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MOLECULAR CHARACTERISATION AND DISSOLUTION PROFILE OF SOLID FORMULATIONS OF METFORMIN

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ABSTRACT

In the present study, three novel multi-component salts of the anti-diabetic drug metformin, one with anti-inflammatory drug diclofenac and two with pharmaceutically active co-crystal formers i.e., succinic acid and fumaric acid have been synthesized. The new solid phases have been preliminarily characterized by differential scanning calorimetry (DSC) and FT-IR analyses. The Single Crystal X-ray Diffraction (SCXRD) reveals the proton transfer from carboxylic acid group of co-formers to guanidine moiety metformin confirming the formation of new molecular salts. The molecular packing of all the salts is mainly supported by N–H···O– and N–H···N and hydrogen bonding interactions. The diclofenac salt of metformin interestingly shows a dissolution profile in a sustained release manner.

KEYWORDS

Molecular salts; metformin; diclofenac; single crystal X-ray analysis; dissolution studies

1. INTRODUCTION

In the pharmaceutical industry salt formation is a widely used method to modulate the physico-chemical properties of active pharmaceutical ingredients (APIs) [1,2]. An active pharmaceutical salt is a combination of an API with the GRAS (generally regarded as safe by US FDA) listed co-former [3]. In recent years salts or co-crystals formed by two APIs or drugs are of great interest to the crystal engineering community [4-7].

Metformin (MET) is an oral antidiabetic drug in the biguanide class. MET is the first-line drug of choice for the treatment of type 2 diabetes (formerly known as noninsulin- dependent diabetes mellitus (NIDDM) or adult-onset diabetes) [8,9]. MET also increasingly being used polycystic ovary syndrome (PCOS) non-alcoholic fatty liver disease (NAFLD) and premature puberty [10-12]. In recent years MET has drawn great interest from crystal engineers, due to its tendency to from robust supramolecular architectures with compounds having both phenolic and carboxylic acid functional groups. Thermodynamic properties of metformin salicylate and fluorescence properties of metformin-mangostin molecular salt have been reported. Diclofenac (DF) is a potent, non-steroidal, anti-inflammatory drug [13,14]. Co-crystals of DF with isonicotinamide and co-crystals of DF with various amino pyridines [15,16]. Here in we report the synthesis, SXRD, FT-IR and DSC analyses of new solid forms of MET with DF, SUC and FA. Our endeavours in the present study are syntheses of one drug-drug molecular salt of MET with DF, and two other molecular salts of MET with the GRAS listed compounds succinic acid (SUC) and fumaric acid (FA). The drug-drug combination of MET and DF resulted in a synergistic pharmaceutically active new solid form [17]. The molecular structures of MET, DF, SUC and FA are shown in Figure 1.

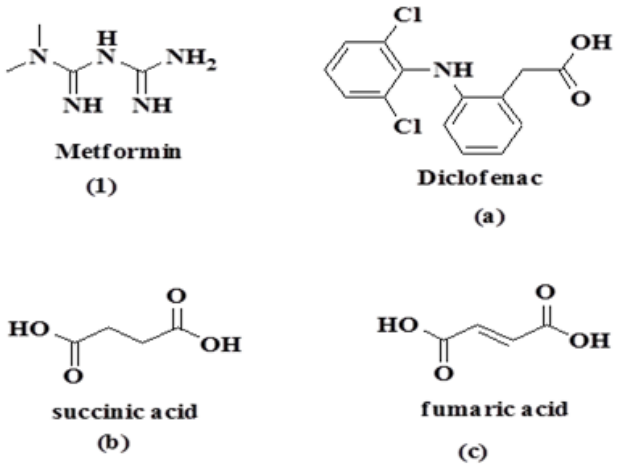

Figure 1: Molecular structures of Metformin (1), Diclofenac (a), Succinic acid (b) and Fumaric acid (c)

Such type of salts would result in entities have synergistic or additive pharmacological activity. Since Metformin is very high soluble in water, the above mentioned salts have been prepared to modulate the solubility.

2. METHODS

2.1 Materials

Metformin hydrochloride with 98% purity and diclofenac with 98% purity were purchased from Merck, India. Succinic acid with 98% purity and fumaric acid with 98% purity were purchased from Spectrochem Pvt. Ltd., India. All the solvents with HPLC grade purity were procured from Merck chemicals, India. The solvents were used without further purification.

