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Research Article

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# Preparation and Evaluation of Amino Acid Based Salt Forms of Model Zwitterionic Drug Ciprofloxacin

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## Abstract

Ciprofloxacin (CIP) is a quinolone derivative which is widely used for the treatment of a number of urinary tract infections. It exhibits its antimicrobial activity by inhibiting bacterial DNA gyrase enzyme. BCS classification of CIP is challenging due to the absence of any linear dose proportionality of AUC in humans. Yet, most of the studies classify CIP as a BCS IV (low solubility & low permeability drug) candidate. This study aims at exploiting the zwitterionic nature of CIP and investigates the ability of acidic and basic amino acids to form new salts with the primary aim of improving its solubility. Two salts were prepared using L-glutamic acid and L-aspartic acid as counter ions which resulted in increasing CIP solubility by $2.9x10^3$ and $2.5x10^3$ folds respectively. On the other hand, cationic amino acids namely (L-arginine, L-lysine & L-histidine) failed to form any salts. To investigate the absence of salt formation with the cationic amino acids, the role of inter- and intra molecular interactions between CIP and amino acids on the salt formation was studied using molecular dynamic simulation. Both the experimental and theoretical results revealed that ionic and hydrophobic interactions are essential for salt formation and that the ionic interaction and/or hydrophilic interactions between CIP and amino acids molecules should be greater than hydrophobic interactions between CIP molecules. Future work will study the effect of the salts on the permeability behaviour of CIP across Caco-2 monolayers.

**Keywords:** Ciprofloxacin; Amino acids; $^1$HNMR; FTIR; Simulation studies

## Introduction

Ciprofloxacin (CIP) (1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-yl-quinoline-3-carboxylic acid) is a member of quinolone antibiotics which are widely used due to their broad spectrum of activity, low resistance and high tolerance [1,2]. CIP is characterised by the presence of two types of rings in its structure (Figure 1); napthyridine ring which contains nitrogen atom at position 17 and 20, the second nucleus known as quinolone nucleus containing one nitrogen at position 1 [3]. Ciprofloxacin contains keto oxygen at C-4 and carboxylic acid side chains at C-3 which are essential for ciprofloxacin activity. It acts by inhibiting bacterial DNA gyrase enzyme; this process depends greatly on the pH and concentration of the acid [4]. Moreover, CIP is classified according to the biopharmaceutical classification system as a class IV drug (BCS IV). Its solubility is pH dependent and ranges between 0.15 mg/mL to 6.19 mg/mL at pH 7 and 5 respectively [5].

As shown in figure 1 CIP is a zwitterionic molecule with pKa's of 6.09 and 8.74 and p*I* of 7.42 and is an ideal candidate for salt formation with both acidic and basic counter ions. Complexation of CIP with multivalent cations such as $Ca^{+2}$, $Al^{+3}$, $Fe^{+3}$ and $Mg^{+2}$ was approved by FDA as alternative for the parent active [6]. Therefore these complexes were commonly used with CIP and other fluroquinolones in order to improve the solubility of these drugs with the aim of increasing their bioavailability [7]. Nevertheless, fluroquinolones metal complexes were found to have negative impact on bioavailability [7,8] as the resultant complexes were too large to be effectively transported across the cell membranes. As a consequence, the efficacy of fluroquinolones was lower [9] and the risk of developing bacterial resistance was greater. Although salt formation is a commonly used approach in order to improve drug solubility, fluroquinolones hydrochloride (HCl) salts (most commonly and widely used for CIP) failed to dissolve completely at intestinal pH of 6.8 [6]. Another study conducted by Romanuk et al. showed an improvement in CIP solubility together with taste masking for a new salt with saccharin [10].

Hence, improving the solubility of fluroquinolones without impairing their permeability behaviour would be a challenging task which if achieved would provide CIP a BCS biowaiver.

The aim of this study was to improve the solubility and dissolution properties of CIP via salt formation. In addition, we investigated the role of inter- and intra molecular interaction between the drug and amino acids by integrating experimental and theoretical approaches. Amino acids were selected for this study as both cationic (arginine, lysine and histidine) and anionic charged molecules (aspartic acid and glutamic acid) can be investigated for any electro static interaction with zwitter ionic CIP. Stable electrostatic interaction would result in salt formation and improving CIP solubility as discussed in our previous work [11,12].

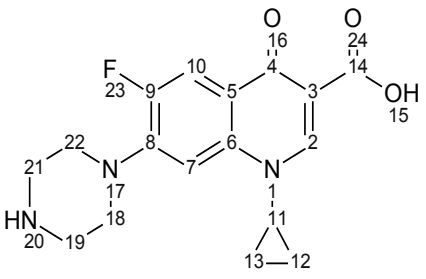


**Figure 1:** Structure of ciprofloxacin. The central structural unit is a quinolone ring with a fluorine atom at the 9-position, a piperazine moiety at the 8-position, a cyclopropyl ring at position 1 and a carboxyl group at position 3. There are two pK values, pKa;COOH = 6.09 and pKa;N20 = 8.7.

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Investigation of published literature has shown that limited work has been done on theoretical studies on CIP complexes as listed in table 1 [13-16]. These studies utilised the simple force field (COMPASS force field) and short simulation time (up to 40 ps) resulting in low precision. The current paper utilises all-atomic molecular dynamics simulation with general AMBER force field (gaff) to study the molecular interaction between CIP molecules and amino acids.

## Materials and Methods

### Materials

Ciprofloxacin, L-Arginine (non-animal source), L-Histidine (Reagent plus™), L-Lysine, L-Glutamic acid (Reagent plus ≥ 99%), L-aspartic acid (≥ 98%) and Potassium bromide (99% FT-IR grade) were all purchased from Sigma Aldrich, UK. Methanol and acetic acid were purchased from Fischer scientific. $D_2O$ (99.9% min) was purchased from Coss scientific instrument Ltd.

### Methods

**Salt preparation:** Equimolar amount of CIP and the free amino acid were solubilised in water, and the solutions were mixed and stirred until equilibration was achieved and filtered. The filtrate was transferred into stainless steel pan and freeze dried for 42 h using Virtis Advantage (wizard 2.0) freeze dryer. Salts were primary dried at -40°C shelf temperature and under -55 mTorr vacuum for 36 h and secondary dried at 20°C for 6 h.

