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Theoretical Investigations on the Separation of Medetomidine Enantiomers

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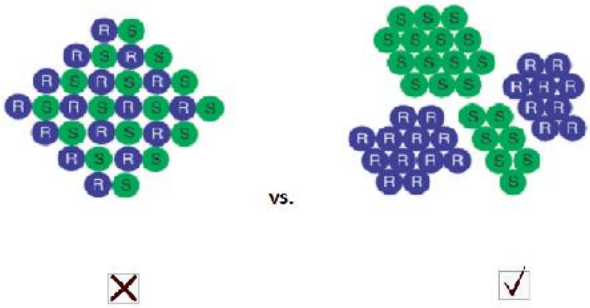
ABSTRACT

The enantiomeric separation of racemic compounds is of special importance. Conglomerate mixture is of considerable interest, since it corresponds to the possibility of spontaneous resolution of the two enantiomers. The aim of this paper is to find the achiral anions causing conglomerate formation of Medetomidine salts. For this purpose, the effect of 9 anion (X) on the heterochiral structure of Medetomidine enantiomers salts have been studied by Material Studio software. The crystal structures of all systems were determined by quantum calculations of CASTEP module. Investigation of the crystal structures and their respective energy show that Medetomidine salts, formed by Oxalic acid, Maleic acid and Fumaric acid crystalize as conglomerate, favoring preferential crystallization. The AIM results confirmed the more stability of conglomerate crystal in these cases while in the presence of other salting agent as Hydrochloric acid, Acetic acid, Carbonic acid, Formic acid, Malic acid and Lactic acid racemic crystal form is calculated as the more stable crystal. Using Forcite module, the total energy of the crystalline systems (calculated as the sum of the energies of the bonded and non-bonded interactions) are in agreement with those predicted by CASTEP module and AIM calculations.

GRAPHICAL ABSTRACT



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Introduction

Despite the existence of methods such as enantioselective synthesis and chromatographic separation, enantiomeric separation of racemic compounds remains the most desirable method for producing pure enantiomers on a larger scale [1-5]. Generally, crystalline racemic compounds can be classified into three different categories: 1) a racemic crystal, in which both enantiomers in the unit cell is in equal stoichiometry, 2) a conglomerate crystal in which there are a mechanical mixture of equimolar quantity of the two homochiral crystals, and 3) more rarely, a racemic solid solution, where both enantiomers are present in the unit cell with no fixed stoichiometry [6-8]. The conglomerate crystals, which is reflected by a mechanical mixture of the two pure single stereoisomers, are of considerable importance, since they corresponds to the possibility of spontaneous resolution of the two enantiomers, [9-11]. In a wide variety of applications, including the agrochemical, pharmaceutical and food industries, it is a necessity to obtain enantiomeric compounds in an optically pure form [12-14]. This is because only one out of a pair of enantiomers has valuable utility and desirable activity. In particular, in the pharmaceutical field there is a special interest in optically active isomeric forms of drugs [15, 16]. Typical examples are levothyroxine and thalidomide drugs [17-21]. Crystallization and liquid chromatography are two widely used methods for separation of enantiomers. Because of the cost and scale of chromatographic separations, the more preferred method for separation of enantiomers is crystallization method [22-25].

Results of previous studies show that the probability of finding conglomerate in the salts form of racemates is 2 or 3 times greater than in their covalent form [24]. Thus, a particular challenge in this regard is to finding appropriate salting reagents for conglomerate crystal formation [24, 25]. Doe to the importance of the subject, computer modeling and simulation techniques have recently been used [1-5, 23]. The crystalline structures of diastereomeric salts of ephedrine and chlocyphos in homo- and hetero-chiral forms are modeled and simulated [23]. For investigation of agent ability in separation of enantiomers, the lattice energies obtained from computational method have been evaluated. Such theoretical studies, by proposing more appropriate separating agents, significantly reduce the experimental effort to finding an especial separating agent.

