Crystal structure of methylene blue citrate

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Abstract: The citrate salt of methylene blue), (Mb)(H$_3$Cit) 0.18H$_2$O (where Mb =methylene blue cation and Cit = citrate anion)have been determined by single-crystal X-ray diffraction. MB$^+$cation are is planar and stacked in an antiparallel fashion, exhibiting π–πassociations at a distance of 3.746(6)Å. The Mb$^+$ cation forms a short contacts of the type C-H..O with citric anion through methyl groups of the NMe$_2$ side substituents. Water molecules occupy voids between stacks of MB$^+$ cations with a very low site occupancies and do not form any short contacts with the citric anion.The intermolecular interactionsand specific weak contacts were analyzed by Hirshfeld surface calculations.

Keywords: methylene blue; citrate; a single crystal X-ray diffraction; π–πstacking; Hirshfeld surface calculation.

1 Introduction

Methylene blue (methylthioninium chloride) (3,7-bis(dimethylamino)phenothia-zin-5-ium chloride, C$_{16}$H$_{18}$SN$_3$Cl) is a tricyclic phenothiazine drug and dye acting as an inhibitor of nitric oxide synthase and guanylate cyclase [1, 2]. It is currently FDA-approved for the treatment of hereditary and acute methemoglobinemia, prevention of urinary tract infections in the elderly, and as a dye for intraoperative tissue visualization [3].

Interest in methylene blue has arisen due to recent information about its therapeutic effect against Covid-19 [1, 4]. Methylene blue (MB·Cl) is a thiazine compound and consists of three condensed six-membered rings and two coplanar NMe$_2$ substituents (Fig. 1).

The aim of our research is to develop pharmaceutical MB`s co-crystals with various suitable organic compounds, since the creation of such MB co-crystals allows the creation of effective MB`s drugs against Alzheimer's disease [5].

Here we discuss the results of X-Ray diffraction analysis of a novel synthesized salt-type co-crystal of MB with citric acid and by Hirshfeld surface calculations analyzed its intermolecular interactions.

2 Results and discussion

The asymmetric unit of (Mb)$^+$·(Cit)$^-$·0.18H$_2$O (**1**), where Mb$^+$ = C$_{16}$H$_{18}$SN$_3$$^+$ and Cit$^-$ = C$_6$H$_7$O$_7$$^-$consists of an MB$^+$ cation, a singly charged [H$_3$Cit]$^-$ anion and disordered over three positions water molecules with summary quantity of0.18 (Fig. 2). The MB$^+$ cation has geometric parameters typical for MB$^+$ cation: C1-C2 (1.367(7) Å), C4-C12 (1.383(6) Å), C8-C9 (1.357(7) Å) and C6-C13 (1.364.5 Å) bonds are shorter than other C-C bonds (1.423 Å (av.)).

The two C–S bonds are equal in length (1.729(4) and 1.731(4) Å), indicating that conjugation occurs via N10. The MB cation is thus planar and the adjacent cations are stacked in an antiparallel fashionwith the sulfur atom disposed alternatively on opposite sides and exhibiting π-π stacking at anaverage distance of 3.746 (8) Å (Fig. 2).This contact is longer thananalogical contact in other MB`ssalts where it lays in the range of 3.326 ÷ 3.550 Å. A bulky citrate anion changespacking of MB$^+$ cation in crystal.In contrast to MBCl·5H$_2$O, MBCl·2H$_2$O, MBNO$_3$·2H$_2$O, MB·CNS, MB·Urat·6H$_2$O(Urat is anion of uric acid)where MB$^+$ anions are packed into one antiparallel stack, in **1**, the stacks of MB$^+$ cations are arranged into two zigzag-shaped stacks, which mutually are related by a glide plane **c** and oriented at an angle of 127.3

(Fig. 3). Similar arrangements of the MB^+ cations also take place in the $MB.HSO_4$ crystal packing.

In contrast to MB chloride, which crystallizes with five lattice water molecules, given crystal contains only 0.18 water molecules disordered over three positions with occupations 0.04, 0.05 and 0.09, which are located between two adjacent stacks of MB^+ cations and they accompany zigzag chains of MB^+ cations and form no contact with citrate anions (Fig. 3).