**HPLC analysis:** The amount of ciprofloxacin (CIP) dissolved in the samples was quantified by HPLC using a Dionex 1100 system fitted with autosampler (AS50), gradient pump (GP50), UV detector (UVD 170U) and RP-C18 analytical column (Phenomenex 110A, 150x4.6 mm, 5 µm). Mobile phase consisted of methanol: water: acetic acid (840:158:2) and pumped at 1.0 mL/min to elute CIP. $\lambda_{max}$ was determined using Unicam UV-Visible Spectrophotometer and the HPLC UV detector was set at $\lambda_{max}$ of 280 nm. CIP was eluted with a retention time of 1.5 ± 0.1 min and a rectilinear calibration curve was established at concentration range between 10- 200 µg/mL with $R^2$ value of 0.998. LOD and LOQ were calculated using standard deviation of response and slope using equations (1 and 2). The standard deviation of response (σ) was found to be 0.056 and the slope of the calibration curve (S= 1.57). Applying these values to the equation above LOD and LOQ were found to be 0.107 and 0.356 respectively.

$$LOD = \frac{3.3\sigma}{S} \quad (1)$$

$$LOQ = \frac{10\sigma}{S} \quad (2)$$

**Phase solubility diagram:** Phase solubility diagram was established by measuring the saturated solubility of CIP free drug with various concentrations of cationic amino acids (L-arginine, L-lysine and L-histidine) and anionic amino acids (L-aspartic acid and L-glutamic acid) as previously discussed by [11,12]. Excess CIP was added into screw-capped tubes containing serial dilutions of the amino acid solutions and agitated at room temperature (Stuart SB 162 stirrer) for 24 h. After equilibrium the supernatant was filtered through 0.45 µm filters and analysed by HPLC to determine CIP concentration. The pH of amino acid solutions was monitored using Xisherbrand Hydrus 500 pH meter.

**Differential scanning calorimetry and thermogravimetric analysis:** Thermal events such as melting point and crystallization exotherms of CIP and its prepared salts were measured using Differential Scanning Calorimeter (Pyris Diamond DSC). Approximately, 2-5 mg of the salts were weighed and transferred to an aluminium sample pan (50 µL capacity) then sealed with aluminium top. Intra cooler 2P system was used to initially cool the samples to 50°C and then sample heated to 300°C at rate of 10°C/min. Nitrogen was used as a purge gas at a flow rate of 20 mL/min, Indium and Zinc were used to calibrate the heat flow and melting point onset (156.6°C for Indium and 419.47°C for Zinc). The obtained thermograms were analysed using Pyris Manager Software (version 5.00.02). The experiment was performed in triplicate and an empty aluminium pan, sealed in the same way of the samples, was used as a reference cell for all the measurements.

Moisture content and decomposition temperature of CIP and its prepared salts were determined using thermogravimetric analyser (Pyris 1 TGA, Perkin Elmer). 5-10 mg of samples were added to an open pan and analysed at temperature range 30- 300°C at 10°C/min scanning rate and under nitrogen stream. The resultant thermograms were analysed using Pyris Manager Software (version 5.00.02).

**FT-infrared (IR) spectroscopy:** Nicolet IR 200 spectrometer (Thermo Electron Corporation) was used to measure FTIR absorbance spectra of CIP and its salts using KBr discs technique. Crystalline KBr was dried at 65°C over night before use. Samples were prepared by mixing CIP or its salts with dry KBr at 1:100 (w/w) ratio and pressed at 8 tones for 5 min (using Specac tablet presser) to form KBr discs. 64 scans were performed over wavelength 4000- 400 $cm^{-1}$ for each sample. FTIR spectra were interpreted using EZ OMNNIC 7.0 software.

**$^1$HNMR:** Samples were analysed with Bruker Avance DPX-250 NMR (at 250.1 MHz) and Bruker Topspin software was used to analyse the data. Approximately, 1-2 mg of the salts were dissolved in 1 mL of deuterated water ($D_2O$) and placed in sample capillary vial up to 5-10 cm height.

**Solubility and dissolution studies:** The method of Higuchi and Connors [17] was used to measure the solubility of CIP salts. An excess amount of CIP or its salts was added to capped test tubes with

**Table 1:** Computer simulation about ciprofloxacin complexes.

| Complexes | Simulation details | References |
|---|---|---|
| Ciprofloxacin/humic substance complexes | InsightII version 2000.1 software package with COMPASS force field, 5-A° layer of water box; up to 40 ps | Aristilde and Sposito [13] |
| Mg/ciprofloxacin complexes | HyperChem and the MM+ force field<br>Langevin dynamics with a simulated annealing method | Turel et al. [14] |
| Ciprofloxacin/metal complexes | Cerius package with COMPASS force field,<br>35 ps simulation, water box | Aristilde and Sposito [15] |
| solid complexes $[Y(CIP)_2(H_2O)_2]Cl_3 \cdot 10H_2O$ and $[ZrO(CIP)_2Cl]Cl \cdot 15H_2O$ | Gaussian98W package by density functional theory (DFT) at the B3LYP/CEP-31G level of theory | Sadeek et al. [16] |

5 mL of deionised water and stirred for 24 hours at room temperature until equilibrium was reached. Subsequently, the suspension was filtered through 0.45 µm syringe filters and suitably diluted and the concentration was measured by HPLC.

Conventional tablets weighing 500 mg and containing 50 mg of CIP or equivalent amounts of the prepared salts were prepared for dissolution studies. Starch was used as a tablet binder and anhydrous lactose was used as a diluent in 1: 8 ratio respectively. All the excipients were geometrically mixed with the active ingredients and compressed (Specac tablet presser) at 8 tones pressure for 10 minutes.

USP II paddle method (ERWEKA DT-600) was used to perform the *in-vitro* dissolution studies. The prepared tablets were placed into dissolution vessels containing 900 mL of phosphate buffer (pH 7.2) and the dissolution media was maintained at 37°C ± 0.5°C and stirred at 50 rpm. 5 mL of samples were collected at predetermined time intervals (1, 5, 10, 15, 20, 25, 30 and 40 min) then filtered through 0.45 µm Millipore filters. The dissolution media was replaced by 5 mL of fresh dissolution fluid in order to maintain a constant volume. The samples were analysed by HPLC as mentioned above.

**Simulation studies:** MD simulations were carried out using the AMBER11 software package [18] with the general AMBER force field (gaff) for all molecules [19]. All molecules were built by Discovery Studio Visualizer 3.1 (http://accelrys.com/products/discovery-studio/index.html). At neutral pH (pH ~ 7), all the primary amines of amino acids were protonated and carboxyl groups of amino acids were negatively-charged. Using the LEAP module in AmberTools 1.5, the amino acids were positioned near the ciprofloxacin molecules (at least 3 Å for enough sampling). The electrostatic interactions were calculated using particle mesh Ewald method [20-25] and the cutoff was 10 Å. Using the LEAP module in Amber Tools 1.5, the complex structure was immersed in a truncated octahedral water box with a solvation shell of 10 Å thickness using TIP3P model for water [26]. This procedure resulted in solvated water structures containing approximately 10,000 atoms which included 164 ciprofloxacin atoms and different numbers of amino acid atoms, with the remainder being water molecules.