In the continuation of our previous researches (experimental and theoretical) concerning the possibility of crystal conglomerate formation of Medetomidine salts [26-31], herein, we are interested to the theoretical investigation of the crystal formation between Medetomidine (free base) and achiral acids.

Medetomidine (4-[1-(2, 3 dimethylphenyl)ethyl]-1*H*-imidazole) (Figure 1) is an α2-adrenoreceptor agonist, which is used as an analgesic and anesthetic drug in veterinary medicine [32-34]. Medetomidine (MED) is a racemic mixture of two optical stereoisomers: S-enantiomer (dexmedetomidine) and R-enantiomer (levomedetomidine). S-enantiomer of MED is the pharmacologically active component, which has been developed for human use as a sedative in anesthesia management in surgery and in intensive care medicine. On the other hand, the R-enantiomer is entirely devoid of pharmacodynamic activity [35-40].

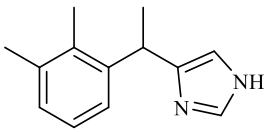

Figure 1: Chemical structure of Medetomidine.

In this study, crystal structure prediction methods were used to study the relative stabilities of enantiopure and racemic crystals of Medetomidine drug. Our aim is finding salting achiral agents for conglomerate formation. In this regards, Nine different acid, Oxalic acid (Ox: $(COOH)_2$), Hydrochloric acid (HCl), Acetic acid (Ac: CH_3COOH), Carbonic acid (Car: (H_2CO_3)), Maleic acid (Mle: ($C_4H_4O_4$)), Formic acid (Fo: HCOOH), Malic acid (Mli: ($C_4H_6O_5$)), Lactic acid (Lac: ($CH_3CHOHCOOH$)) and Fumaric acid (Fu: $(CHCOOH)_2$) were selected for investigation.

Methods

Initial conformational analyses of the molecules had been performed at the B3LYP/6-31G(d,p) level using GAUSSIAN 09 [41]. The electrostatic potential (ESP) were determined by re-minimizing conformations with Dmol3. These calculations were carried out using the generalized gradient approximation (GGA) and the Perdew, Burke and Ernzerhof (PBE) functional, applying progressively more restricted convergence criteria. We used a single effective core potential to treat the core electrons and a double numerical basis set. Monte Carlo simulated annealing procedure implemented in the Polymorph module of Materials Studio 6.1 [42] was employed to collect a variety of packing arrangements for each crystal. The COMPASS force field was used in the calculations. The 8 most common space groups found in organic crystals registered in the Cambridge Structural Database (CSD) were selected (P21, C2/c, P212121, P-1, P21/c, PNA21, PBCA and C2). The five potential packing structures are then subjected to the structural optimization using the DFT-D method with the generalized gradient approximation. These calculations were performed using CASTEP module and the results for the most stable structures are reported. CASTEP module of Material Studio is an ab initio quantum mechanical program employing density functional theory to simulate the properties of systems. Using Forcite module the total energy of the systems was also calculated as the sum of the different states of the bonded and non-bonded interactions. Forcite module of Material Studio is an classical molecular mechanics tool, that allows fast energies calculations and reliable geometry optimization of molecule and periodic systems. The quantum theory of atoms in molecules (QTAIM) analysis [43] was carried out using AIMALL package [44].

Results and Discussion

Molecular electrostatic potential (MEP) is an appropriate method to specify the charge distributions of molecules as three dimensional. This counter map is very useful to determine the reactive sites of molecules in both nucleophilic and electrophilic reactions for investigation of molecules. The molecular electrostatic potentials for both enantiomers of Medetomidine (cationic form) and anions of acids were calculated and presented in Figure 2 and 3, to characterize the nucleophilic and electrophilic sites. The positive regions of MEP which are suitable sites for nucleophilic reactivity are specified with blue color. The negative area that is the preferred site for electrophilic reactivity is presented by red color. As can be seen from Figure 2, the color of R/S-enantiomers of Medetomidine are blue. The MEP maps of acid molecules (anions) show that the red colors are observed around of the O and Cl atoms. Based on MEP results different initial structures were prepared and optimized using gaussian software. For example, different initial structures for S/S-enantiomers of Medetomidine in presence of oxalic acid (SS-Ox) are presented in Figure 4. The most stable structures were selected for crystal structure prediction.