Zigzag chains of citrate anions are oriented along the c axis, which are linked by O4C-H4…O2C and O7C-H7…O3C hydrogen bonds into a two-dimensional network.

In crystals of **1**, as in crystals of $MBCl·5H_2O$ and $MB·NO_3·2H_2O$, the MB^+ cation is hydrated similarly through the O1W…N10 hydrogen bond (Table 2).
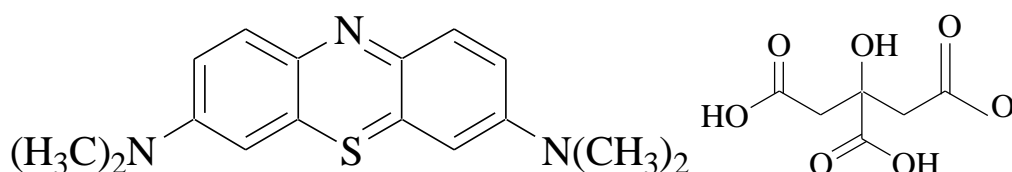

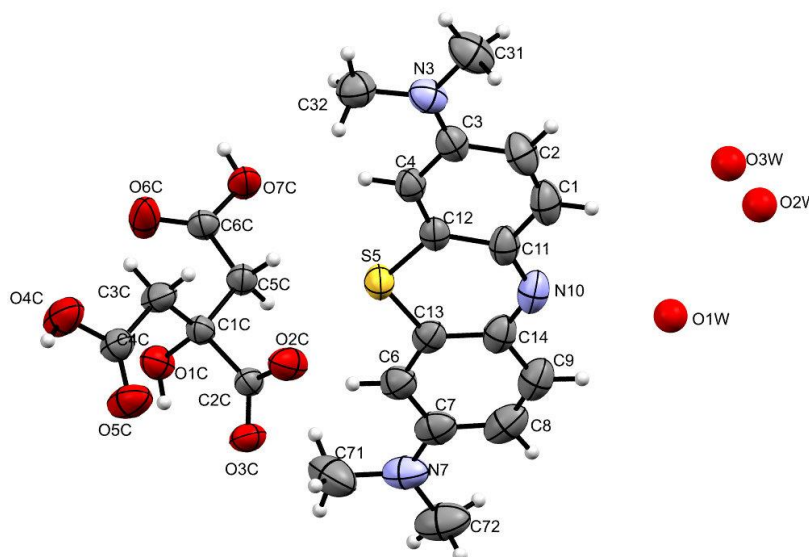Figure 1. Chemical formula of Mb citrate

Figure 2. Molecular structure and atom-numbering scheme for **1**. Displacement ellipsoids are drawn at the 50% probability level.
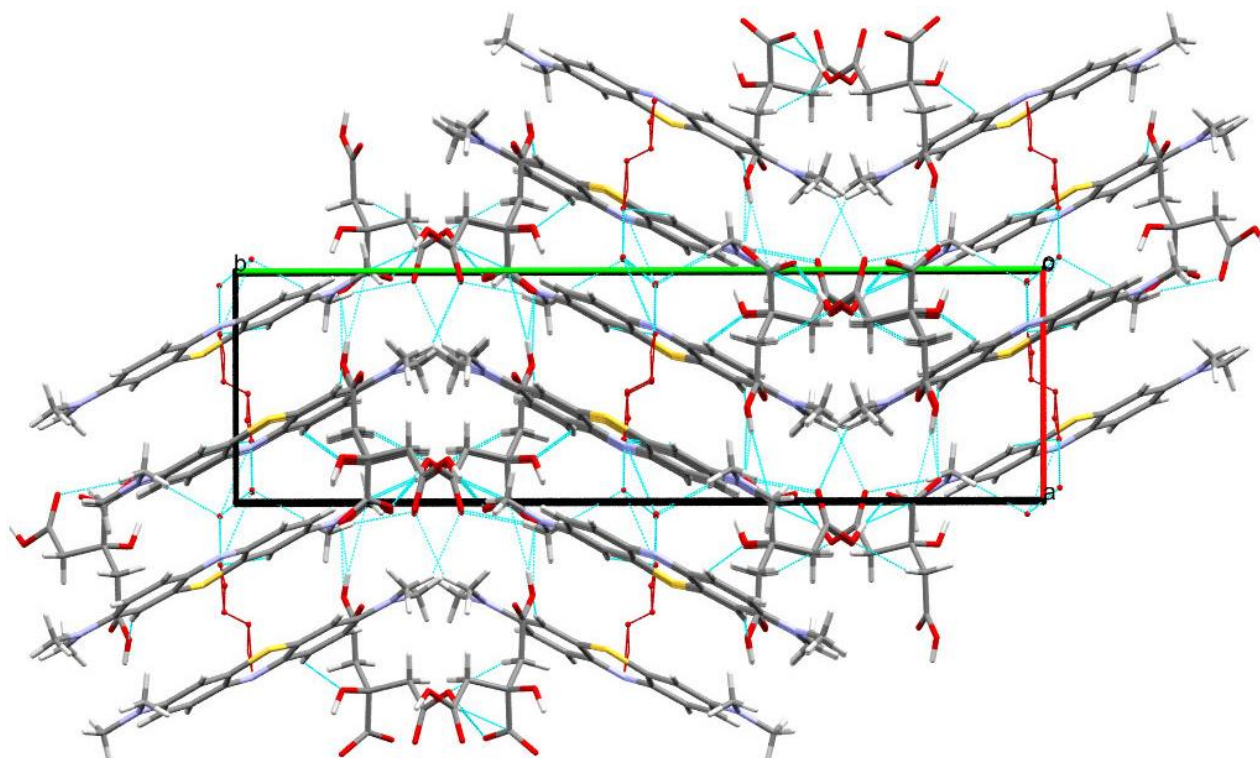
Figure 3. A view of a packing diagram for **1** showing arrangement of the zigzag chains of MB[+] cations. Short contacts and hydrogen bonds are shown as dashed lines.