The minimization procedure for solvated complex consisted of two steps. In the first stage, the complex was fixed and positions of water and ions were minimized. The solvated structures were then subjected to 500 steps of steepest descent minimization followed by 500 steps of conjugate gradient minimization [27,28]. During this minimization process the complex was fixed in its starting conformation using harmonic constraints with a force constant of 500 kcal/mol/Å$^2$. In the second stage, the entire system was minimized by 1000 steps of steepest descent minimization followed by 1500 steps of conjugate gradient minimization without the restraints.

The minimized structure was then subjected to 20 ps of MD, using a 2 fs time step for integration. During the MD simulation the system was gradually heated from 0 to 300 K using 10 kcal/mol/Å$^2$ weak positional restraints on the complex. The SHAKE algorithm was used in which all bonds involving hydrogen are constrained [29]. After the system was heated at constant volume with weak restraints on the complex, MD was performed for 1 ns with a time step of 2 fs under constant pressure/constant temperature (NPT ensemble) at 300 K with an average pressure of 1 atm without positional restraints. The random number seed of every restart was changed [30]. Isotropic position scaling [31] was used to maintain the pressure and a relaxation time of 2 ps was employed. SHAKE was used to constrain bonds involving hydrogen and the temperature was kept at 300 K with Langevin dynamics [32] using a collision frequency of 1.0 ps$^{-1}$.

## Results and Discussion

### Ciprofloxacin phase solubility profile

CIP is a zwitterionic molecule as it has a positively charged nitrogen atom (N20) and negatively charged carboxylic group (Figure 1). Therefore, the ability of CIP to form new salts with cationic and anionic amino acids was investigated in this study.

Figure 2 shows the phase solubility diagram of CIP with three basic amino acids namely; L-arginine, L-lysine and L-histidine and two acidic amino acids; L-aspartic acid and L-glutamic acid. The phase solubility diagram indicates that both aspartic acid and glutamic acid ionised CIP to a greater extent while the extent of ionisation for CIP was lower with basic amino acids such as arginine and lysine. No ionization was observed upon inclusion of histidine. Therefore, four salts were prepared in this study and further investigated by FT-IR, $^1$HNMR, DSC and TGA to confirm salt formation.

CIP solubility in water was found to be 7.22 ± 1.02 µg/mL at 25°C. The use of acidic amino acids considerably improved the solubility of CIP. CIP solubility increased significantly upon solubilising in L-glutamic acid solution especially at high concentrations of the counter ion. Around 1.2 mg of CIP was solubilised in 1 mL of 1000 µg/mL of glutamic acid. These results suggest that CIP acts as strong base in glutamic acid solution possibly due to the large difference between the pKa values of CIP and glutamic acid (8.74 and 2.19 respectively). Therefore, the basic amino group is completely ionised in glutamic acid solution favouring salt formation between the basic group of CIP and the acidic group of the amino acid. Similarly, the solubility of CIP increased in L-aspartic acid solution and could be potentially due to salt formation between the two ionised species (these data were further confirmed using FT-IR and $^1$HNMR as discussed below).

On the other hand, solubilizing CIP in various concentrations of L-histidine solutions showed no improvement in solubility with a

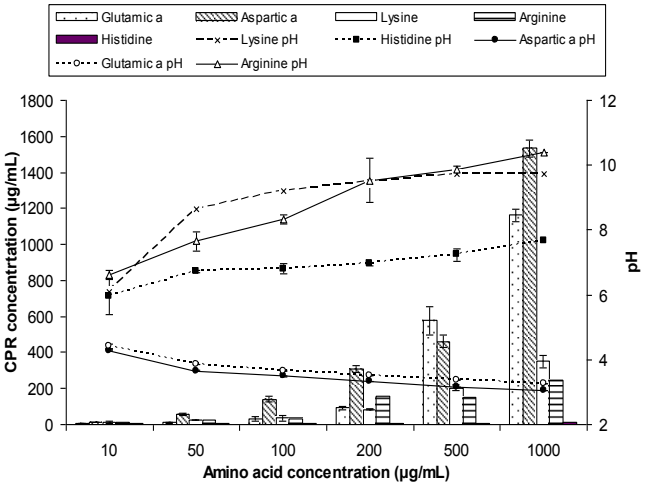


**Figure 2:** Phase solubility diagram of CIP in the presence of different concentrations of arginine, lysine, histidine, glutamic acid and aspartic acid at different pH (n=3).

maximum solubility of 9.65 ± 0.28 µg/mL at 1000 µg/mL of histidine solution. Poor solubilisation of CIP can potentially be due to similar pKa of basic group on histidine (pKa 6) and the carboxylic group of CIP resulting in no ionisation of the parent drug.

Interestingly, increasing the concentration of L-arginine solution was found to improve the solubility of CIP. At a concentration of 10µg/mL arginine, CIP solubility was 10.7 ± 2.8 µg/mL and the solubility increased linearly upon increasing the concentration of arginine solution where CIP solubility was 247.8 ± 4.2 µg/mL at 1000 µg/mL of arginine solution (Figure 2).

Although the pKa of $-NH_2$ side chain of L-arginine (12.48) is much higher than the pKa of CIP acidic side chain, CIP was ionised to a lower degree. A similar trend was observed when L-lysine was used as CIP solubility increased from 11.62 ± 5.8 µg/mL to 350.7 ± 34 µg/mL when lysine concentration increased from 10 µg/mL to 1000 µg/mL.

## Characterisation of CIP salts

**Fourier transform infrared spectroscopy (FT-IR):** Phase solubility diagram has shown that CIP possibly dissociates in acidic amino acids solution and some of the basic amino acids. The next stage of the work was to prepare five salts of CIP amino acids and investigate and characterise the salt formation using physico-chemical methods such as FT-IR, $^1$HNMR and DSC.