2.2 Metformin free base synthesis

Metformin hydrochloride (5g, 0.0303 mol) and sodium hydroxide (1.2g, 0.0303 mol) were dissolved in 2-propanol and stirred for 3h at 50 °C. After completion of the reaction, the resulting solution was filtered and the solvent was removed under reduced pressure to obtain metformin free base as a white solid [18,19].

2.3 Molecular salts synthesis

MET-DF(1a): A mixture of MET (129 mg, 1 mmol) and DF (296 mg, 1 mmol) was dissolved in 15 mL of methanol by heating at 60° C for 10 minutes and the resulting clear solution was kept for crystallization at room temperature. Suitable square block crystals were appeared at ambient conditions after 3-4 days.

MET-SUC(1b): A mixture of MET (129 mg, 1 mmol) and SUC (118 mg, 1 mmol) was dissolved in 15 mL of methanol by heating at 60° C for 10 minutes and the resulting clear solution was kept for crystallization at room temperature. Suitable small square block crystals were appeared at ambient conditions after 2-3 days.

MET-FA(1c): A mixture of MET (129 mg, 1 mmol) and FA (116 mg, 1 mmol) was dissolved in 15 mL of methanol by heating at 60° C for 10 minutes and the resulting clear solution was kept for crystallization at room temperature. Suitable rectangular block crystals were appeared at ambient conditions after 2-3 days.

2.4 Infrared spectroscopy (FTIR)

A Bruker Alpha-T Fourier transform infrared spectrophotometer in the spectral range 4000 to 600 cm−1 with resolution of 2cm-1 was used to record the infrared spectra of the starting materials and the molecular salts 1a, 1b and 1c with the KBr pellet making technique.

2.5 Differential scanning calorimetry (DSC)

Thermal properties of the starting materials and the molecular salts 1a, 1b and 1c were determined by differential scanning calorimeter (DSC 200 F3, Netzsch, Germany). Samples equivalent to 5 mg were placed in aluminium crucibles sealed with lid and DSC analyses were carried out at a nitrogen flow of 50 mL/min and a heating rate of 10°C/min in the heating range of 30 to 250°C.

2.6 Single crystal X-ray diffraction (SCXRD)

Single-crystal X-ray diffraction data (Table 1) were collected at 293 (2) K on Bruker axs kappa apex2 CCD Diffractometer using a single wavelength Enhance X-ray source with Mo Kα radiation (λ = 0.71073 Å) . Data reduction was performed with the SAINT program and the structure was solved using the SIR92 [20]. The structure refinements were performed by full-matrix least-squares on F2 with SHELXL97 [21]. PLATON was used to check the result of the X-ray analysis [22]. All programs used during the crystal structure determination process are included in the WINGX software [23].Structure invariant direct methods were used for primary atom site locations and the secondary atom site locations were found from the Fourier map. All the H positions bound to C atoms were calculated after each cycle of refinement using a riding model C–H = 0.93 Å and Uiso(H) = 1.2Ueq(C). All the H atoms bound to N and O atoms were located in difference Fourier maps and freely refined.

Complete structural details in cif format for structures 1a, 1band 1c were deposited with the Cambridge Crystallographic Data Centre as CCDC 950401, CCDC 881966 and CCDC 950402 respectively. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Table 1: Crystallographic data for metformin salts

	MET-DF	MET-SA	MET-FA
Formula	$C_{18}H_{22}C_{12}N_6O_2$	$C_6H_{14}N_5O_2$	$C_6H_{13}N_5O_2$
Molecular weight	425.32	188.22	186.21
Crystal system	Monoclinic	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/n$	$P2_1/n$
a/Å	8.8750(2)	9.551(5)	9.615(4)
b/Å	10.1742(2)	8.821(5)	8.722(3)
c/Å	22.3978(6)	10.851(5)	10.818(4)
α/(°)	90	90	90
β/(°)	94.170(2)	92.481(5)	90.97(2)
γ/(°)	90	90	90
Volume/Å^3	2017.08(8)	913.3(8)	907.1(6)
Z	4	4	4
D_x/ g cm^{-3}	1.402	1.369	1.364
F(000)	888.0	404.0	396
μ(Mo-Kα)/mm^{-1}	0.349	0.105	0.106
Crystal size/ mm	0.35 × 0.35 × 0.30	0.20 × 0.20 × 0.15	0.40 × 0.30 × 0.20
Temp. (K)	293(2)	293(2)	296(2)
θ range for data collection	2.20–25.00	2.78–24.99	2.81–28.45
R_1	0.0371	0.0323	0.0630
wR_2	0.0996	0.0882	0.1671
Goodness-of-fit	1.044	1.052	1.075
Parameters	282	153	144
Reflections collected	17307	7809	6784
Unique reflections	3547	1618	2264
CCDC No.	950401	881966	950402