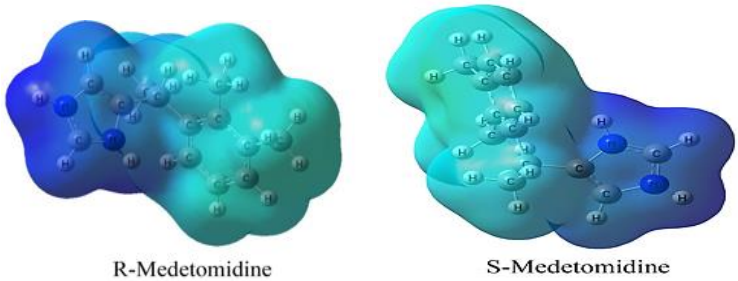

Figure 2: Molecular electrostatic potentials on the 0.001 (electron/Bohr3) electron density of S/R-enantiomers of Medetomidine in cationic form

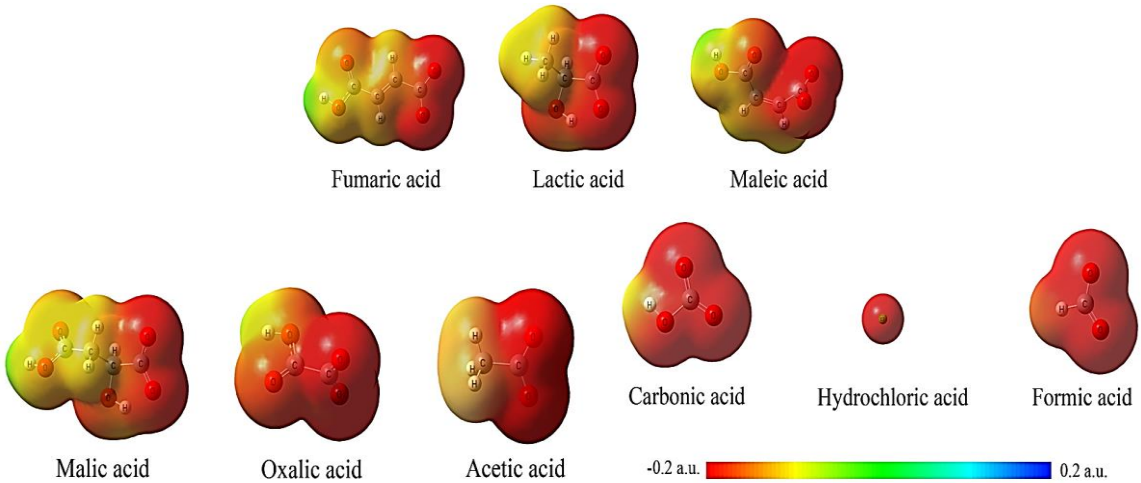

Figure 3: Molecular electrostatic potentials on the 0.001 (electron/Bohr3) electron density of anions

