Electronic Supporting Information

Multidrug Salt Forms of Norfloxacin with Non-Steroidal Anti-Inflammatory Drugs: Solubility and Membrane Permeability Studies

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Table S1 p^{Ka} Value of NOR (p^{Ka} = 8.75; piperazine fragment) and other NSAIDs, and the calculated Δp^{Ka} values.

Compound	DIC	DIF	MEF	IND
p^{Ka}	4.51	2.96	3.89	4.50
$\Delta p^{Ka} = p^{Ka(NOR)} - p^{Ka(Other\ API)}$	4.24	6.06	4.86	4.25

FTIR Spectra

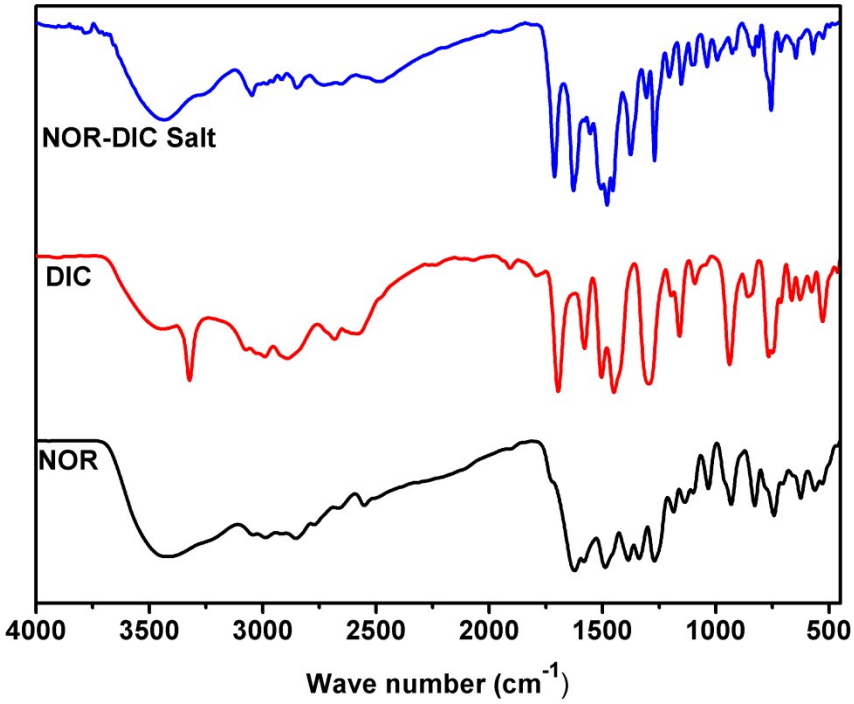


Fig. S1 Comparison of FTIR spectra of NOR, DIC and its NOR-DIC salt.

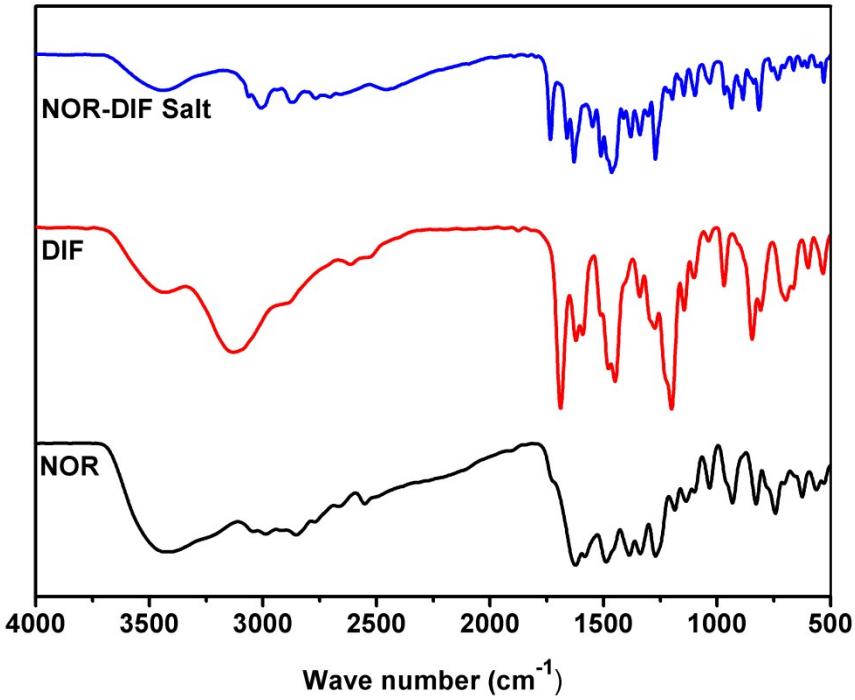


Fig. S2 Comparison of FTIR spectra of NOR, DIF and its NOR-DIF salt.

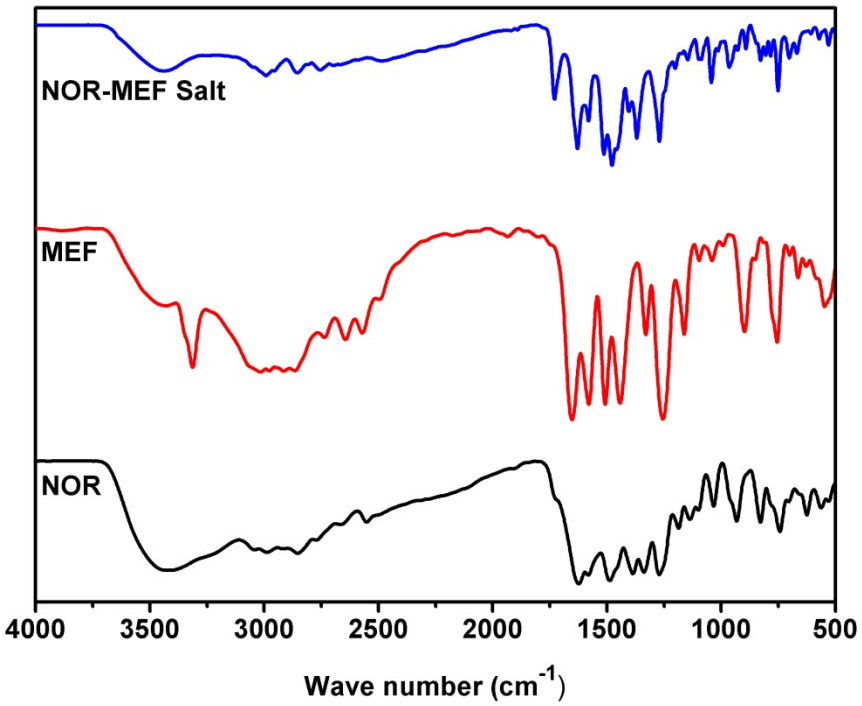


Fig. S3 Comparison of FTIR spectra of NOR, MEF and its NOR-MEF salt.