Figure 3 shows the FT-IR spectra of CIP, CIP aspartate and CIP glutamate. CIP had characteristic absorption peaks at a 3044 which can be assigned to C-H olefinic and aromatic chains, 1617.8 corresponding to C4O16 keto group. Since CIP is in the zwitterionic form, the carboxylic group C14=OOH does not appear in CIP FT-IR spectrum [10]. Interestingly, this carboxylic group was present in CIP glutamate and aspartate salts at 1720 and 1719 respectively suggesting that the zwitterionic nature of CIP was reverted by proton transference from L-aspartic acid and L-glutamic acid. These results are in line with results obtained by [33]. On the other hand, FT-IR data for basic amino acids with CIP does not show the C4O16 keto group at 1617.8 but had absorption band at 1579.55 and 1646.4 corresponding to carboxylic group C=O of lysine and arginine respectively [34,35]. Upon comparing CIP lysine and lysine free amino acid, similar spectrum was observed which support our findings (Figures 4C and 4D). Our results were further confirmed using $^1$HNMR as discussed below.

**$^1$HNMR:** To further confirm the salt formation and investigate the molar ratio of interaction between CIP and the counter ions, the total number of protons in the prepared salts was studied using $^1$HNMR.

Figure 5 shows the $^1$HNMR data for CIP aspartate. Both C12H2 and C13H2 of the cyclopropyl group of CIP appeared at 1.08 and 1.3 ppm, while the C11H group was deshielded at higher (δ) due to the deshielding effect exerted by N1. Piperazine ring 8 protons C22H2, C21H2, C18H2 and C19H2 appeared as multiplet at around 3.79 ppm. C2H, C7H, C10H appeared deshielded at 8.5, 7.4 and 7.33 respectively. The total integration of CIP was found to be 16, similar to what was previously reported by Wetzstein et al. [36].

CIP aspartate salt total proton integration was found to be 19 as two new bands appeared at 2.67 and 3.81 ppm corresponding to three protons of one molecule of aspartic acid (C28H and C30H2) i.e. this data shows that one molecule of CIP has formed a salt with one molecule of aspartic acid in 1:1 ratio.

Similarly, the $^1$HNMR spectrum of CIP glutamate (Figure 6) showed 5 new proton assignments between 1.98 and 2.3 ppm which correspond to the 5 alphatic protons of L-glutamic acid. However,