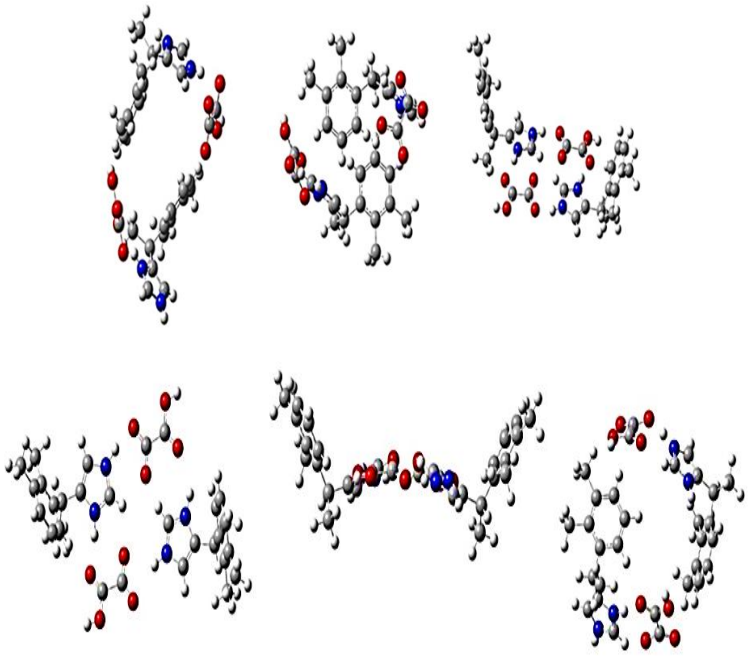

Figure 4: Different initial structures for SS-Ox system

Table 1 show energy, space group, and electron densities of the predicted more stable structure of studied systems calculated by CASTEP and Forcite modules and AIM method. In this Table RS refers to racemic structures and SS or RR refers to

homochiral crystal. The CASTEP results show that for Medetomidine in the presence of Oxalic acid, Maleic acid and Fumaric acid, the homochairl crystals are more stable than the racemic crystals (ΔE=-0.789 eV, ΔE=-0.404 eV and ΔE= -0.455 eV for Oxalic acid, Maleic acid and Fumaric acid, respectively). These results are conforms to a conglomerate crystal forming system in presence of Oxalic acid, Maleic acid and Fumaric acid, favoring their enantiomeric purification by preferential crystallization. The predicted crystal structures for Medetomidine in presence of Oxalic acid, Maleic acid and Fumaric acid, are shown in Figure 5. For Medetomidine in the presence of other acids, the racemic crystals are more stable than the homochiral crystals, which mean that these acids are not suitable for separation of Medetomidine enantiomers. These results are in agreement with previous experimental study, which binary phase diagram of MED-Ox system shows a racemic conglomerate behavior [27].

Table 1: The energy (CASTEP and Forcite modules results), space group and electron densities (AIM results) of predicted structures for homochiral and heterochiral crystallization of medetomidine salts formed by Oxalic acid (Ox), Hydrochloric acid (HCl), Acetic acid (Ac), Carbonic acid (Car), Maleic acid (Mle), Formic acid (Fo), Malic acid (Mli), Lactic acid (Lac), Fumaric acid (Fu).

System	**Energy**							**Electron Densities**	
	CASTEP module (eV)		Forcite module (kcal/mol)				Space group	ρ	$\Delta\rho$
	Total Energy	ΔE	Valence energy[a]	Non-bond energy[b]	Total Energy	ΔE			
RS-Ox	-4902.253	-0.789	81.129	-205.865	-124.736	-11.22	P212121	0.3574	0.1423
SS-Ox	-4903.042		77.272	-213.227	-135.955		P212121	0.4997	
RS-HCl	-3235.550	0.701	76.225	-430.112	-353.887	5.48	PBCA	0.2716	-0.0103
SS- HCl	-3234.849		75.478	-423.887	-348.409		P-1	0.2613	
RS-Ac	-4061.875	0.011	62.956	-464.647	-401.691	3.25	P212121	0.3381	-0.0066
SS-Ac	-4061.864		63.875	-462.312	-398.437		P212121	0.3315	
RS-Car	-4310.897	2.514	61.256	-490.09	-428.834	21.8	P21/C	0.4140	-0.026
SS-Car	-4308.383		64.321	-471.355	-407.034		C2	0.3880	
RS-Mle	-5243.163	-0.404	71.496	-482.165	-410.669	-6.29	P212121	0.2553	0.0854
SS-Mle	-5243.567		68.025	-484.984	-416.959		P21	0.3407	
RS-Fo	-3874.355	0.054	75.644	-420.53	-344.886	3.4	PBCA	0.3728	-0.0082
SS-Fo	-3874.301		74.358	-415.844	-341.486		P-1	0.3646	
RS-Mli	-5679.322	0.223	72.875	-493.785	-420.91	7.01	P212121	0.3353	-0.0646
SS-Mli	-5679.099		69.45	-483.35	-413.9		P212121	0.2907	
RS-Lac	-4685.501	15.724	75.954	-478.074	-402.12	23.8	P21	0.4081	-0.0366
SS-Lac	-4669.777		73.865	-452.185	-378.32		C2/C	0.3715	
RS-Fu	-5243.068	-0.455	73.535	-481.840	-408.305	-7.46	P-1	0.2756	0.0947
SS-Fu	-5243.523		68.392	-484.157	-415.765		P-1	0.3703	