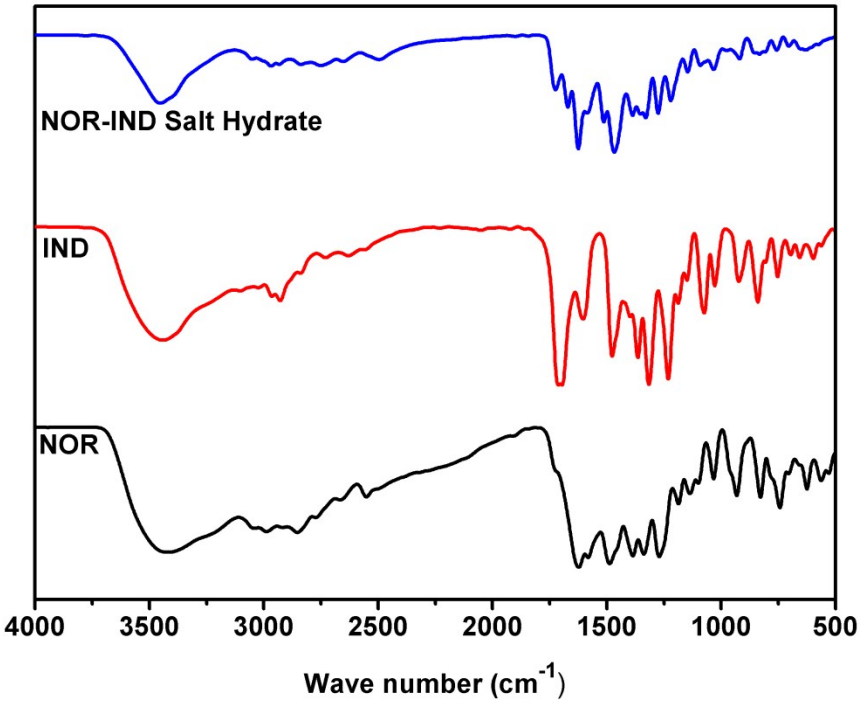


Fig. S4 Comparison of FTIR spectra of NOR, IND and its NOR-IND salt hydrate.

PXRD

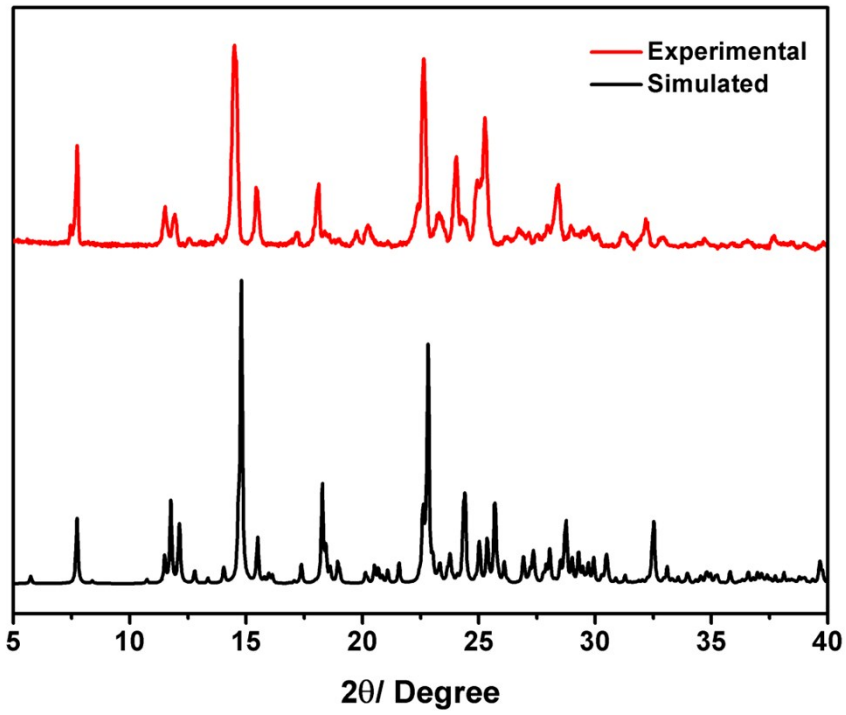


Fig. S5 PXRD comparison of simulated pattern and experimental pattern for NOR-DIC salt.

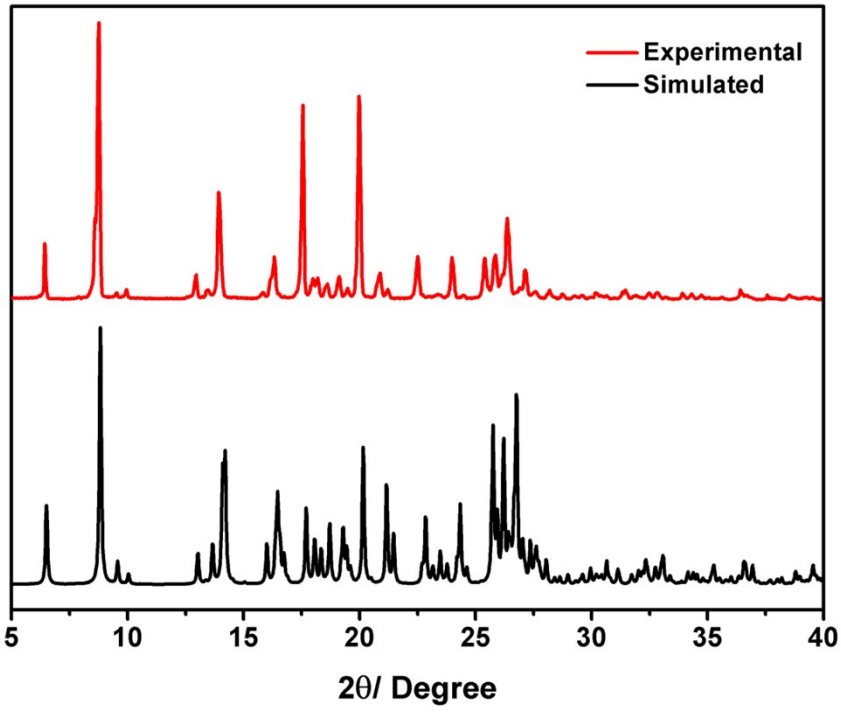


Fig. S6 PXRD comparison of simulated pattern and experimental pattern for NOR-DIF salt.

