

## Original Research Article

# Studies on the interaction between ciprofloxacin hydrochloride and diclofenac sodium

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### Abstract

**Purpose:** To study the interaction between ciprofloxacin hydrochloride (Cipro) and diclofenac sodium (DS) in the presence and absence of metal ions.

**Methods:** Complexes were prepared in the aqueous phase at different molar ratios ( $r$ ) of Cipro:DS (ranged from 0.2 – 2.0). The complexes were characterized by Fourier transform-infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), and high pressure liquid chromatography (HPLC). Their properties, i.e., solubility, dissolution and partition coefficient ( $\log P$ ), were studied along with their permeability across Caco-2 cells. Furthermore, the antimicrobial activity of Cipro and its complexes was determined using standard broth dilution method and expressed as minimum inhibitory concentration (MIC).

**Results:** Cipro formed an ion pair with DS. The product was confirmed to be a combination of the two drugs, DS and Cipro, but in a ratio that is dependent on the added amounts of each component ( $r = 1:1$  or  $1:2$ ). The  $1:1$  product was more lipophilic than the individual components leading to a lower aqueous solubility and a higher octanol/water partition coefficient  $\log P$  (6.7 vs. 0.77). The presence of DS within the dissolution medium appeared to modify the dissolution of Cipro depending on the concentration. Moreover, ternary complexes involving Cipro, DS and metal ions (iron and/or calcium) exhibited improved antimicrobial effect (MIC, 0.016  $\mu\text{g/ml}$  compared to 0.258  $\mu\text{g/ml}$  for Cipro). Caco-2 cell permeation data indicate that the presence of DS significantly improved the apparent permeability coefficient ( $P_{app}$ ) of Cipro ( $20.6 \times 10^{-6}$  cm/s) which was three times higher than that of free Cipro ( $p < 0.05$ ). DS also appeared to counteract the well-known negative effect of metal ions on the bioavailability of Cipro.

**Conclusion:** There is a clinically relevant interaction between DS and Cipro at the absorption level as a result of ion pair formation, which might even counteract the negative effect of metals on the absorption of Cipro. These findings should aid the design of new Cipro ion pairs that provide higher bioavailability than free Cipro.

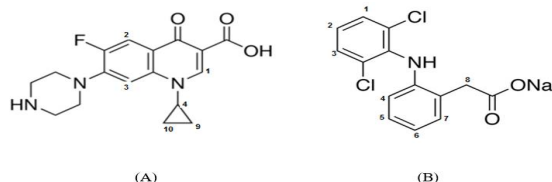
**Keywords:** Ciprofloxacin, Diclofenac, Interaction, Ion pair, Permeability coefficient, Bioavailability, Absorption

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## INTRODUCTION

Ciprofloxacin hydrochloride (Cipro) is a widely used antibacterial fluoroquinolone compound (Figure 1). The drug is approved for the treatment of several infections and perhaps most commonly employed for the treatment of urinary tract infections [1-3]. Also, the oral bioavailability of Cipro is reported to be about 70 % [4].



**Figure 1:** Chemical structure of Cipro (A) and DS (B)

The most extensively studied drug-drug interactions with Cipro are those with metal ions [5-7]. However, there are a few other studies documenting potentially significant interactions between Cipro and other drugs, e.g., paracetamol, phenazopyridine and zolpidem [8-10].

Of particular interest was the report in which the co-administration of diclofenac sodium (DS, Figure 1) and Cipro was shown to lead to about a 50 % increase in the bioavailability of the latter warning against the concomitant use of DS with Cipro [11]. However, the authors suggested that the absorption phase, metabolism and elimination rate were the potential stages of interaction. Therefore, there is a need to further explore the mechanism by which the observed DS-Cipro interaction occurred. In this work, it has been hypothesized that the negatively charged DS may form an ion pair with the zwitterionic Cipro (predominantly positively charged at a slightly acidic pH) leading to a more lipophilic form that is more permeable than the negative hydrophilic Cipro. Thus, the sought ion pairs were prepared and characterized, and their permeability through Caco-2 monolayer cells was evaluated in comparison to Cipro. Because of the well-known negative effect of metal ions on the absorption of Cipro [5,7], attempts were made to prepare complexes of Cipro with DS in the presence of calcium and iron to further characterize their influence on the cellular permeation of Cipro.