[a] valence energy (sum of bond, angle, torsion and inversion energy)

[b] nan-bond energy (sum of hydrogen bond, van der waals, long range correction and electrostatic energy).

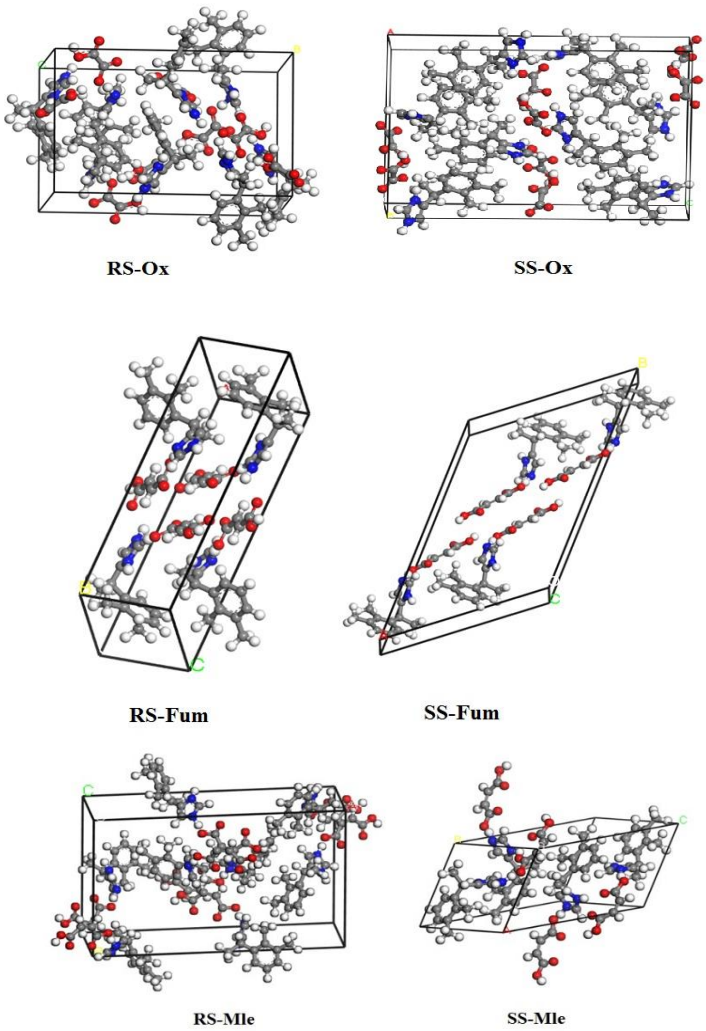

Figure 5: Predicted structure for Medetomidin in presence of Oxalic acid, Maleic acid and Fumaric acid.