Table 1. Hydrogen bonds and the short contacts

H bonds	D-H, Å	A…H, Å	D…A, Å	∠D-H…A, °	Symmetry
C1-H1…O1C	0.93	2.45	3.127(8)	129	x, y, z-1
C4-H4…O7C	0.93	2.47	3.378(8)	165	x, y, z
C6-H6 …O2W	0.93	2.58	3.40(3)	148	x, y, 1+z
O1C-H1C…O3C	0.82	2.07	2.576(6)	119	x,y,z
O4C-H4C…O2C	0.82	1.75	2.559(8)	169	x, -y+1/2, z+1/2
O7C-H7C…O3C	0.82	1.77	2.569(6)	162	x+1, y, z
C3C-H3CB…O6C	0.97	2.36	3.031(8)	126	x,y,z
C5C-H5CB…O4C	0.97	2.51	3.405(9)	154	x, -y+1/2, z-1/2
C71-H71B…O1W	0.96	2.32	3.282(7)	175	-x, -y+1, -z+1
C71-H71C…O5C	0.96	2.54	3.495(8)	171	-x, -y+1, -z+2
C32-H32A…O5C	0.96	2.50	3.296(8)	140	x+1, -y+1/2, z-1/2
C5C-H5CB…S5	0.97	3.043	3.847(4)	141	x, y, z
O1W…N10[*]			2.92(5)		x, y, z
O1W...O2W[*]			2.96(7)		x,y,z
O2W…O1W[*]			2.89(6)		-x, 1-y, -z
O2W…O3W[*]			2.73(6)		1-x, 1-y, -z
S5…O2W**			3.34(3)		x, y, z

[*]Hydrogen bonds with disordered water molecules where the positions of H atoms are not defined.

**Secondary weak interaction C-S…O.

The water O2W atomforms the short contacts:O2W...O1W(-x, 1-y, -z) (2.96(7) Å), C5-H5CB (x, y, -1+z)…O2W (2.868 Å) and H6-C6 (x, y, -1+z)…O2W (H…O 2.58 Å)which correspond to hydrogen bonds. The water atom O3Whas only one closest contact: O3W…O2W (1-x, 1-y, -z) (2.73 Å) (Table 1).

There is a short contactS(5)…O2Wwhich may be explained as a specific weak interaction of the anti-bonding orbital of the C13-S5 bond of the phenothiazine ring with the O2W atom. The O2W atom is located at the distance 3.34 Å from the S(10) atom and almost on the continuation of the C(13)-S(5) bond: the ∠C-S…O angle is 156.6(7)°. Interatomic S…O distanceis comparable with the sum of the van-der-Waals radii of the S and O atoms (3.32 Å).