## EXPERIMENTAL

### Materials and equipment

Cipro and DS (purity > 98 %) were donated from the Jordanian Pharmaceutical Manufacturing

Company JPM (Naur, Jordan). All other reagents were purchased from Sigma (USA). A Cary UV spectrophotometer was used for all UV measurements. FTIR spectra were recorded using a Shimadzu FTIR spectrophotometer. For proton NMR spectra, a Perkin 350 MHz NMR spectrophotometer was employed. Chromatographic analysis was carried out using an isocratic Merck Hitachi HPLC pump system equipped with a UV detector.

### Preparation and characterization of Cipro-DS complexes

Potential ion pairs (complexes) of Cipro and DS were prepared at different ratios of Cipro to DS (in the range of 0.2 – 2.0). Increasing volumes of Cipro solutions (100 mM in distilled water) were added to 600 ml of 6.7 mM DS solutions so that the nominal ratio between Cipro and DS would be in the range 0.2 – 4.0. Solutions containing equimolar amounts of the metal (iron or calcium) and Cipro were mixed with solutions containing an equimolar amount of DS. The formed precipitates were filtered, dried and kept for further analysis.

### Effect of ion pair formation on pH of solution

Solutions containing 50 mg of DS alone or together with equimolar amounts of each of the employed metals were titrated with Cipro solutions (75 mg/100 ml distilled water). The pH values were measured after each addition of 1 ml of the Cipro solution.

### High pressure liquid chromatography

Cipro was determined in all relevant experiments using a validated HPLC method that was capable of separating DS from Cipro. The method utilized a phenyl column (150 x 4.5 mm ID, 5  $\mu$ m particle size) and a mobile phase composed of 25 % acetonitrile in 50 mM phosphate buffer (pH 6.5) and containing 0.2 g % citric acid. Flow rates of 1.5 ml/min were employed with 277 nm as the detection wavelength.

### Determination of saturation solubility and partition coefficient (log *P*)

The excess solid of each compound to be tested was added to 4 ml of a phosphate buffer (pH 6.8) and left on a shaker water bath (37 °C) for 24 h. One ml was centrifuged for 20 min, and then 100  $\mu$ l of the supernatant were diluted with 400  $\mu$ l of 50 mM phosphate buffer before being injected into the HPLC. Two ml of the supernatant solutions were transferred to a new tube containing 2 ml of octanol in order to determine

an apparent partition coefficient. Mixtures containing the two layers of octanol and water were kept on a shaker water bath (37 °C) for 24 h. Aliquots of the aqueous layer were diluted with phosphate buffer before they were injected into the HPLC.

### Dissolution studies

Dissolution experiments were performed for commercial 500 mg Cipro tablets using a Copley Scientific dissolution apparatus, DIS 6000 (Copley, UK). The tests were performed according to pharmacopoeial specifications using Apparatus 2 (paddle method). The two media employed for testing were distilled water and a phosphate buffer (pH 6.8). Tests were carried out in 500 ml of the dissolution medium with a temperature maintained at 37 °C. Paddle rotation was set at 75 rpm.

Four vessels were employed for each experiment, and samples (1 ml) were withdrawn at 15, 30, 45, 60 and 120 min. Experiments included testing the dissolution of Cipro tablets alone and in the presence of DS. For experiments involving the presence of DS, 50 mg of the DS powdered raw material was added to the dissolution medium 20 min before the Cipro tablets were added in order to ensure the complete dissolution of DS.

### Permeation studies

Caco-2 permeability studies were carried out for Cipro and the complexes by applying a previously described method [5]. Caco-2 cells were supplemented with 10 % heat-inactivated fetal bovine serum (FBS) and 1 % penicillin-streptomycin. When cells became 80 % confluent, the medium was aspirated and the attached cells were washed out with a phosphate buffered saline (PBS). Then, 2 ml of trypsin-EDTA was added to the flask and incubated at 37 °C for 10-15 min until the cells detached. A 5 ml medium was added and the resulting suspension was aspirated and transferred to a conical tube. Appropriate volumes of the cell suspensions were used for the seeding of transwell plates. Monolayer integrity was assured by recording the trans-epithelial electrical resistance (TEER) during culture. Membranes were used in the experiments when the TEER<sub>final</sub> value reached more than 1000 Ω.cm<sup>2</sup>. Lucifer yellow rejection assay was done on the day before the transport study and only inserts showing more than 99 % rejection were used.