In order to further study, the nature of the interactions between MED enantiomers and oxalic acid were investigated by using the quantum theory of atoms in molecules (QTAIM). The AIM analysis of the electron density shows the existence of bond critical points (BCPs) between MED enantiomer and oxalic acid (see Figure 6). The results of calculations including the electron density (ρ_b) and its Laplacian ($\nabla^2\rho_b$), kinetic electron energy density (G_b) and potential electron energy density (V_b) are given in Table 2 and 3. The electron densities at BCPs between MED enantiomer and oxalic acid range from 0.0006 to 0.0630 au. As can be seen from Table 2 and 3, all value of $\nabla^2\rho b$ are positive, which indicates depletion of electronic charge density in the interatomic surface and means a closed-shell interaction. The summation of electron density for RS-Ox and SS-Ox systems are 0.3574 and 0.4997, respectively. The results demonstrate that the strength of interactions in SS-Ox system are greater than that in RS-Ox system. Therefore it can conclude that the more stability of SS-Ox system is due the intermolecular interactions. The overall electron densities obtained from AIM calculations for different structures of hetero and homochiral crystals of Medetomidine-anions are presented in Table 1. These results show that the structure of homochiral crystals of Medetomidine in the presence of Oxalic, Maleic, and Fumaric acids are also more stable than heterochiral crystals and for

other anions, structures of heterochiral crystals of Medetomidine-anion are more stable than homochiral crystals. The computational results of AIM are in accordance with the computational results of energy for different structures of hetero and homochiral crystals of Medetomidine-anion and confirm those results. The total energy of the crystalline systems obtained by the Forcite module is calculated as the sum of the energies of the bonded and non-bonded interactions. Since the crystals have a salt structure, it is observed that the energy contribution of the non-bonded interaction, especially the electrostatic interaction, is greater than of other interactions in the total energy of the crystal. The results obtained by Forcite module showed that the non-bonding interaction energies for Oxalic, Maleic, and Fumaric acids in the SS-Medetomidine structure are greater than in the RS-Medetomidine structure (Table 1). The results of Forcite module for the Medetomidine crystals in the presence of other achiral acids show that RS-Medetomidine crystals have higher non-bonded interaction energies than SS-Medetomidine. Thus, results of the Forcite module for the total energy of these systems are in agreement with the corresponding results obtained from the CASTEP module and AIM method.