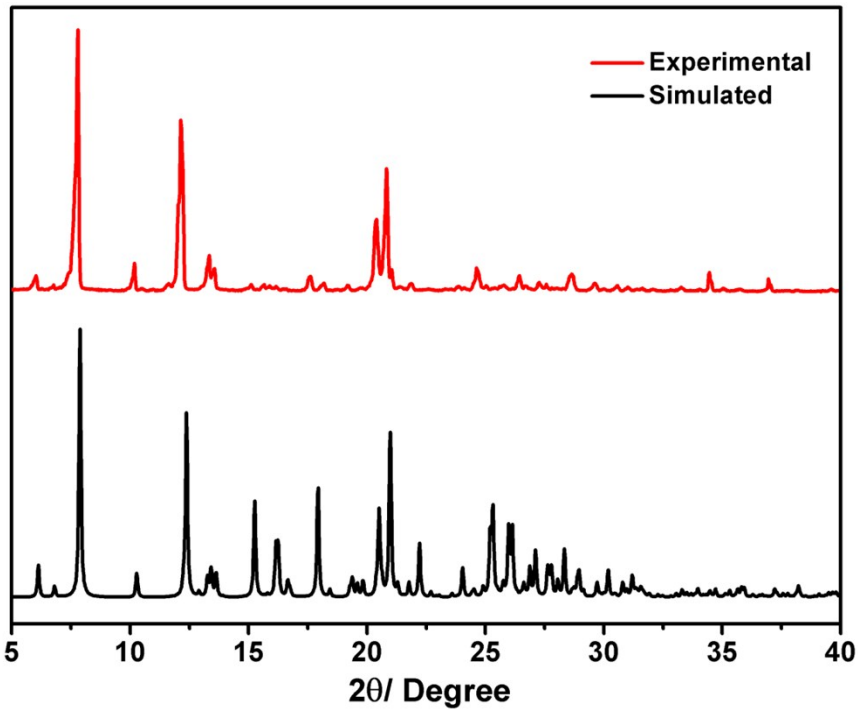


Fig. S7 PXRD comparison of simulated pattern and experimental pattern for NOR-MEF salt.

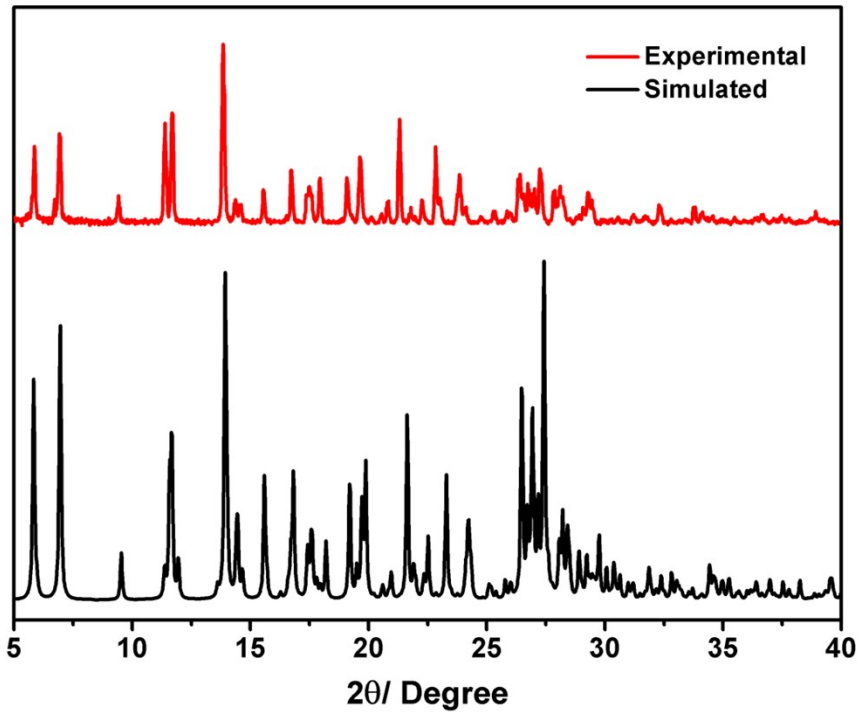


Fig. S8 PXRD comparison of simulated pattern and experimental pattern for NOR-IND salt hydrate.

Table S2 Geometrical parameters of hydrogen bonds in all the compounds.

NOR-DIC

D—H···A	D···H (Å)	H···A (Å)	D···A (Å)	D—H···A (deg)	Symmetry code
O2-H2···O3	0.8200	1.7300	2.496(4)	155.00	X, Y, Z
N4-H4···O5	0.8600	2.2700	2.992(5)	142.00	X, Y, Z
N3-H3A···O5	0.9000	1.8400	2.737(6)	176.00	2-X,2-Y,2-Z
N3-H3B···O4	0.9000	1.8000	2.682(7)	167.00	-1+X,-1+Y,Z
C1-H1···O1	0.9300	2.3100	3.218(5)	164.00	2-X,3-Y,1-Z
C7-H7···O3	0.9300	2.4200	3.216(5)	143.00	3-X,2-Y,1-Z
C22-H22···O1	0.9300	2.4100	3.297(6)	159.00	2-X,3-Y,1-Z