2.7 Dissolution studies

2.7.1 In-vitro release studies

The metformin salts prepared by complexation method with 1:1 ratio of conformers Viz., succinic acid, fumaric acid and diclofenac were filled in capsules and the percentage of drug release obtained was compared with pure drug (Metformin HCl) at the end of fourth hour to estimate the release effect of drug from the complexes. The capsules were placed into the basket of USP type I apparatus (Electro lab, TDT 08L) and dipped into the dissolution chamber containing 900 mL of water as dissolution media with the bath temperature of 37 ±0.5ºC. The study was carried at 100 rpm and the samples of 10 mL were withdrawn using a syringe at predetermined time intervals such as 5, 10, 15, 30, 45, 60, 90, 120, 150, 180 and 240 mins). The collected samples were analyzed using UV-Vis Spectrophotometry (EL150, Electrolab) at 254 nm and the rate of dissolution was calculated using standard calibration curve method. The data was fitted with kinetic models such as zero order and Higuchi to understand the mechanism of drug release.

3. RESULTS AND DISCUSSION

Salts can be distinguished from the co-crystals by the location of the proton between an acid and a base. The proton transfer can be evaluated from their SCXRD and FT- IR spectroscopic analysis. Salt formation was revealed from the single-crystal SCXRD analysis of the molecular salts 1a, 1b and 1c where the transferred hydrogen was located from a difference Fourier map [24].

3.1 Single crystal X-ray analysis

3.1.1 Description of the crystal structure of 1a

The crystal structure of salt 1a was solved in monoclinic space group P21/c. The asymmetric unit of salt 1a consists of one MET cation and one DF anion, resulting in a 1:1 salt of MET and DF. The atom numbering schemes for (1a) (Figure 2).

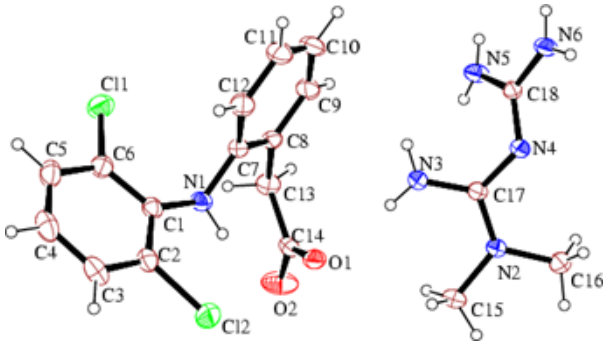

Figure 2: Molecular structure of (1a) with the atom-labelling scheme. Displacement ellipsoids are shown at the 30% probability level

Layered type supramolecular architectures are formed by tetramers in which the molecules are connected by N (5)– H(5A) ·O(2) and N(5) –H(5B) ·O(2) hydrogen bonding interactions. Each tetramer is interacting with four tetramers by N–HN and N–HO– hydrogen bonding interactions. In the molecular salt, two MET molecules form N–H ·N dimer of R^2_2[8] homosynthon and MET and DF molecules also form N–HO– dimer of R^2 [8] heterosynthon. The overall crystal packing resulted in extended layer type supramolecular arrangement is shown in Figure 3.

The metformin cation can exist in three tautomeric structures a, b, and c, all of them fully delocalized. The X- ray structure of 1a corresponds to tautomer c. All reported molecular salts of MET also belong to tautomer c [25-28].