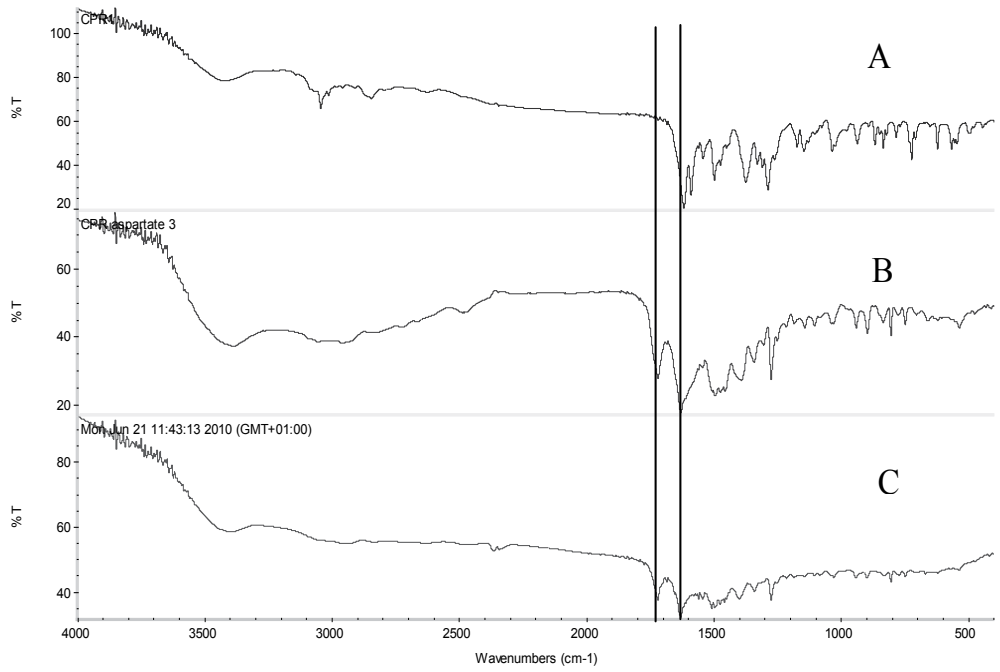


**Figure 3:** FTIR spectra of (A)Ciprofloxacin, (B) CIP L-aspartate and (C) CIP L-glutamate.

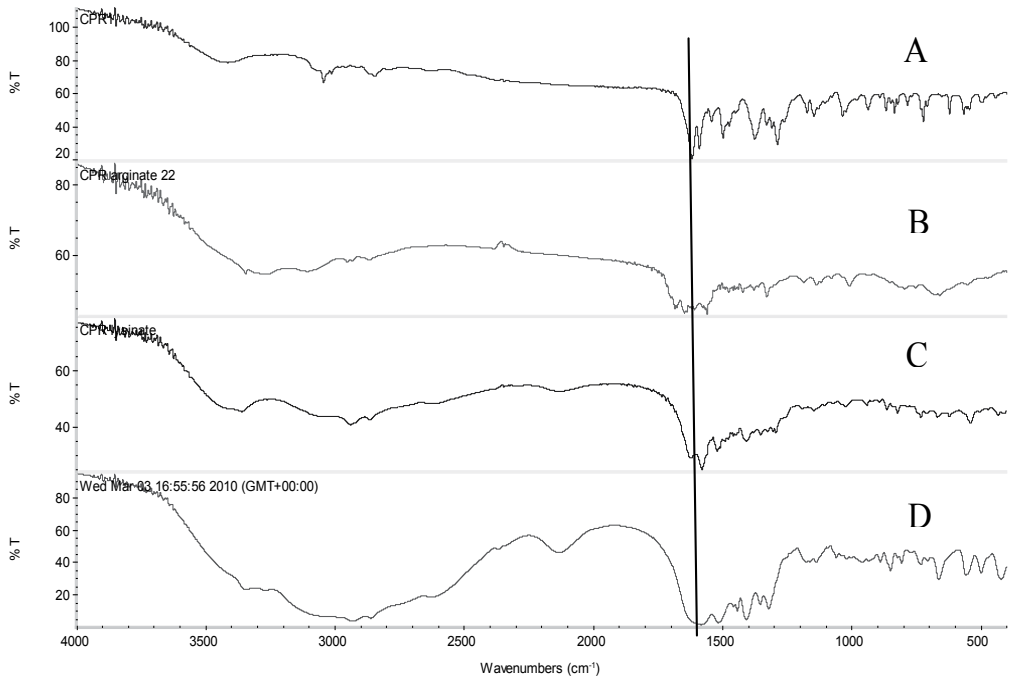


**Figure 4:** FTIR spectra of (A) Ciprofloxacin, (B) CIP arginine, (C) CIP lysine, (D) lysine free base.

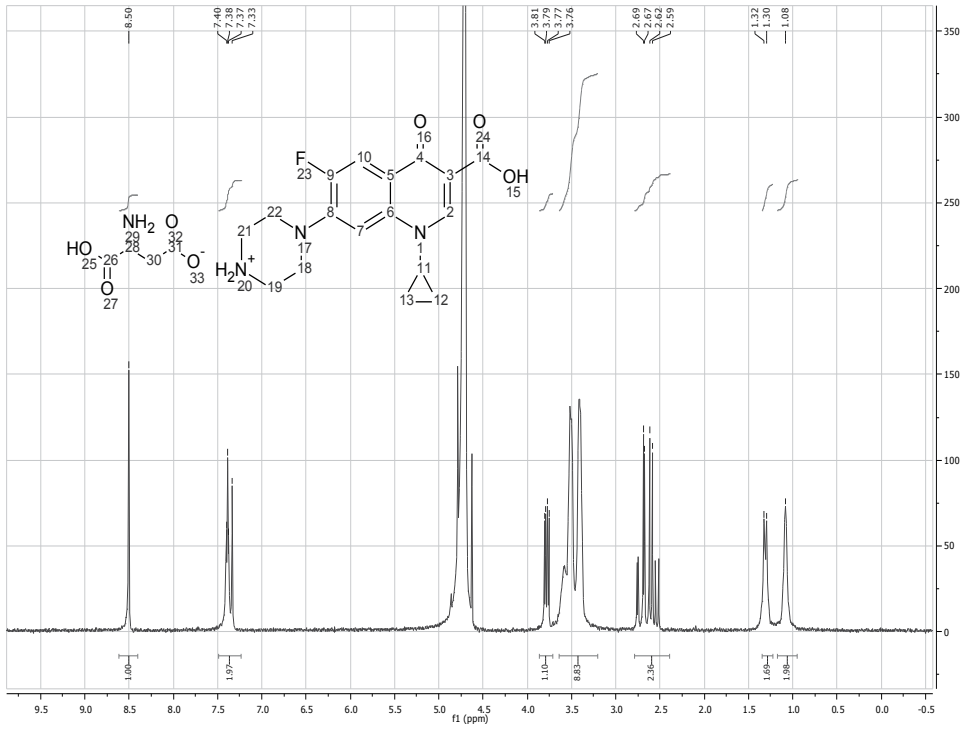


**Figure 5:** $^{1}$HNMR spectra for Ciprofloxacin Aspartate dissolved in $D_2O$.

the $^{1}$HNMR data for CIP basic amino acid preparations show no salt formation and only protons of the free basic amino acids were detected by the $^{1}$HNMR (Figures 7 and 8).

These data suggest that only acidic amino acids were capable of forming salts with CIP, while the basic amino acids failed to form of salts as suggested by the FT-IR studies above.