In citrate anion, an α-carboxylate group is deprotonated and both C-O bonds are nearly aligned (1.248(4) and 1.243(4) Å) and the O-C-O angle is enlarged, while for both remaining COOH-groups that are 122.1(4) and 122.9(3)°. There is an intramolecular short contact O1C-H…O3C[H…O 2.07 Å, O1…O32.67 Å, ∠O1-H…O3 is 118.9°) between an α-OH and deprotonated α-COO group which has, most likely,ionic character. In both beta-carboxyl groups, the C-OH and C=O bonds are mutually distinct: both 1.318(4) Å and 1.185(5)/1.200(5) Å, respectively.

The C3C, C1C and C5C atoms form the skeletonof the citrate anion. The rotational state of C3C–C1C and C1C–C5C bonds, referred to as "centralbonds", are characterized by two dihedral angles, $φ1=C4C – C3C – C1C – C5C = 179.4(6)°$and $φ2 = C3C – C1C – C5C – C6C = -58.2(2)°$. The values of the torsion angles correspond to *trans*and*gauche* conformations of the skeleton fragment(Table 2). The different values of the torsion angles, presented in Table 1, show that terminal carboxyl and carboxylate groups have widely conformational flexibilities.
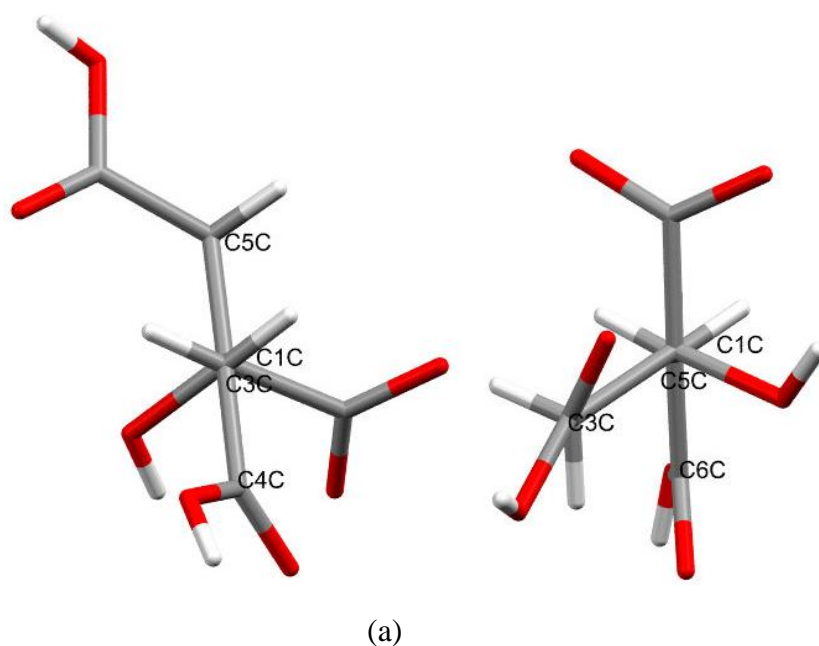

(a) (b)

Figure 4. Representation of the *trans* (a) – and *gauge* (b) - conformationsof the citrate anion around the C3C – C1C central bonds

The C3C – C4C and C5C –C6C bonds (1.499(5) and 1.515(5) Å) attached to β-COOHgroup and C1C –C2C (1.554(5) Å) bond attached to α-COO⁻group are distinguished. The C-C bondlengths around the central carbon atom C1C also are differentiated: 1.524(5) and 1.531(5) Å for the C-C bonds linked to the β-COOH groups and 1.554(5) Å for the C-C bond linked to the deprotonated α-COO⁻ group.
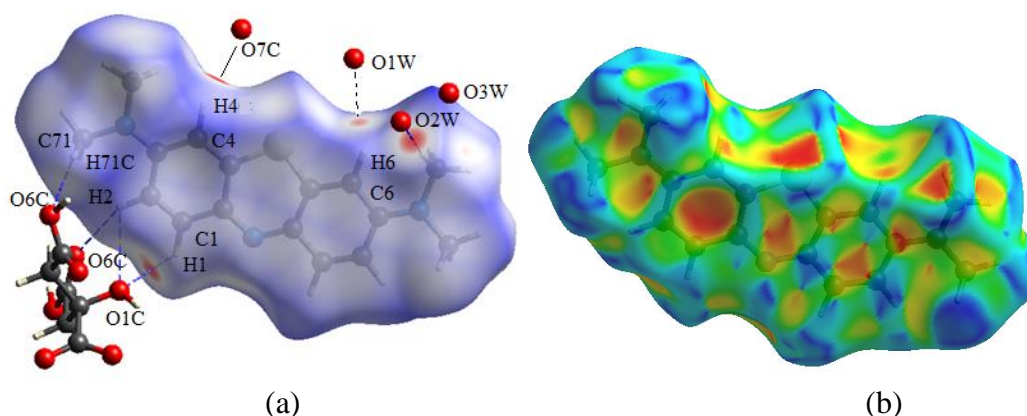
Hirshfeld surfaces