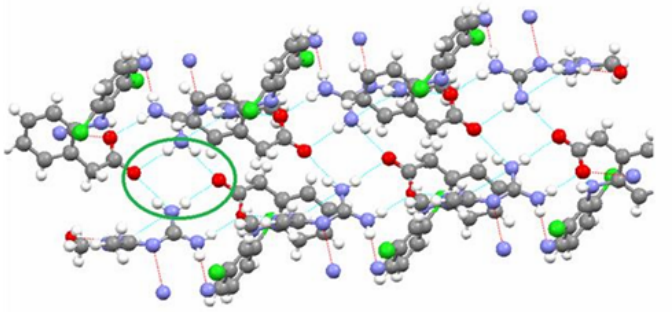

Figure 3: Crystal packing of the molecular salt (1a)

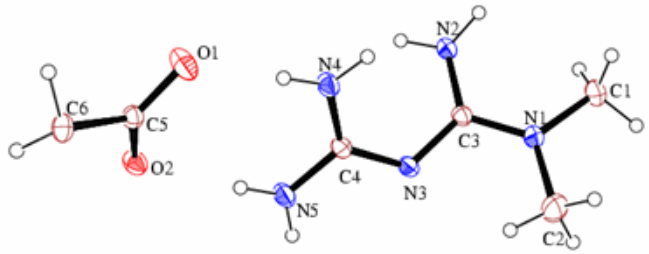

Figure 4: Molecular structure of (1b) with the atom-labelling scheme. Displacement ellipsoids are shown at the 30% probability level

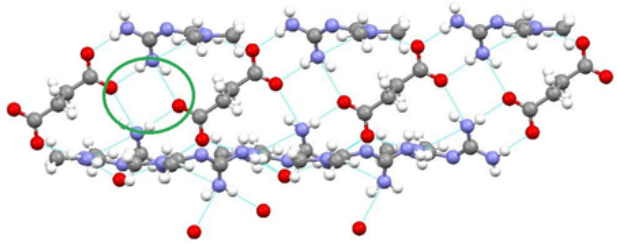

Figure 5: Crystal packing of the molecular salt (1b)

3.1.2 Description of the crystal structure of 1b

The asymmetric unit in the monoclinic space group P21/n consists of one MET cation and one-half SUC anion, resulting in a 1:0.5 salt of MET and SUC. The atom numbering schemes for (1b) (Figure 4).

Layered type supramolecular architectures are formed by tetramers in which the molecules are connected by N (4)– H(4A)·O(1) and N(4)–H(4B O(1) hydrogen bonding interactions (Figure 5).

Each tetramer is interacting with four tetramers by N– H ·N and N –H·O – hydrogen bonding interactions. In the molecular salt, two MET molecules form N–H·N dimer of R^2_2 [8] homosynthon and MET and SUC molecules also form N–H·O – dimer of R^2_2 [8] heterosynthon. MET molecule in the X-ray structure of 1b corresponds to tautomer c.

3.1.3 Description of the crystal structure of 1c

The crystal structure of salt 1c was solved in monoclinic space group P21/c. The asymmetric unit of salt 1c consists of one MET cation and one-half FA anion, resulting in a 1:0.5 salt of MET and FA. The atom numbering schemes for (1c) (Figure 6).

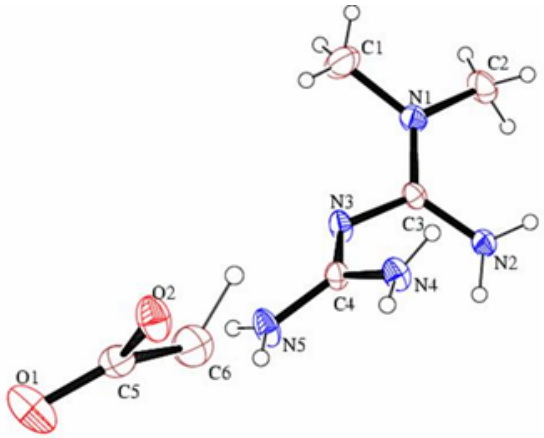

Figure 6: Molecular structure of (1b) with the atom-labelling scheme. Displacement ellipsoids are shown at the 30% probability level

Layered type supramolecular architectures are formed by tetramers (Figure 7.) in which the molecules are connected by N(4)–H(3)·O(1) and N(4) –H(4)·O(1) hydrogen bonding interactions. Each tetramer is interacting with four tetramers by N–H ·N and N –H ·O – hydrogen bonding interactions. The detailed geometrical parameters of the hydrogen bonding are listed in Table 2.