NOR-DIF

D—H···A	D···H (Å)	H···A (Å)	D···A (Å)	D—H···A (deg)	Symmetry code
O2-H2···O3	0.8200	1.7900	2.551(2)	154.00	X, Y, Z
N3-H3A···O5	0.9000	1.8500	2.730(2)	165.00	1-X,2-Y,1-Z
N3-H3B···O4	0.9000	1.8000	2.665(3)	162.00	X, Y, Z
O6-H6···O5	0.8200	1.7800	2.513(2)	148.00	X, Y, Z
C21-H21···O3	0.9300	2.5200	3.386(3)	156.00	3/2-X,1/2+Y,1/2-Z
C26-H26···O6	0.9300	2.4000	3.296(3)	161.00	5/2-X,-1/2+Y,1/2-Z

NOR-MEF

D—H···A	D···H (Å)	H···A (Å)	D···A (Å)	D—H···A (deg)	Symmetry code
O2-H2···O3	0.8200	1.7500	2.521(2)	155.00	X, Y, Z
N3-H3A···O4	0.9000	1.8300	2.636(2)	148.00	X, Y, Z
N3-H3B···O5	0.9000	1.8100	2.692(2)	164.00	-X,1-Y,2-Z

NOR-IND-H_2O

D—H···A	D···H (Å)	H···A (Å)	D···A (Å)	D—H···A (deg)	Symmetry code
O2-H2···O3	0.8400	1.7400	2.519(2)	154.00	X, Y, Z
N3-H3A···O4	0.9200	1.8100	2.692(3)	160.00	X, Y, Z
N3-H3B···O5	0.9200	1.9000	2.724(3)	148.00	1-X,2-Y,1-Z
O8-H8A···O5	0.8500	1.9700	2.778(2)	159.00	X, Y, Z
O8-H8B···O4	0.8500	2.0400	2.876(3)	169.00	1-X,1-Y,1-Z
C1-H1···Cl1	0.9500	2.8200	3.682(3)	152.00	1+X,1+Y,Z
C34-H34···O1	0.9500	2.3900	3.336(3)	172.00	-1+X,-1+Y,Z
C35-H35···O8	0.9500	2.5600	3.480(3)	163.00	X, Y, Z

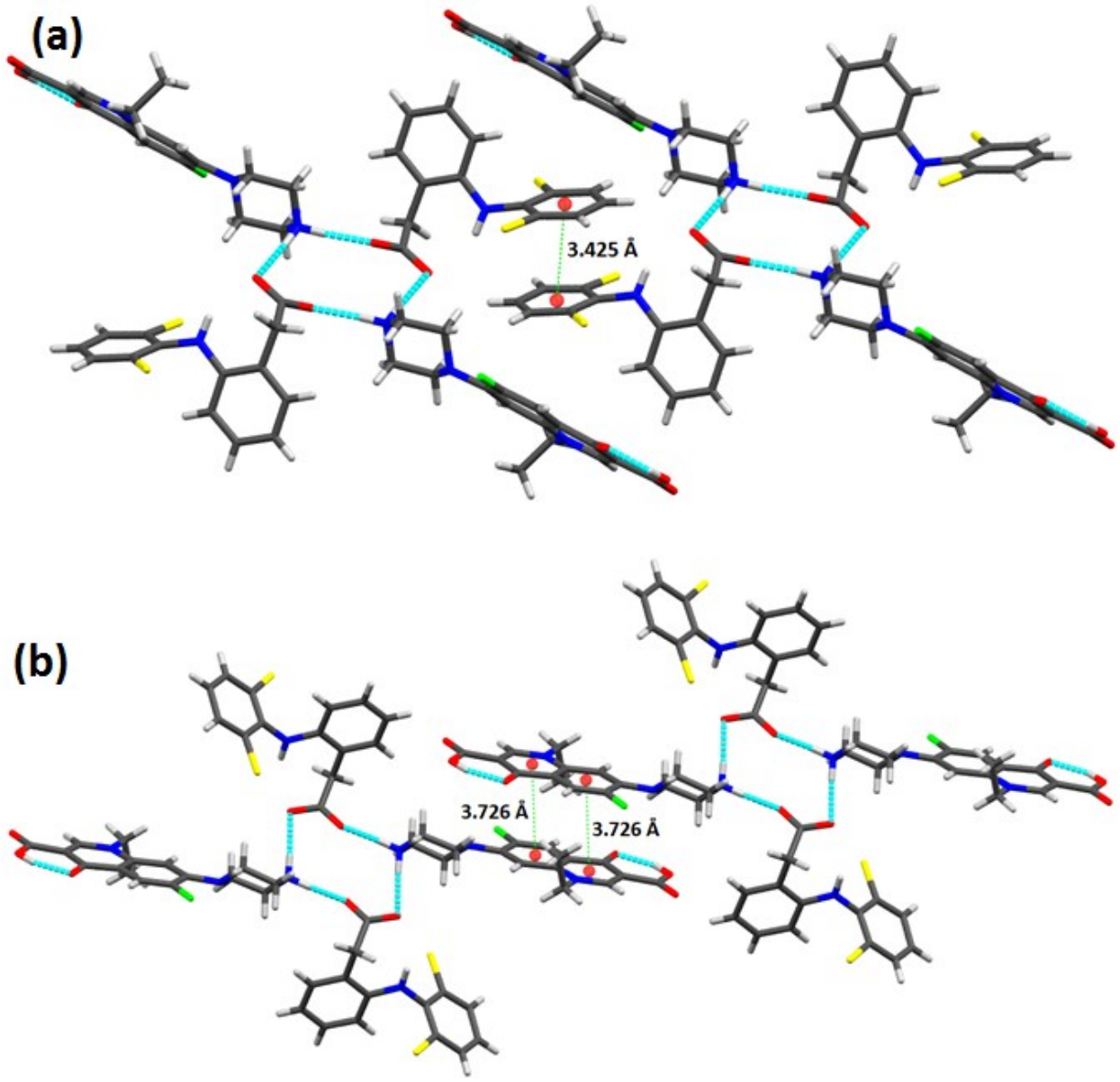


Fig. S9 (a) π···π Interactions in NOR-DIC salt between phenyl rings of DIC anions in adjacent centrosymmetric tetrameric motif. (b) The quinolone moieties of norfloxacin molecules stack *via* π···π interactions in NOR-DIC salt.