The three-dimensional Hirshfeld surfaces generated for structure (**1**) are presented in Fig. 3 and percentage contributions of each interaction to the Hirshfeld surface are given in Table 3. They are used to quantify and visualize the closed intermolecular atomic contacts responsible for the hydrogen bonds and the specific non-bonded interactions in the crystal (**1**). Intermolecular contacts closer than the sum of their van der Waals radii are highlighted in red on the d_{norm} surface, contacts with a distance greater than the sum of the van der Waals radii are highlighted in blue and contacts around the sum of van der Waals radii are white.

Table 2. Selected torsion angles (°) in the citric anion of compound (1) and citric acid monohydrate [22]

| Torsion angle	Given work	Citric acid anhydrate	Citric acid monohydrate	Trisodium citrate hydrate	Citrate nicotinamide	K$_4$Zn(H$_4$Cit-H)
O6C=C6C-C5C-C1C	-0.8(6)	8.24	116.8(5)	28.5	23.0(1)	174.7(2)
O5C=C4C-C3C-C1C	29.2(6)	50.42	48.2(4)	140.2	-95.4(1)	33.0(3)
C3C-C1C-C5C-C6C	-58.2(3)	-174.7	-174.1	159.4	146.3(1)	-172.5(2)
C4C-C3C-C1C-C5C	179.4(6)	171.1	174.1	-55.6	75.6(1)	179.9(2)
C4C-C3C-C1C-O1C	59.4(6)	50.7	62.0	-68.4	163.6(2)	58.9(2)
C2C-C1C-C3C-C4C	-60.3(4)	-68.7	-56.1	174.1	176.3(2)	-59.8(2)
C3C-C1C-C2C-O2C	-54.7(3)	129.6	120.0	-77.1	66.3(1)	-48.0(2)
O1C-C1C-O2C-O3C	8.4(3)	12.3	3.3	3.8	-28.4(2)	
C2C–C1C– C5C–C6C	178.6(4)	64.5	-56.2	57.4	57.6(2)	
C4C–C3C–C1C–C5C	179.4(6)	171.1	-174.2	176.9	-76.6(2)	

(a)   (b)