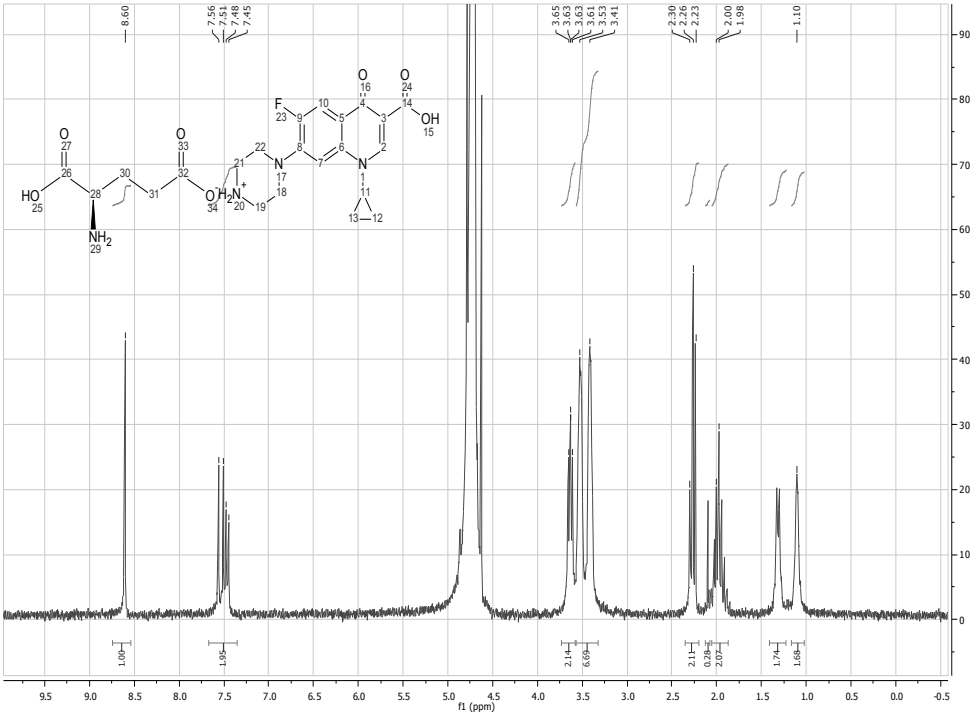


**Figure 6:** $^{1}$HNMR spectra for Ciprofloxacin glutamate dissolved in $D_2O$.

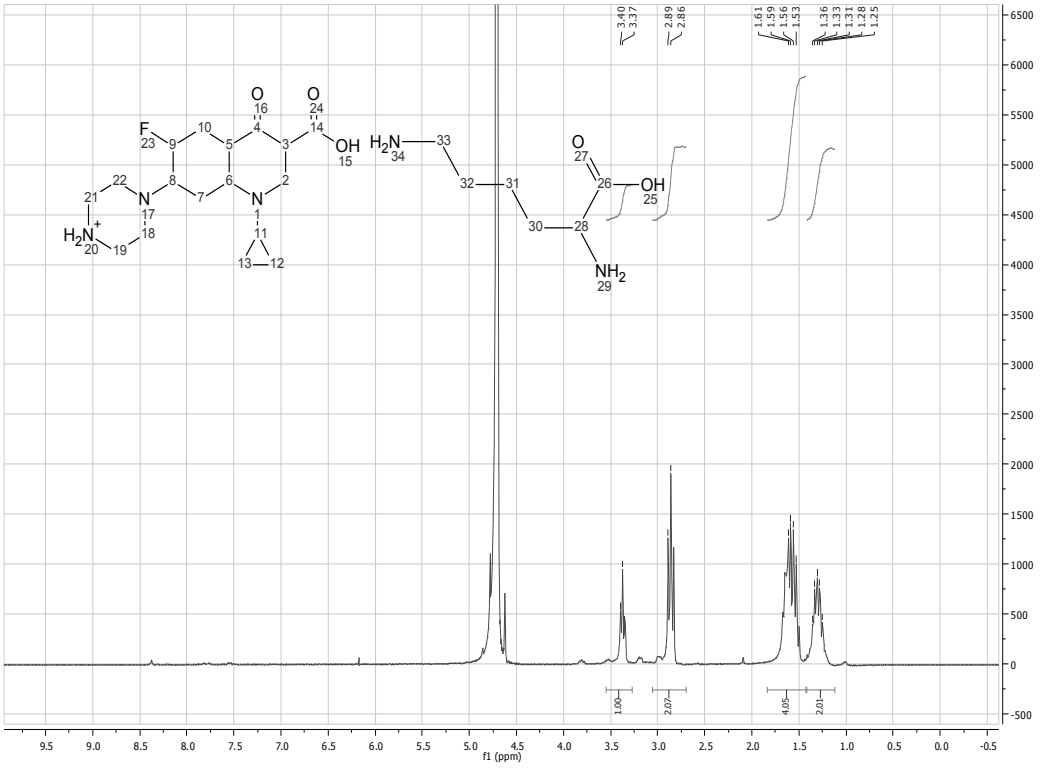


**Figure 7:** $^{1}$HNMR spectra for Ciprofloxacin lysine dissolved in $D_2O$.

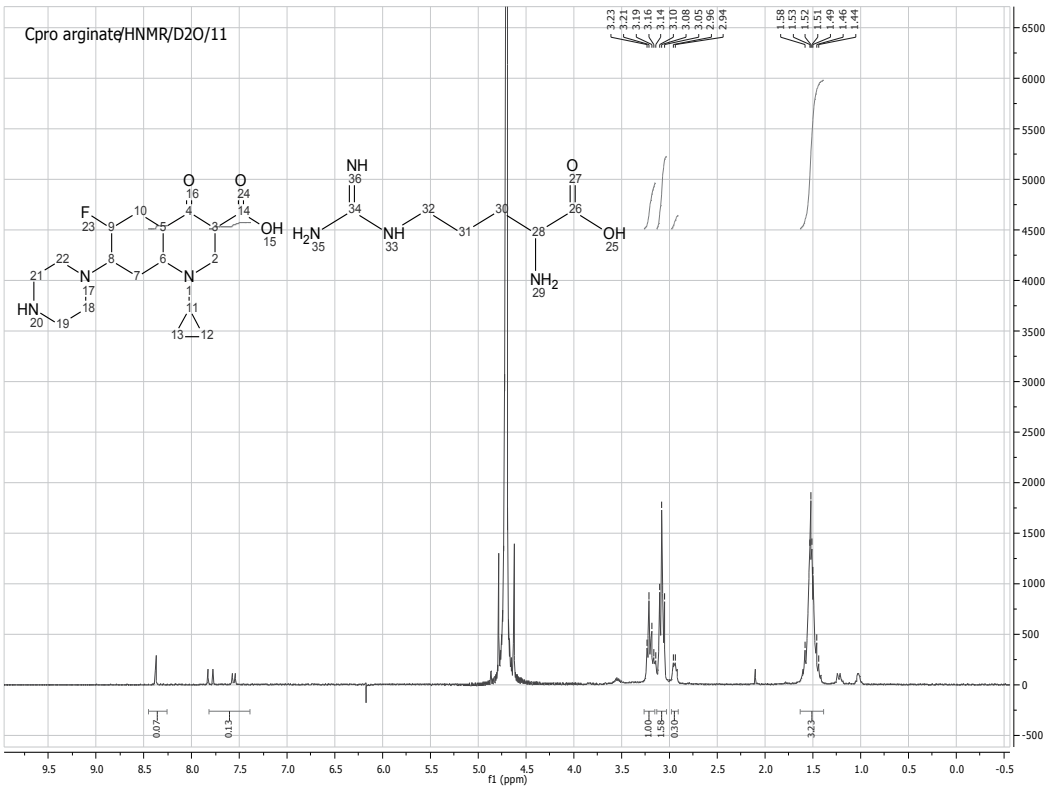


**Figure 8:** $^{1}$HNMR spectra for Ciprofloxacin arginine dissolved in $D_2O$.

**Differential scanning calorimetry and thermogravimetric analysis:** New salts have different physical behaviour from their parent components. Therefore, the melting point and degradation temperature of CIP and its salts were investigated using DSC and TGA. CIP free drug was in the crystalline form and showed an endothermic peak at 272.15°C (ΔH= 173.19 J/g) corresponding to its melting endotherm. TGA data showed a start of drug degradation upon completion of its melt. On the other hand, CIP aspartate salt had two endothermic peaks at 224.63 and 237.8°C suggesting that CIP aspartate salt has two forms. On the other hand, CIP glutamate salt showed a single melting endotherm at 203.35°C. Arginine CIP formulations had a melt at 227.31°C which was found to be similar to that of arginine free base and the absence of CIP melting endotherm or any other new melts confirms the failure of salt formation between CIP and basic amino acids as discussed previously.

## Simulation studies

Characterisation studies showed that anionic amino acids form new salts with CIP while the cationic amino acids failed to form any salts with the drug. This was contrary to the generally accepted hypothesis that a 3 unit difference in pKa between the parent compound and the counterion results in salt formation. Therefore, the role of inter and intra molecular interaction between CIP and the amino acid was investigated using molecular dynamic simulation studies in order to gain further understanding of CIP in different solutions of amino acids. Two amino acid models were used in this study; aspartic acid and histidine as aspartic acid resulted in CIP salt formation while histidine failed.