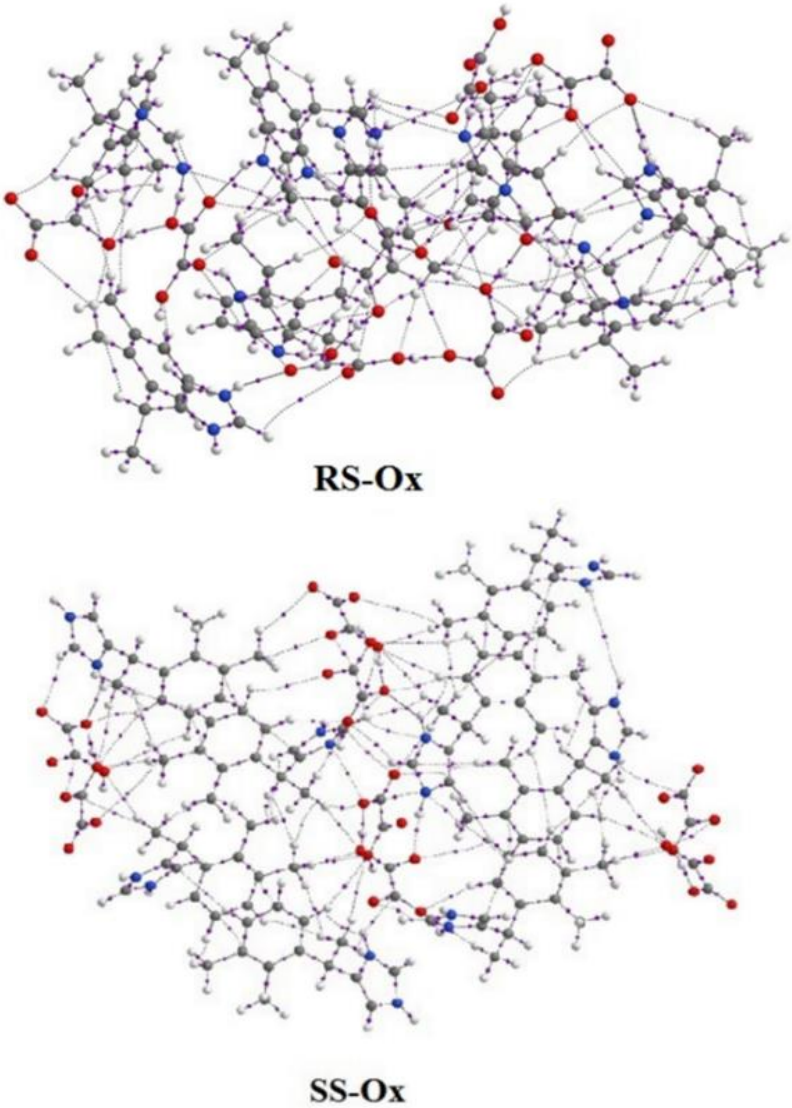

Figure 6: AIM graph for Medetomidine salts formed by Oxalic acid (RS-Ox and SS-Ox)

Table 2: AIM results for medetomidine salts formed by medetomidine enantiomers (R & S) and oxalic acid (RS-Ox). All units are a.u.

V_b	G_b	$\nabla^2\rho_b$	ρ_b	V_b	G_b	$\nabla^2\rho_b$	ρ_b
-0.0010	0.0022	0.0134	0.0024	-0.0010	0.0021	0.0127	0.0024
-0.0047	0.0072	0.0388	0.0069	-0.0026	0.0047	0.0274	0.0049
-0.0028	0.0049	0.0282	0.0049	-0.0032	0.0053	0.0293	0.0048
-0.0032	0.0053	0.0298	0.0049	-0.0022	0.0029	0.0141	0.0037
-0.0071	0.0097	0.0491	0.0088	-0.0074	0.0083	0.0370	0.0097
-0.0075	0.0084	0.0372	0.0098	-0.0045	0.0058	0.0287	0.0060
-0.0020	0.0034	0.0193	0.0032	-0.0052	0.0073	0.0380	0.0068
-0.0028	0.0049	0.0282	0.0049	-0.0052	0.0073	0.0380	0.0068
-0.0032	0.0053	0.0298	0.0049	-0.0031	0.0047	0.0252	0.0046
-0.0071	0.0097	0.0491	0.0088	-0.0331	0.0315	0.1199	0.0263
-0.0069	0.0083	0.0390	0.0087	-0.0004	0.0009	0.0055	0.0006
-0.0075	0.0084	0.0372	0.0098	-0.0191	0.0216	0.0966	0.0170
-0.0010	0.0021	0.0128	0.0024	-0.0030	0.0052	0.0295	0.0046
-0.0030	0.0045	0.0243	0.0045	-0.0077	0.0102	0.0510	0.0097
-0.0045	0.0058	0.0286	0.0060	-0.0004	0.0009	0.0053	0.0006
-0.0077	0.0086	0.0380	0.0099	-0.0330	0.0311	0.1169	0.0265
-0.0022	0.0028	0.0138	0.0037	-0.0021	0.0032	0.0175	0.0043
-0.0011	0.0022	0.0135	0.0024	-0.0047	0.0072	0.0388	0.0069
-0.0054	0.0082	0.0437	0.0078	-0.0022	0.0028	0.0138	0.0037
-0.0404	0.0327	0.1000	0.0341	-0.0045	0.0058	0.0286	0.0060
-0.0079	0.0106	0.0536	0.0101	-0.0049	0.0072	0.0379	0.0069
-0.0077	0.0086	0.0380	0.0099	-0.0013	0.0023	0.0134	0.0022
-0.0054	0.0081	0.0435	0.0077	-0.0032	0.0042	0.0209	0.0059
				-0.0078	0.0106	0.0535	0.0099

Table 3: AIM results for medetomidine salts formed by medetomidine enantiomers (SS) and oxalic acid (SS-Ox). All units are a.u.