(c)   (d)
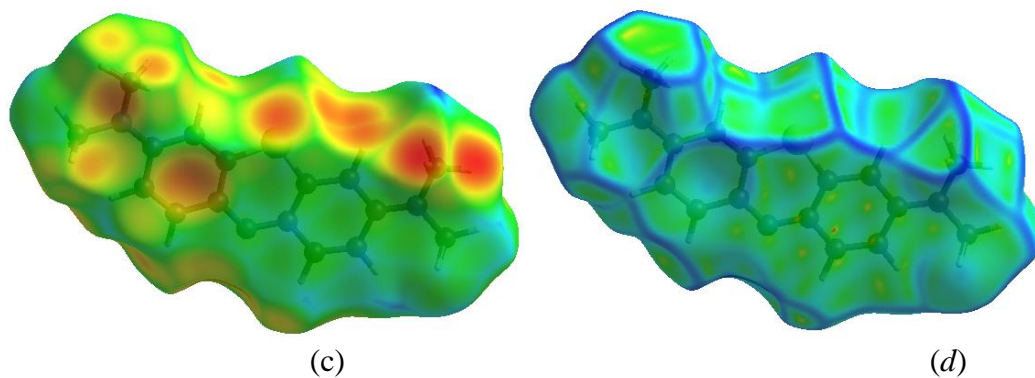
Figure 3. View of the three-dimensional Hirshfeld surface of the title compound mapped over (*a*) d_{norm}, (*b*) shape index, (*c*) d_e (*c*) and curvedness (*d*).