Figure 9 shows the interaction between CIP molecules; two CIP molecules in water aggregate together after 400 ps due to the hydrophobic interaction thereby further confirming the role of hydrophobic forces in the low solubility of CIP. The lab based experiments showed that CIP solubility in aspartic acid increased significantly especially at high concentrations of aspartic acid solution. Thus the interaction of two CIP molecules with one aspartic acid molecule in water (low concentration), and the interaction of two CIP molecules with four aspartic acid molecules in water (high concentration) was studied using molecular simulations. As shown in figure 10, the aggregation of CIP molecules is delayed until 800 ps in the presence of one amino acid (aspartic acid) molecule. On the other hand, aggregation of CIP molecules was hindered in the solution with high concentration of amino acid (four aspartic acid molecules) as shown in figure 11. Data obtained from the simulation studies was in agreement with the results of experiments where high concentrations of amino acid solutions (e.g. 500 and 1000 µg/mL) resulted in significant increase in solubilization capability, while the low concentration of amino acid solutions have lower effect. However, it was interesting to see that inclusion of histidine did not prevent aggregation of CIP molecules even at high concentrations (with four histidine molecules), as shown in figure 12. It further confirms experimental results of the solubilization incapability of histidine. These results indicated that two factors influence the inter- and intra-action between CIP molecules and amino acids. On one hand, CIP is an amphoteric electrolyte and acts as the base in aspartic acid solution due to the difference between the pKa values of -NH group of CIP (8.74) and -COOH group of aspartic acid (3.86).

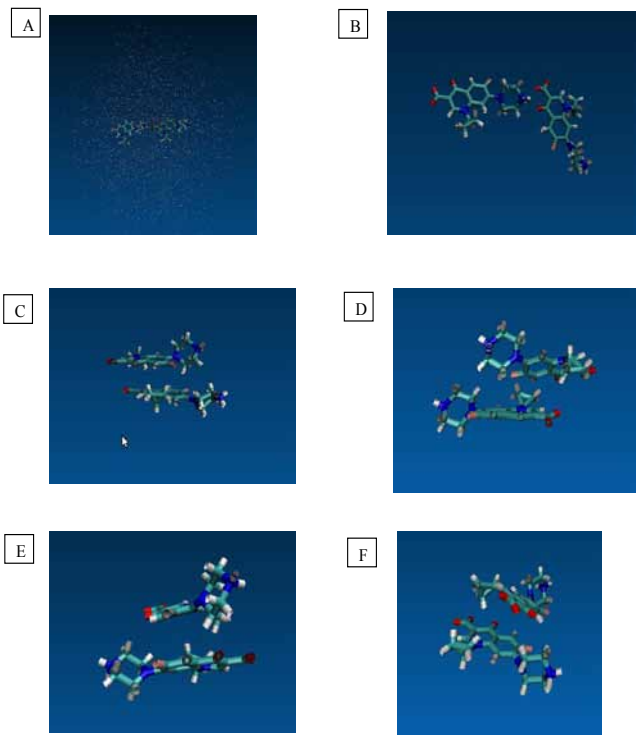


**Figure 9:** Snapshots of two ciprofloxacin molecules in water: (A) at 0 ns, (B) after 200 ps, (C) after 400 ps, (D) after 600 ps, (E) after 800 ps and (F) after 1 ns. (Water is only shown in A) and is ignored from B) to F) for clarity).

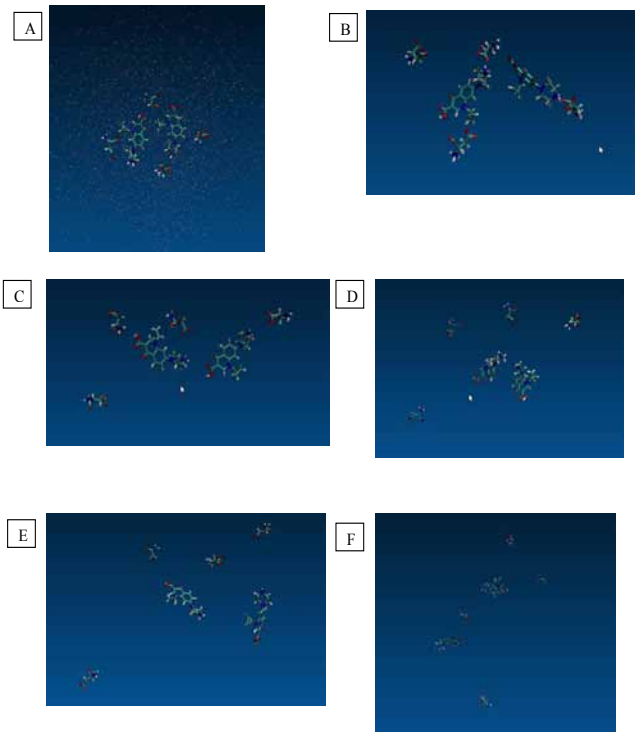


**Figure 11:** Snapshots of two ciprofloxacin molecules with four aspartic acid molecules in water: (A) at 0 ns, (B) after 200 ps, (C) after 400 ps, (D) after 600 ps, (E) after 800 ps and (f) after 1 ns. (Water is only shown in A) and is ignored from B) to F) for clarity).

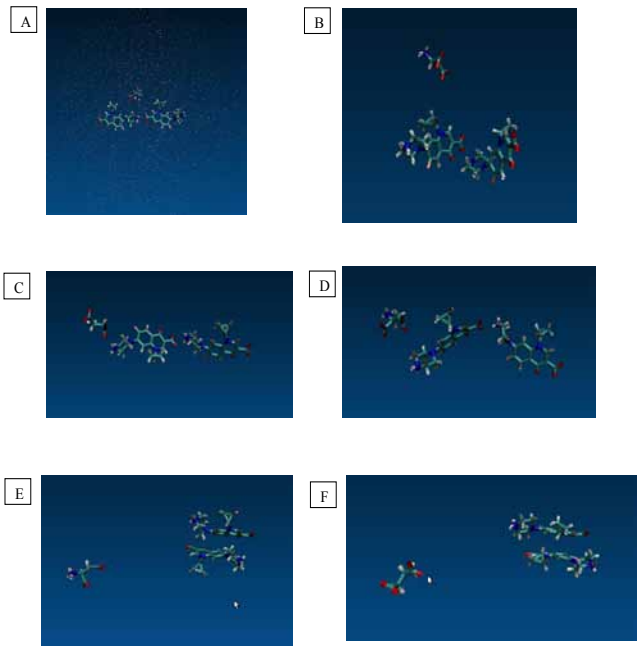


**Figure 10:** Snapshots of two ciprofloxacin molecules with one aspartic acid molecule in water: (A) at 0 ns, (B) after 200 ps, (C) after 400 ps, (D) after 600 ps, (E) after 800 ps and (F) after 1 ns. (Water is only shown in A) and is ignored from B) to F) for clarity).