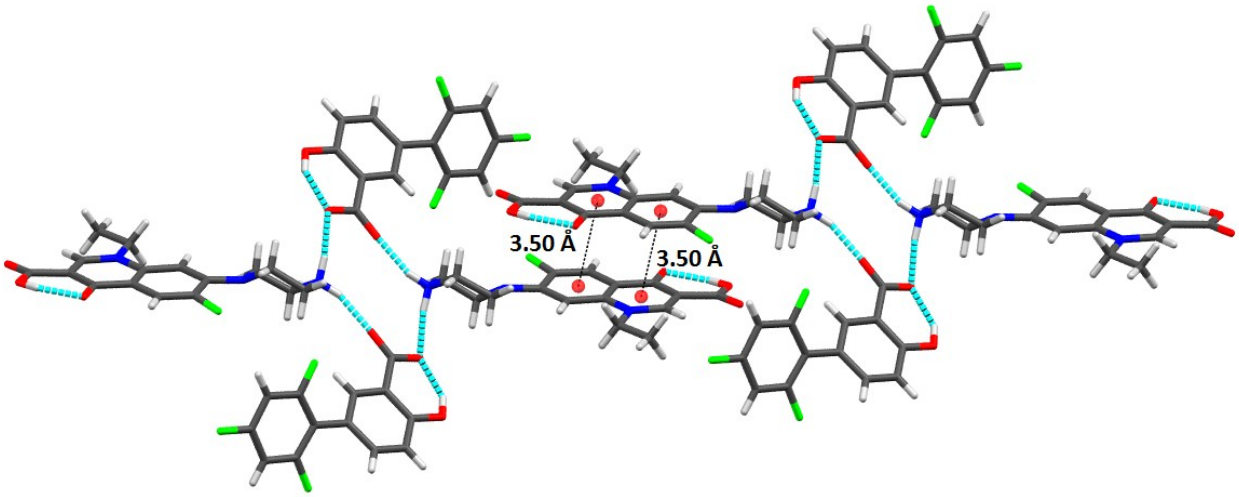


Fig. S10 The quinolone moieties of norfloxacin molecules stack *via* π···π interactions in NOR-DIF salt.

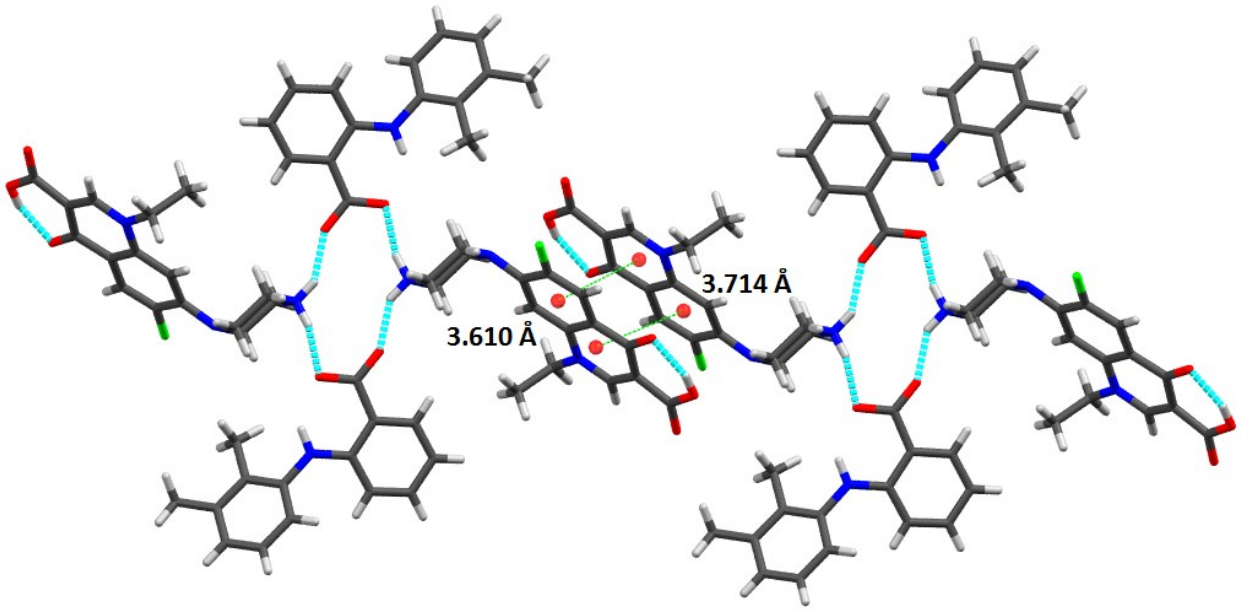


Fig. S11 π···π Interactions in NOR-MEF salt between quinolone moieties of norfloxacin cations in adjacent centrosymmetric tetrameric motif.

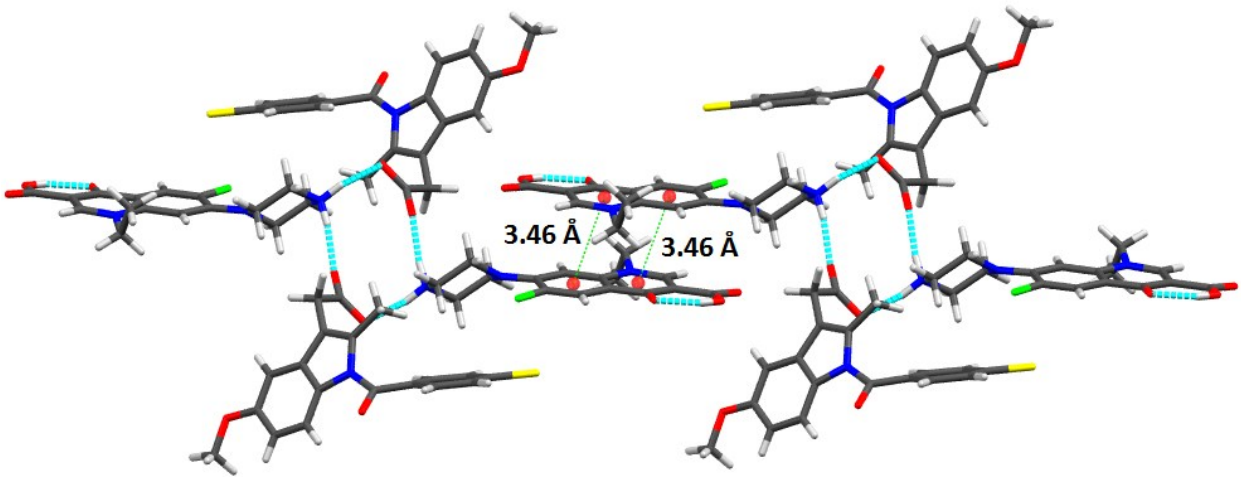


Fig. S12 The quinolone moieties of norfloxacin molecules stack *via* π···π interactions in NOR-IND-H_2O salt hydrate.