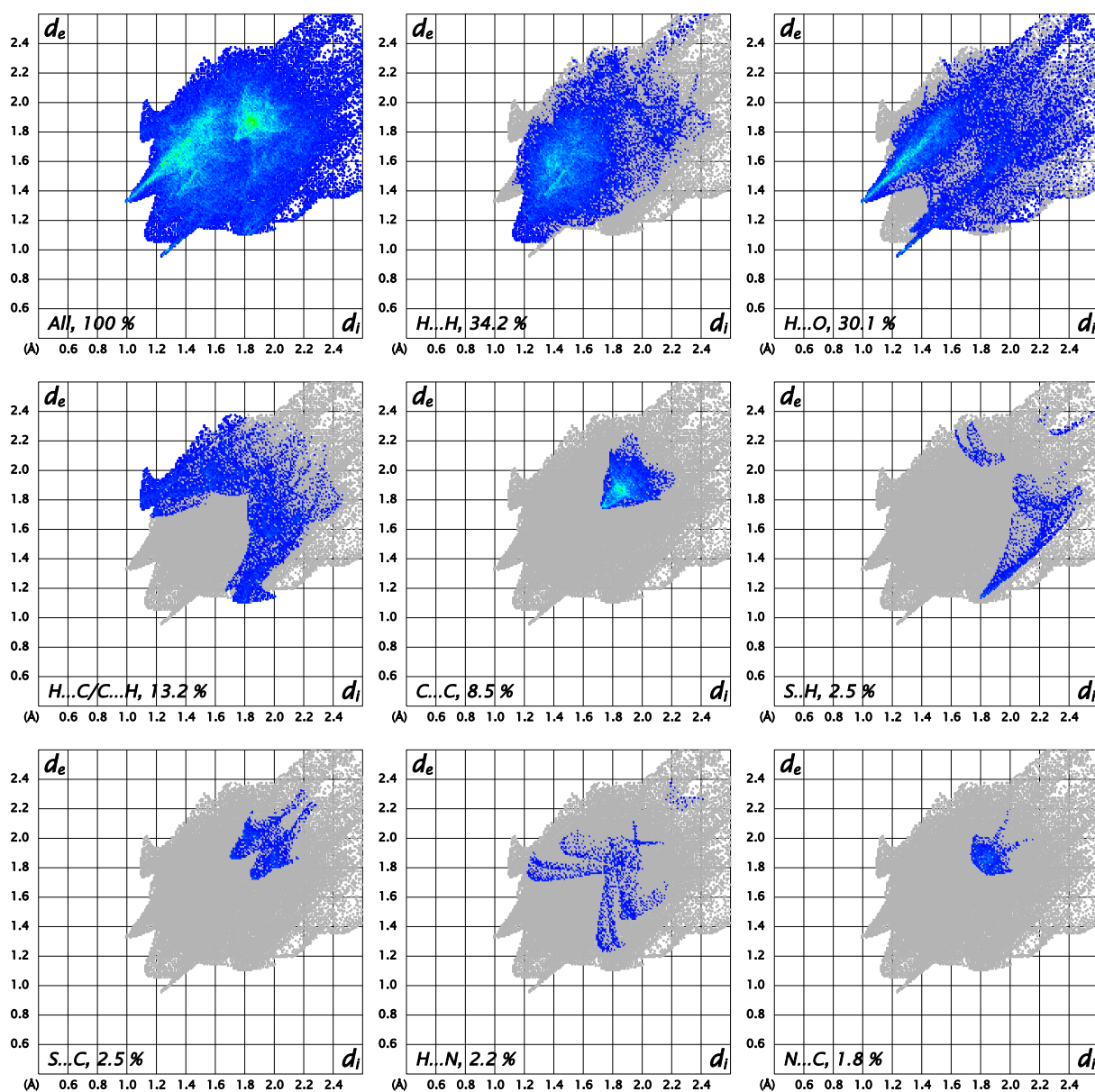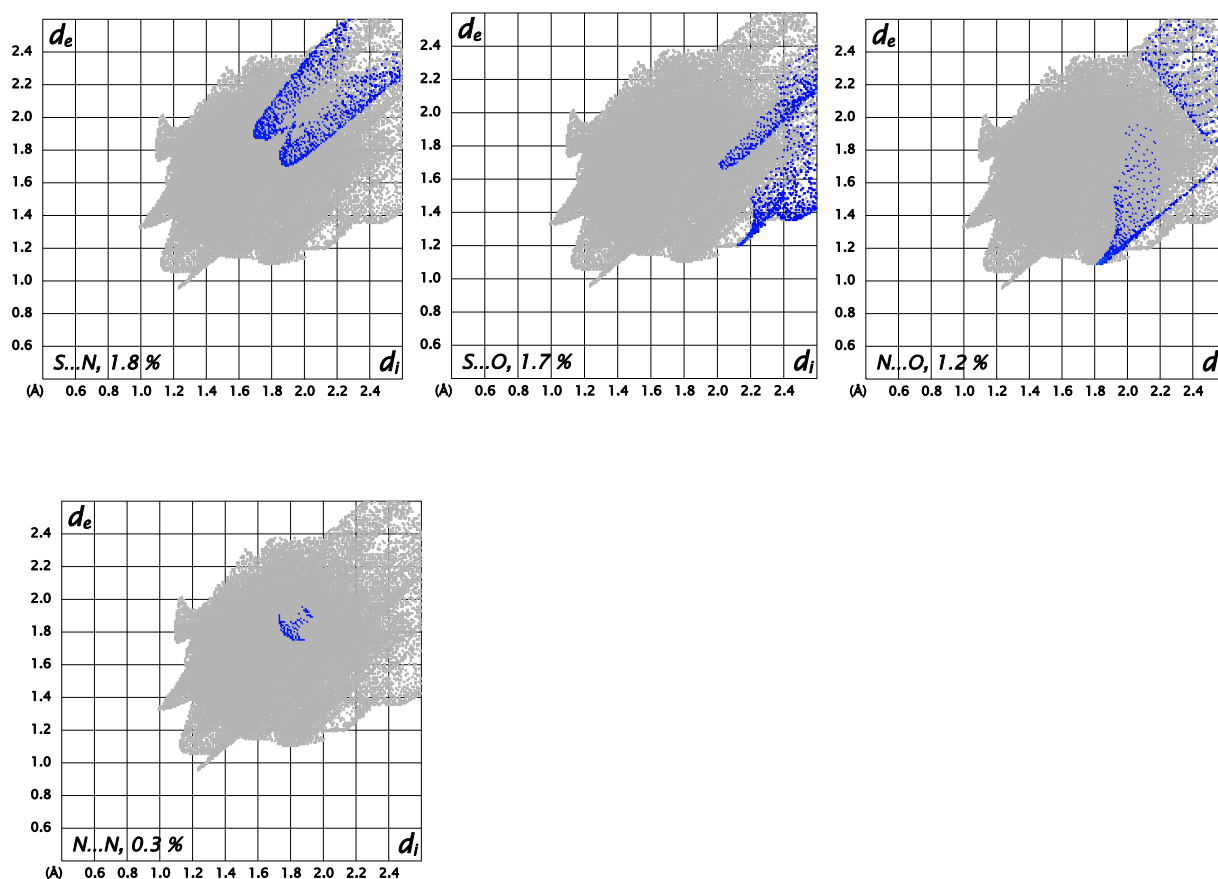
Figure 3. Two dimensional fingerprint plots for 1 with the contributions of a single interaction to the total intermolecular interaction
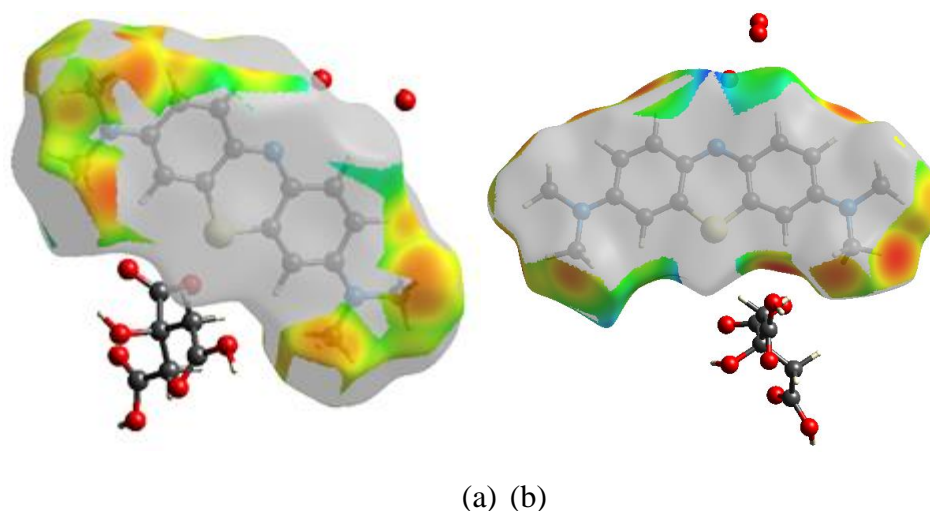