Table 2: The hydrogen bonding geometrical parameters for the metformin molecular salts (1a) (1b) and (1c)

D–H···A	*d*(D–H)/Å	*d*(H···A)/Å	*d*(D···A)/Å	? (D–H···A)/°	Symmetry transformations
MET-DF[1]					
C(15)–H(15A)···Cl(1)	0.96	2.80	3.7522(2)	172.5	x,–y+1/2,z+1/2
C(15)–H(15B)···O(1)	0.96	2.65	3.213(3)	118.3	intramolecular
C(15)–H(15C)···N(2)	0.96	2.69	3.546(3)	148.4	–x+1,–y+1,–z+2
C(16)–H(16B)···Cl(2)	0.96	2.91	3.490(2)	119.9	–x+1,–y+1,–z+2
N(1)–H(1A)···O(1)	0.848(16)	2.31(2)	2.979(2)	136(2)	intramolecular
N(1)–H(1A)···Cl(2)	0.848(16)	2.48(2)	2.963(2)	116.6(19)	intramolecular
O(8)–H(8)···O(5)	0.82	1.79	2.5232(19)	148.4	intramolecular
N(5)–H(5B)···O(2)	0.834(15)	2.090(16)	2.921(3)	174(2)	x+1,y,z
N(5)–H(5A)···O(2)	0.856(15)	2.034(18)	2.832(2)	155(2)	–x+1,–y,–z+2
N(6)–H(6B)···O(1)	0.842(15)	2.058(15)	2.897(2)	174(2)	x+1,y,z
N(6)–H(6A)···N(4)	0.830(16)	2.254(16)	3.083(3)	176(2)	–x+2,–y+1,–z+2
N(3)–H(3B)···N(5)	0.825(15)	2.55(2)	2.944(3)	110.7(19)	intramolecular
N(3)–H(3A)···O(1)	0.833(15)	2.103(16)	2.931(2)	173(2)	intramolecular
MET-SA[2]					
N(2)–H(2D)···O(2)	0.886(13)	2.013(14)	2.896(2)	174.7(16)	x,y+1,z
N(2)–H(2E)···O(2)	0.879(13)	2.172(13)	3.0394(19)	168.8(15)	–x+1/2,y+1/2,–z+1/2
N(4)–H(4A)···O(1)	0.887(13)	2.051(15)	2.8405(18)	147.6(14)	–x,–y+2,–z
N(4)–H(4B)···O(1)	0.867(13)	2.052(13)	2.9099(19)	170.4(15)	intramolecular
N(5)–H(5A)···O(2)	0.878(13)	2.069(13)	2.9460(19)	176.8(15)	intramolecular
N(5)–H(5B)···N(3)	0.858(13)	2.232(14)	3.090(2)	177.1(15)	–x,–y+2,–z+1
MET-FA[3]					
N(2)–H(1)···O(2)	0.86(4)	2.11(4)	2.957(4)	172(4)	x–1/2,–y+3/2,z–1/2
N(5)–H(2)···O(2)	0.89(4)	2.08(4)	2.908(3)	180(5)	–x+1,–y+2,–z+2
N(4)–H(3)···O(1)	0.81(4)	2.11(4)	2.912(4)	169(3)	–x+1,–y+2,–z+2
N(4)–H(4)···O(1)	0.84(4)	2.13(4)	2.879(4)	148(4)	x,y–1,z
N(5)–H(5)···N(3)	0.82(4)	2.23(4)	3.044(3)	175(4)	–x+1,–y+1,–z+1
N(2)–H(6)···O(2)	0.85(4)	2.07(4)	2.913(3)	171(4)	–x+1,–y+1,–z+2
C(1)–H(8)···N(3)	0.86(4)	2.32(4)	2.712(5)	108(3)	intramolecular
C(6)–H(12)···N(2)	1.12(4)	2.61(4)	3.247(4)	116(2)	–x+1,–y+1,–z+2

In the molecular salt, two MET molecules form N–H·N dimer of R^2_2 [8] homosynthon and MET and FA molecules also form N–H·O – dimer of R^2_2 [8] heterosynthon. MET molecule in the X-ray structure of 1c adopts the structure of tautomer c.

3.2 FT-IR spectral analysis

FT-IR spectroscopy is widely used technique in the characterization and identification of different solid-state forms. The FT-IR spectra of (1a), (1b) and (1c) are shown in (Figure 8).