Table S3 Selected torsion angles of norfloxacin ion in the crystal structures of salt, salt hydrate and norfloxacin molecule in its parent structure.

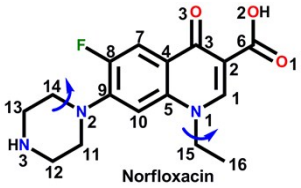
Norfloxacin molecule with atom numbering	**Solid form**	**Color code**	**τ_1 (C1-N1-C15-C16)**	**τ_2 (C9-N2-C14-C13)**
	NOR	Red	107.9°	172.2°
	NOR-DIC	Green	102.4°	166.1°
	NOR-DIF	Blue	-1.4°	148.7°
	NOR-MEF	Magenta	98.5°	91.4°
	NOR-IND-H_2O	Cyan	99.8°	138.5°

Table S4 Melting points of APIs, drug-drug salts and salt hydrate

	Melting point of APIs[a] (°C)	Melting point of drug-drug salt or salt hydrate (°C)
NOR-DIC	227/283	229
NOR-DIF	227/210	270
NOR-MEF	227/230	242
NOR-IND-H_2O	227/311	160

[a]Melting point values as reported in Sigma-Aldrich chemical catalog

Methodology

Analytical method for quantification of drug(s) in solubility and permeability studies

High performance liquid chromatography (HPLC) method was used for analysis of solubility and permeability of different drug and drug-drug cocrystal salts. The analytical HPLC method was used as prescribed by United States Pharmacopoeia (USP), with slight modification as described below.

Norfloxacin: Mobile phase used was 0.1 % orthophosphoric acid (85 parts) and acetonitrile (15 parts) at a flow rate of 2 ml per minute and detected using photodiode array (PDA) detector at 275 nm in 150 mm C18 column.

Diclofenac: Mobile phase used was 0.5 g/L of phosphoric acid and 0.8 g/L of sodium dihydrogen phosphate adjusted to pH 2.5 with phosphoric acid (34 parts) and methanol (66 parts) at a flow rate of 1 ml per minute and detected using photodiode array (PDA) detector at 254 nm in 150 mm C18 column.

Diflunisal: Mobile phase used was HPLC grade water (44 parts), acetonitrile (53 parts) and acetic acid (3 parts) at a flow rate of 1 ml per minute and detected using photodiode array (PDA) detector at 254 nm in 150 mm C18 column.

Mefenamic acid: Mobile phase used was 50 mM monobasic ammonium phosphate pH adjusted with 3 M ammonium hydroxide to 5.0 (40 parts), acetonitrile (46 parts) and tetrahydrofuran (14 parts) at a flow rate of 1 ml per minute and detected using photodiode array (PDA) detector at 286 nm in 150 mm C18 column.

Indomethacin: Mobile phase used was 10 mM monobasic sodium phosphate and 10 mM dibasic sodium phosphate (50 parts) and acetonitrile (50 parts) at a flow rate of 1 ml per minute and detected using photodiode array (PDA) detector at 320 nm in 150 mm C18 column.

Solubility studies

The solubility of norfloxacin and its different salts with NSAIDs were determined at different pH (pH 1.2, 4.5 and 7.4) buffer solution in order to understand and control transformations for achieving required solubility and bioavailability of the multicomponent crystalline new drug salts in aqueous media.[1] pH1.2 (0.1 N HCl), pH 4.5 (acetate buffer) and pH 7.4 (phosphate buffer), were prepared using standard pharmacopoeial method as prescribed by USP. The study was conducted using shake flask method.[2] In this method, supersaturated solution of the drug was prepared in the respective buffer by dissolving excess amount of drug/drug salts in small quantity of buffer in conical flask (10 mL) and placed firmly in water bath shaker (Remi, Mumbai, India) at 37 °C. Shaking was allowed for 48 h and after that samples were filtered, diluted suitably and analyzed using HPLC (Waters, 1525, Massachusetts, USA).

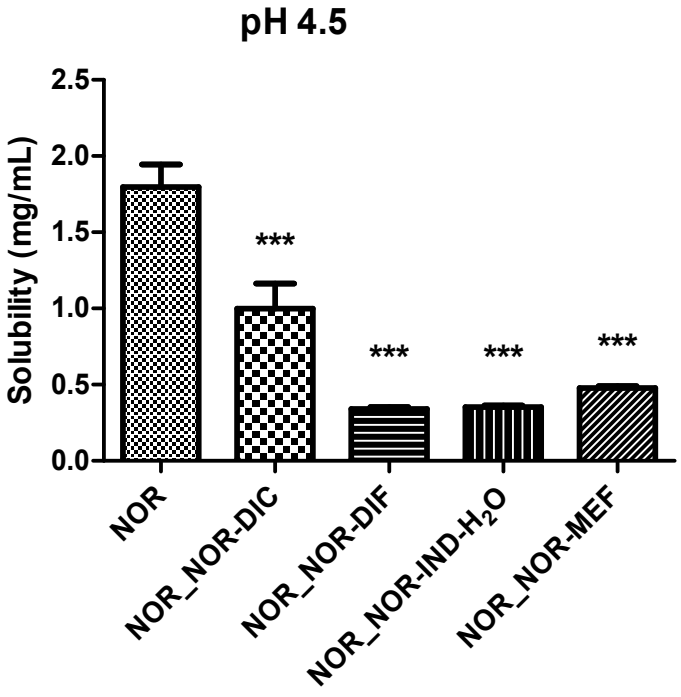


Fig. S13 Solubility comparisons of NOR in its pure form, and in salts, NOR_NOR-DIC, NOR_NOR-DIF, NOR_NOR-MEF and salt hydrate NOR_NOR-IND-H_2O in pH 4.5 buffer solution. Data were statistically analyzed using Graph pad Prism (Version 5, Graph Pad software, Inc., California corporation) using one way ANOVA ($p<0.05$).