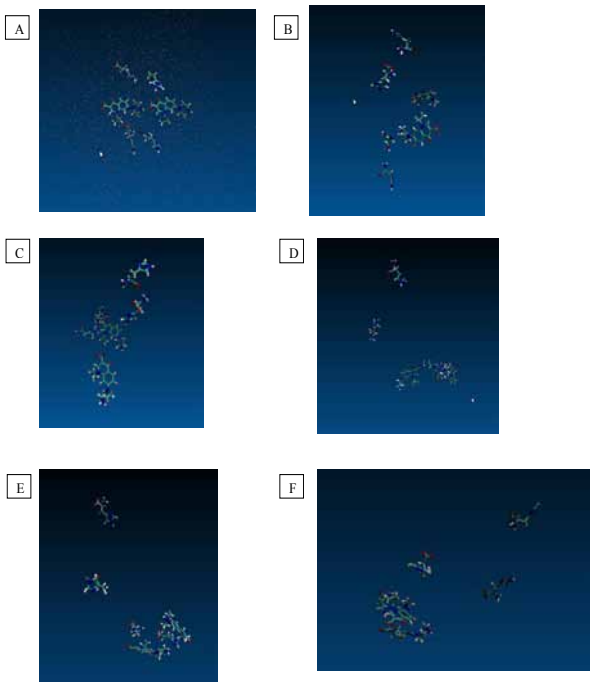


**Figure 12:** Snapshots of two ciprofloxacin molecules with four histidine molecules in water: (A) at 0 ns, (B) after 200 ps, (C) after 400 ps, (D) after 600 ps, (E) after 800 ps and (f) after 1 ns. (Water is only shown in A) and is ignored from B) to F) for clarity).

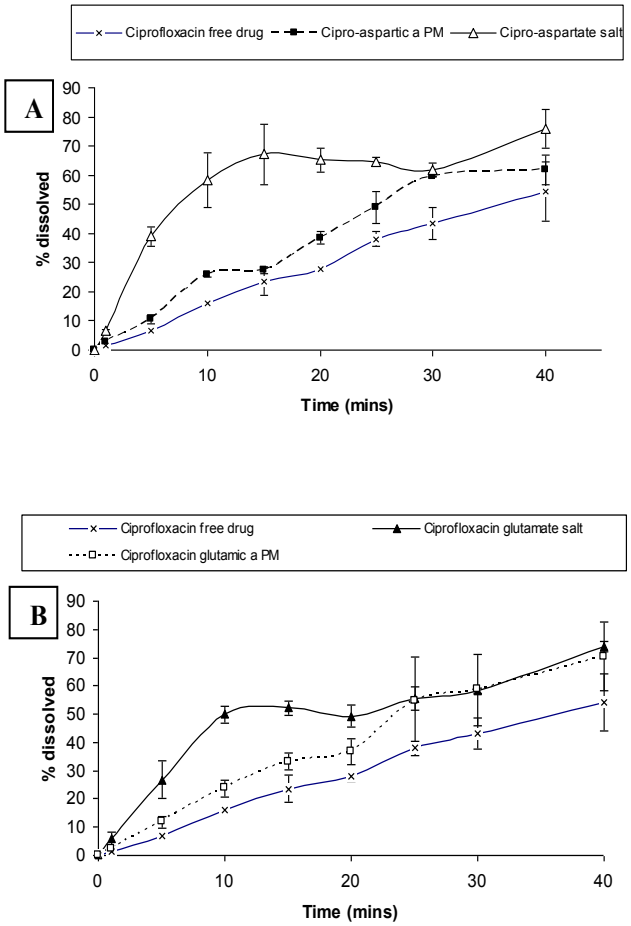


**Figure 13:** (A) Dissolution profile for CIP free drug, CIP aspartic acid physical mixture and CIP aspartate, (B) Dissolution profile for CIP glutamic acid physical mixture and CIP glutamate, data are represented as mean ± SD (n=3).

The positively-charged -NH group of CIP and negatively-charged -COOH group of aspartic acid form the electrostatic interaction. The pKa of the basic amino group of L-histidine (6) is almost the same as that of CIP carboxylic group (6.09) possibly resulting in no enhancement in solubility. Besides, hydrophobic interaction is also an important factor in CIP aggregation. When ionic interaction and/or hydrophobic interactions between CIP molecules and amino acid molecules is stronger than hydrophobic interaction between CIP molecules, the solubilization process is accelerated.

## Solubility and dissolution studies

In order to assess the saturated solubility of CIP and its prepared salts, excess amount of the drug and its prepared salts were stirred in 2 mL of deionised water and stirred for 12 hours until equilibrium was achieved. Drugs were adequately diluted and their solubility was measured using HPLC. The highest solubility was exhibited by CIP glutamate salt which was found to be 215 ± 13.3 mg/mL while the solubility of CIP aspartate was 185.4 ± 8.6 mg/mL. As demonstrated by DSC study the melting point of CIP aspartate salt was lower than that of the parent drug which requires less energy to break the crystalline structure of the salt and hence solvate the drug. Other factors such as gravitation index, charge on the most negative atom, and number of hydrogen bonding acceptors might also have played a role in favour of higher water solubility of the salt form [37].

*In vitro* dissolution studies of CIP free drug were compared against it salts and amino acids physical mixtures (Figure 13). After 10 minutes of dissolution test, only 16.09 ± 1.15% of CIP was released from CIP containing tablets while 58.3 ± 10.37 of CIP was released from CIP aspartate salt tablets. More than 75% of CIP was dissolved from CIP salt tablets after 40 minutes of the dissolution study while only 54.3 ± 10 and 61.8 ± 5.02 were released from CIP and CIP aspartic acid physical mixture respectively (Figure 13A).

Figure 13B shows the dissolution profiles of CIP glutamate, CIP and CIP glutamic acid physical mixture. The highest dissolution profile was exhibited by CIP glutamate salt as 26.7 ± 6.7% of CIP was released from this formulation during the first 5 minutes of dissolution studies. These results suggest that CIP aspartate and glutamate salts have relatively high buffering capacity which in turn improves the dissolution profile by increasing the pH of the surrounding diffusion layer.

## Conclusion

CIP is a BCS IV drug with low solubility and permeability. Due to its zwitterionic nature the use of basic and acidic amino acids to formulate new CIP salts was investigated. Acidic amino acids formed two novel salts with high solubility when compared to CIP alone. Phase solubility diagrams have shown high degree of ionisation of CIP in presence of L-aspartic and L-glutamic acid. Characterisation studies confirmed the formation of salt which was supported by FTIR, NMR and DSC studies. $^{1}$HNMR demonstrated that the molecular interaction between CIP and the acidic counter ions was a 1:1 ratio. Moreover, the molecular dynamic simulations have shown for the first time that the hydrophobic interaction between CIP molecules decrease in the presence of the acidic amino acids and in turn form a stable salt.

### Conflict of Interest

The authors declare no conflict of interest.

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