven T, Kudin KN, Burant JC, Millam JM, Iyengar SS, Tomasi J, Barone V, Mennucci B, Cossi M, Scalmani G, Rega N, Petersson GA, Nakatsuji H, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Klene M, Li X, Knox JE, Hratchian HP, Cross JB, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Ayala PY, Morokuma K, Voth GA, Salvador P, Dannenberg JJ, Zakrzewski VG, Dapprich S, Daniels AD, Strain MC, Farkas O, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Ortiz JV, Cui Q, Baboul AG, Clifford S, Cioslowski J, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Challacombe M, Gill PMW, Johnson B, Chen W, Wong MW, Gonzalez C, Pople JA (2003) Gaussian 03 W, Revision B.02. Gaussian Inc, Pittsburgh, PA
23. Becke AD (1993) J Chem Phys 98:5648–5652
24. Lee C, Yang W, Parr RG (1988) Phys Rev B 37:785–789
25. Raghavachari K, Pople JA, Replogle ES, Head-Gordon M (1990) J Phys Chem 94:5579–5596
26. Frisch MJ, Pople JA, Binkley JS (1984) J Chem Phys 80:3265–3269
27. Avci D, Atalay Y (2009) Int J Quantum Chem 109:328–341
28. Boys SF, Bernardi F (1970) Mol Phys 19:553–566
29. Davidson ER, Chakravorty SJ (1994) Chem Phys Lett 217:48–54
30. Van Duijneveldt FB (1994) Van Duijneveldt-van de Rijdt JGCM, Van Lenthe JH. Chem Rev 94:1873–1885
31. Jeziorski B, Moszynski R, Szalewicz K (1994) Chem Rev 94:1887–1930
32. Xantheas SS (1996) J Chem Phys 104:8821–8824
33. Bader RFW, Anderson SG, Duke AJ (1979) J Am Chem Soc 101:1389–1395
34. Bader RFW, Slee TS, Cremer D, Kraka E (1983) J Am Chem Soc 105:5061–5068
35. Bader RFW, MacDougall PJ (1985) J Am Chem Soc 107:6788–6795
36. Bader RFW, MacDougall PJ, Lau CDH (1984) J Am Chem Soc 106:1594–1605
37. Cioslowski J (1993) Chem Phys Lett 203:137–142
38. Cioslowski J (1992) Chem Phys Lett 194:73–78
39. Cioslowski J, Surjan PR (1992) THEOCHEM 255:9–33
40. Jemal M, Ouyang Z, Powell ML (2000) J Pharm Biomed Anal 23:323–340
41. Zhao JJ, Xie IH, Yang AY, Roadcap BA, Rogers JD (2000) J Mass Spectrom 35:1133–1143
42. Kaufman MJ (1990) Int J Pharm 66:97–106
43. Kearny AS, Crawford LF, Mehta SC, Radebaugh GW (1993) Reductase Inhibitor, CI-981. Pharm Res 10:1461–1465
44. Carroll MT, Bader RFW (1988) Mol Phys 63:387–405
45. Platts JA, Howard ST, Bracke BRF (1996) J Am Chem Soc 118:2726–2733
46. Perez-Lustres JL, Mosquera M, Klark T (2001) Phys Chem Chem Phys 3:3569–3579