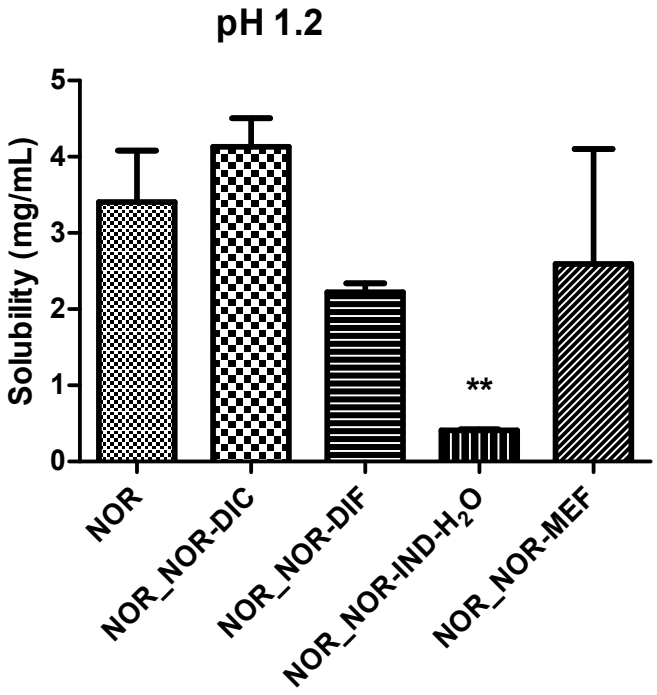


Fig. S14 Solubility comparisons of NOR in its pure form, and in salts, NOR_NOR-DIC, NOR_NOR-DIF, NOR_NOR-MEF and salt hydrate NOR_NOR-IND-H_2O in pH 1.2 buffer solution. Data were statistically analyzed using Graph pad Prism (Version 5, Graph Pad software, Inc., California corporation) using one way ANOVA ($p<0.05$).

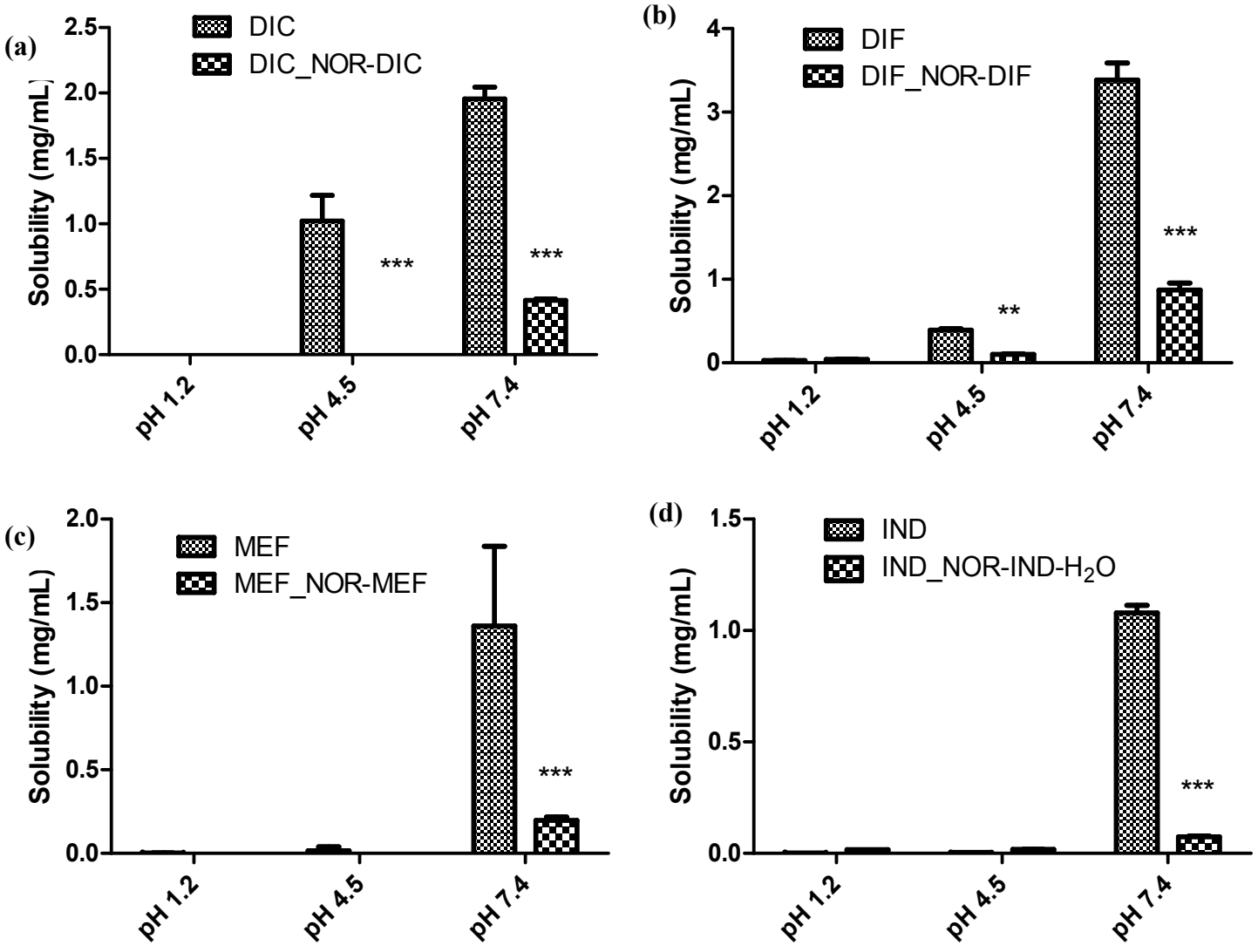


Fig. S15 Solubility comparisons at different pH (1.2, 4.5 and 7.4) conditions for (a) DIC, DIC_NOR-DIC, (b) DIF, DIF_NOR-DIF, (c) MEF, MEF_NOR-MEF and (d) IND, IND_NOR-IND-H_2O salt hydrate. Data were statistically analyzed using Graph pad Prism (Version 5, Graph Pad software, Inc., California corporation) using two way ANOVA ($p>0.05$ for pH 1.2), ($p<0.01$ for pH 4.5) and ($p<0.05$ for pH 7.4).