V_b	G_b	$\nabla^2\rho_b$	ρ_b	V_b	G_b	$\nabla^2\rho_b$	ρ_b
-0.0018	0.0033	0.0192	0.0031	-0.0034	0.0058	0.0326	0.0057
-0.0068	0.0088	0.0434	0.0069	-0.0007	0.0017	0.0105	0.0017
-0.0040	0.0053	0.0266	0.0054	-0.0016	0.0032	0.0192	0.0031
-0.0066	0.0093	0.0477	0.0085	-0.0749	0.0544	0.1357	0.0567
-0.0036	0.0051	0.0260	0.0056	-0.0043	0.0069	0.0377	0.0067
-0.0053	0.0081	0.0437	0.0079	-0.0406	0.0352	0.1193	0.0329
-0.0039	0.0062	0.0337	0.0060	-0.0051	0.0078	0.0424	0.0077
-0.0087	0.0115	0.0571	0.0106	-0.0059	0.0079	0.0400	0.0085
-0.0410	0.0357	0.1219	0.0329	-0.0056	0.0081	0.0425	0.0078
-0.0042	0.0068	0.0375	0.0065	-0.0038	0.0061	0.0335	0.0061
-0.0752	0.0550	0.1395	0.0564	-0.0090	0.0117	0.0575	0.0113
-0.0059	0.0080	0.0403	0.0085	-0.0342	0.0309	0.1105	0.0290
-0.0016	0.0032	0.0191	0.0031	-0.0009	0.0013	0.0066	0.0016
-0.0008	0.0018	0.0111	0.0018	-0.0007	0.0015	0.0089	0.0012
-0.0046	0.0065	0.0338	0.0065	-0.0015	0.0027	0.0154	0.0026
-0.0007	0.0014	0.0085	0.0011	-0.0007	0.0015	0.0089	0.0012
-0.0019	0.0036	0.0208	0.0038	-0.0082	0.0114	0.0583	0.0096

-0.0018	0.0034	0.0201	0.0022	-0.0072	0.0092	0.0447	0.0091
-0.0009	0.0013	0.0066	0.0016	-0.0057	0.0082	0.0427	0.0078
-0.0008	0.0017	0.0107	0.0017	-0.0008	0.0018	0.0109	0.0018
-0.0017	0.0033	0.0195	0.0035	-0.0018	0.0033	0.0197	0.0035
-0.0029	0.0043	0.0230	0.0057	-0.0037	0.0060	0.0332	0.0058
-0.0036	0.0059	0.0329	0.0057	-0.0057	0.0082	0.0427	0.0078
-0.0070	0.0090	0.0445	0.0091	-0.0814	0.0579	0.1372	0.0630
				-0.0032	0.0050	0.0270	0.0035

Conclusions

In order to find salting reagents for separation of Medetomidine drug enantiomers, nine different acid including Oxalic acid, Hydrochloric acid, Acetic acid, Carbonic acid, Maleic acid, Formic acid, Malic acid, Lactic acid and Fumaric acid were investigated. The results of calculations by CASTEP module indicated that for Medetomidine in presence of Oxalic acid, Maleic acid and Fumaric acid, the homochiral crystals are more stable than the racemic crystals. These results conforms to a conglomerate crystal forming system in presence of Oxalic acid, Maleic acid and Fumaric acid. The AIM results demonstrate that the strength of interactions in homochiral crystals of Medetomidine in the presence of Oxalic, Maleic, and Fumaric acids are also more stable than heterochiral crystals. The results obtained by the Forcite module are also in agreement with those of CASTEP module and AIM calculations and predicts the conglomerate crystal formation in presence of Oxalic acid, Maleic acid and Fumaric acid.

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