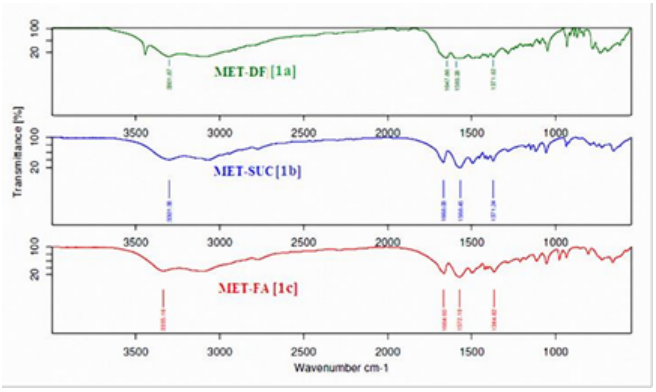

Figure 8: FT-IR spectra of the molecular salts (1a), (1b) and (1c) in spectral range of 400–4000 cm-1 using KBr pellet method

FT-IR spectra based on the vibrational modes of the compound and are extremely sensitive to structure, hydrogen bonding, molecular conformations, and environment of the API [29]. From the FT-IR spectra of 1a, the IR asymmetric stretching frequency of 1° N –H shifted in MET to 3301 cm–1 from 3371 cm^{-1} and N–C=N (*Asym Str*) IR band in MET appeared at 1647 cm^{-1} which was shifted from 1668 cm^{-1}. The carbonyl IR band of –COOH functional group in DF observed at 1697 cm–1 which was absent in salt 1a. The proton transfer from the –COOH functional group in DF is evidenced by the appearance of two carboxylate IR bands at 1589 ($O–C–OA_{sym}$) and 1371 cm^{-1} ($O–C–O_{Sym}$) [30]. From the FT-IR spectra of 1b, primary N–H (*Asym Str*) IR frequency in MET shifted from 3371 to 3301 cm^{-1} and also N–C=N (*Asym Str*) in MET shifted from 1633 to 1668 cm–1 indicating these moieties are interacting with SUC. The carbonyl (C=O) IR absorption frequency of carboxylic acid in SUC observed at 1695 cm^{-1} which was absent in salt 1b. Moreover the appearance of two characteristic carboxylate IR absorption vibrations at 1571 and 1370 cm^{-1} for 1b due to asymmetric and symmetric O–C–O stretch respectively confirmed the proton transfer from the –COOH group in SUC.

Similarly in salt 1c, primary N–H (*Asym Str*) frequency in MET shifted from 3371 to 3335 cm^{-1} and also N–C=N (*Asym Str*) in MET shifted from 1633 to 1664 cm^{-1} indicating these moieties are interacting with FA. The carbonyl (C=O) IR absorption frequency of carboxylic acid in FA observed at 1675 cm^{-1} which was absent in salt 1c. Moreover the appearance of two characteristic carboxylate IR absorption vibrations at 1572 and 1364 cm^{-1} for 1c due to asymmetric and symmetric O–C–O stretch respectively confirmed the proton transfer from the –COOH group in FA.

3.3 Thermophysical properties

The thermal stability and phase transition of drugs are important to study physicochemical and pharmacokinetic properties [31]. The DSC curves are shown in (Figure 9).

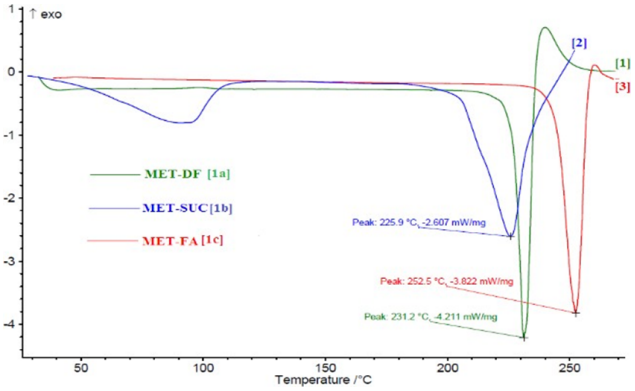

Figure 9: DSC thermo grams of the molecular salts (1a), (1b) and (1c) in the heating range of 30–250 0C with heating rate 10 0C/min

DSC of the salts 1a and 1c exhibited single endotherms at 231 °C and 252 °C respectively confirming thermal stability of the new solid phases. Where as in salt 1b a second endotherm was appeared before melting point, perhaps due to phase transition or decomposition. The melting points of MET, coformers and the new solid phases are listed in Table 3.