Table S5 Solubility values (in mg/ml) of four new salts and salt hydrate along with their parent APIs in different pH buffers.

solubility medium	pH = 7.4	pH = 4.5	pH = 1.2
NOR	0.179	1.797	3.407
DIC	1.956	0.010	0.000
DIF	3.385	0.393	0.027
MEF	1.360	0.016	0.002
IND	1.079	0.004	0.001
NOR_NOR-DIC	0.461	0.998	4.131
DIC_ NOR-DIC	0.415	0.000	0.000
NOR_NOR-DIF	0.263	0.340	2.222
DIF_ NOR-DIF	0.871	0.100	0.041
NOR_ NOR-MEF	0.140	0.479	2.595
MEF_NOR-MEF	0.198	0.000	0.000
NOR_ NOR-IND-H_2O	0.244	0.353	0.409
IND_NOR-IND-H_2O	0.075	0.000	0.017

Membrane diffusivity studies

Diffusion studies for norfloxacin and its different salts with NSAIDS were studied using modified Franz diffusion cell (Zenith Glass, Kolkata, India). The diffusion cell is fabricated in such a manner that it consists of two chambers, upper part contains the drug also called as donor chamber, while lower is receptor chamber. The system is jacketed with recirculating water at 37 °C, so as to mimic the human body temperature. Basically, this method is used to get insight related to the relationship between the skin/membrane and the drug or its formulations, before proceeding towards *ex-vivo* skin permeation or *in-vivo* studies.[3] Due to this advantage, the use of Franz diffusion cell is widely used for the preliminary estimation in permeation studies by a major group of researchers. In the current study, diffusion cell used was having the capacity of 50 ml and 3.78 cm^2 as the effective surface area.

The dialysis membrane (14 KDa, HiMedia, Mumbai, India) used for the study was soaked overnight in phosphate buffer pH 7.4. After the equilibration of membrane with media, dialysis

membrane was fixed on the vertical static diffusion cell and assured for no leakage. The donor compartment contained 50 mg of drug suspended in 1 ml of buffer and receptor compartment was filled with 50 ml of media, temperature was equilibrated at 37 °C. The uniformity of temperature and drug concentration in the receptor compartment was ensured by stirring at 50 rpm using magnetic bead and stirrer. An aliquot of 1 ml samples were withdrawn at predetermined time intervals and replaced with fresh media. The samples were analyzed in HPLC (Waters 1525, Massachusetts, USA) equipped with PDA detector, with priorly validated method.

The amount of norfloxacin, from norfloxacin and its salts diffused from the dialysis membrane at each time interval was calculated and graphically represented as cumulative percentage drug diffused ± S.D. v/s time. Flux was also calculated at each time interval using the equation (1) and reported graphically as flux at each time interval ± S.D. v/s time.[4,5] All the studies were conducted in triplicate and their mean and ± SD values were calculated.

$$J = \frac{Q_T}{t \times S} \qquad (1)$$

Where, J is the flux, S is surface area of the membrane, t is time and Q_T is the amount of drug permeated at each time point.

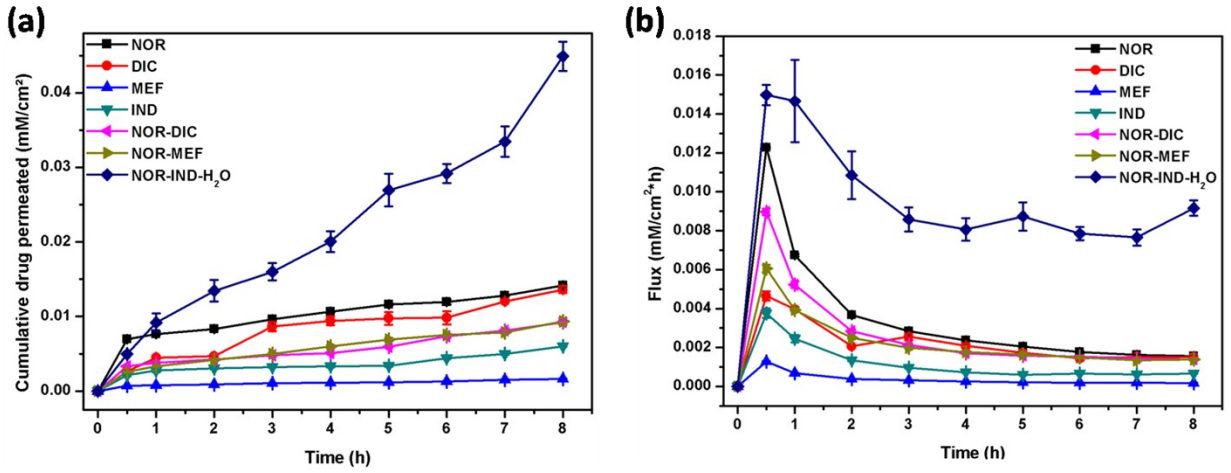


Fig. S16 (a) Cumulative amount of NOR and the NOR_salts (excluding NOR-DIF) and salt hydrate diffused vs time plot. (b) Flux of NOR_salts and salt hydrate vs time.

References

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2. A. Glomme, J. Marz and J. B. Dressman, *J. Pharm. Sci*, 2005, **94**, 1-6.
3. T. J. Franz, *Skin-Drug Application and Evaluation of Environmental Hazards*; Karger Publishers, 1978, **7**, 58-68.
4. A. C. Sintoy and S. Botner, *Int. J. Pharm,* 2006*,* **311**, 55-62.
5. B. Mandal, A. P. Rameshbabu, S. R. Soni, A. Ghosh, S. Dhara and S. Pal, *ACS Appl Mater Inter*, 2017, **9**, 36583-36595.