(a) (b)

Figure 4. The Hirshfeld surface mapped on d_i show the groups which takes place in the H…H (a)and H..O (b) contacts of MB$^+$ cation.

On the d_{norm} surfaces of the crystals **1,** white and blue spots predominate.On the shape-index, convex blue regions represent hydrogen-donor groups and concave red regions represent hydrogen-acceptor groups.

The Hirshfeld surface analysis also shown that π…π the stacking interactions between an adjacent MB$^+$ cations exist, as can be inferred from inspection of the adjacent red and blue triangles (highlighted by yellow circles) on the shape-index surface. Indeed,

the pattern of red and blue triangles in the same region of the shape-index surface is characteristic of π-π stacking interactions; the blue triangles represent convex regions resulting from the presence of ring carbon atoms of the molecule inside the surface, while the red triangles represent concave regions caused by carbon atoms of the π-π stacked molecule above. The relatively bright red spots on d_e map clearly displays the atoms of the MB$^+$cation taking participation on the short contacts. The shape of the blue outline on the curvedness surface unambiguously delineates the contacting patches of the molecules.

Two dimensional fingerprint analysis for 1

Table 2 shows that in given structure, the leading interactions can be grouped in two classes: hydrogen bonds and π–π stacking. The first involves MB$^+$ as a donor by means of three aromatic C–H…O bonds (Table 2), and as an acceptor by means of the central N(10) atom, whose σ lone pair pointing out of the molecule is readily exploited by a water molecule to form a strong hydrogen bond [N···O distance = 2.92 (5) Å]. Hirshfeld surface analyses have shown that weak interactions of the H…H and H …O type make the main contribution to the interatomic interactions of the MB$^+$ cation. Others interactions are significantly inferior to them. However, the calculated interatomic distances show, that all C–H…H–C contacts with the distance of H…H (≤2.4 Å) are intramolecular contacts. The C–H…O contacts between MB$^+$ cation and citrate anion may be considered as the intermolecular hydrogen bonds of MB$^+$ cation. The contribution of these contacts to the total intermolecular bonds is 30.1%.

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3. Experimental section

3.1. Procedure

MBCl·5H$_2$O (409.85 mg, 1.0 mmol) ("Pharmaceuticals Ltd", India) and Na(H$_3$Cit).2H$_2$O (294,10 mg) (Sigma-Aldrich) were dissolved in 5.0 mL of the hydroalcoalcohol solute (in the ratio 1:1) with stirring at magnetic stirrer about 1.0 h at a temperature of ≈60°C. Crystals of (MB)$^+$(H$_3$Cit)$^-$ were obtained by slow evaporation of the reaction solution at a room temperature.

No disordered positions have been fully occupied. These position have been determined in the latest stages of the refinement of structure. The populations of the disordered positions vary in the range 0.06-0.07. Inclusion of these residual electron density in the refenenent of structure lowered the R-factor of refinement from R=0.095 until 0.078. During the refinement of the structure, these positions are clearly

manifested on the different electron maps. Additionally, these disordered positions lined up in a zigzag chain, which is similar to that in the structure of MB pentachloride. The disordered and partially occupied positions of the water molecule form chains passing near the N(11) and S(5) atoms of the column formed by the MB$^+$ cations (Fig. 2). These contacts of water molecules are not at all unexpected, it was expected that they form hydrogen bonds with the O atoms of citric acid, and there are as many as seven of them in the acid anion.

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