Table 3: Melting points of api (metformin), co- formers and molecular salts (1a, 1b, 1c)

	mp of API (°C)	mp of coformer (°C)	mp of salts (°C)
MET	110-114		
MET-DF [1a]		194-198	231
MET-SUC [1b]		182-184	225
MET-FA [1c]		284-286	252

The salts 1a and 1b have exhibited higher melting points than both the API and coformer; whereas the melting point of salt 1c was in between the API and the coformer.

3.4 Dissolution studies

The dissolution study data reveals that the Metformin crystals prepared with FA, SA, and the pure Metformin HCl show more than 95% dissolution with one hour whereas the crystals prepared with DF show the dissolution in sustained manner and attained only around 75% after 4th hour of dissolution study (Figure 10)..

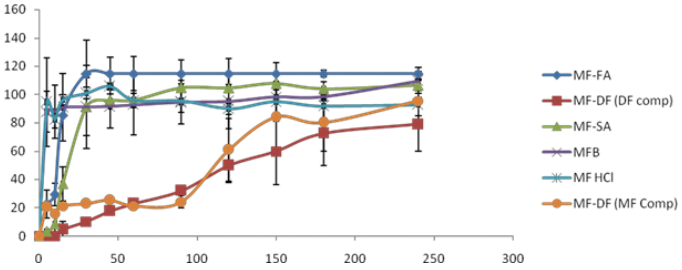

Figure 10: Intrinsic dissolution rate curves of Metformin salts in pure water

It confirms that the crystals prepared with DF provide sustained release of drug. The release kinetics of zero order and Higuchi models found the regression coefficient of (r2) 0.9781, 0.8587 for zero order and 0.8577, 0.8270 for Higuchi model of MF-DF (DF comp) and MF-DF (MF Comp) respectively (Figure 11, Figure 12, Figure 13, Fig 14), it confirms that it follows zero order that is independent of concentration of the drug.

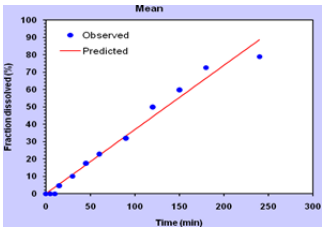

Figure 11: Zero order kinetic plot for MF-DF (1a) (DF comp)

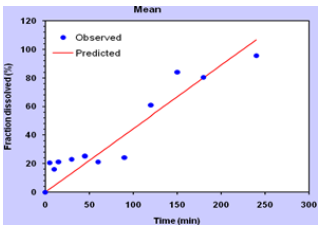

Figure 12: Zero order kinetic plot for MF-DF (1a) (MF comp)

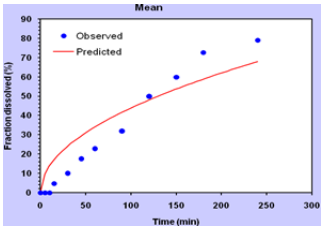

Figure 13: Higuchi kinetic plot for MF-DF (1a) (DF comp)

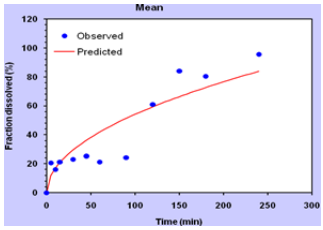

Figure 14: Higuchi kinetic plot for MF-DF (1a) (MF comp)

4. CONCLUSIONS

To summarize, we have reported the synthesis and X- ray crystal structure analysis of three supramolecular complexes of anti-diabetic drug MET with an anti- inflammatory drug diclofenac, succinic acid and fumaric acid respectively. The formation of salts were further characterized and confirmed by FT-IR and DSC analyses. The proton transfer from –COOH functional group in coformers to the guanidine

moiety in MET was evidenced by the appearance of two characteristic carboxylate IR bands in the FT-IR spectra. All the new solid forms exhibited higher thermal stability than the API. The supramolecular architectures are mainly stabilized by N–H···O– and N–H···N hydrogen bonding interactions which resulted in the formation R2 ring motifs.

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