Both sides of the monolayers were washed with Hank's balanced salt solution (HBSS) and pre-

incubated with the transport buffer (HBSS pH 7.4) for 60 min at 37 °C. Then 500 µl of Cipro solutions alone (15 µg/ml) or in the presence of equimolar amounts of DS and/or the metal ion were added to the apical compartment. The receiver chambers were filled with 1500 µl of fresh buffer. Samples (150 µl) were withdrawn from the receiver compartments at 30, 60, 120 and 240 min and were replaced with fresh buffer to maintain sink conditions. At the last time point (240 min), samples were taken from the donor compartments as well in order to confirm mass balance.

### Assessment of antibacterial activity

Cultures of *Staphylococcus aureus* (*S. aureus*; ATCC<sup>®</sup> 29213) and *Escherichia coli* (*E. coli*; ATCC<sup>®</sup> 8739) were used to assess the minimal inhibitory concentration (MIC) values of the test compounds using the two fold broth micro dilution method in 96-well plates. A double-strength medium (100 µl) of Mueller Hinton broth (Oxoid, UK) was used to fill the first experimental well. The other wells were filled with single-strength medium (100 µl). A 100 µl volume of Cipro (66 µg/ml), Cipro:DS (32 µg/ml), Cipro:Fe<sup>2+</sup>:DS (32 µg/ml), or Cipro:Ca<sup>2+</sup>:DS (32 µg/ml) was added to the first well. Cultured microorganisms (10 µl) were used to inoculate each well to achieve an inoculum size of ca. 1.5 × 10<sup>6</sup> CFU/ml. Negative controls were performed with only sterile broth and positive controls were performed with only bacterial culture in the wells.

## RESULTS

### Effect of ion pair formation on pH of solution

When DS was titrated with Cipro in the presence or absence of Ca<sup>2+</sup>, the pH generally decreased. However, the profile for DS when titrated with Cipro was obviously different than that obtained in the presence of calcium. The estimated ratio of potential complexes could be estimated from the breaking points on the obtained curves. Accordingly, the estimated stoichiometry is shown in Table 1.

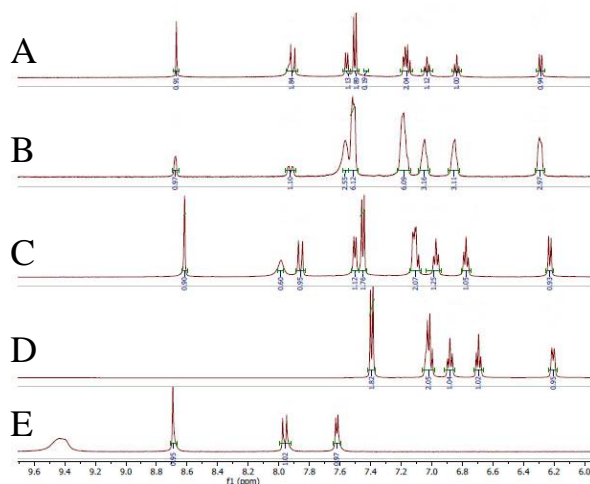
**Table 1:** Stoichiometry of binding of Cipro to DS alone or in the presence of metal ions.

Cipro:drug	DS	DS+Fe <sup>2+</sup>	DS+Ca <sup>2+</sup>
Ratio A	1:2	1:1	1:1
Ratio B	1:1	3:2	3:2

### NMR spectra

The obtained NMR spectra for DS, Cipro and complexes are shown in Figure 2. Generally, the

spectra accorded well with those previously reported [12, 13]. Thus, aromatic protons number 1, 2 and 3 in Cipro resonated at 8.7, 7.9 and 7.6, respectively, while protons in DS appeared at 7.50 ppm (protons 1 + 3, d), 7.13 (protons 5 + 7, t), 7.03 (proton 2, t), 6.82 (proton 6, t), and 6.25 (proton 4, d). The major observation in the NMR spectra for the product of DS with Cipro was the obvious presence of protons that were characteristic of both DS and Cipro.



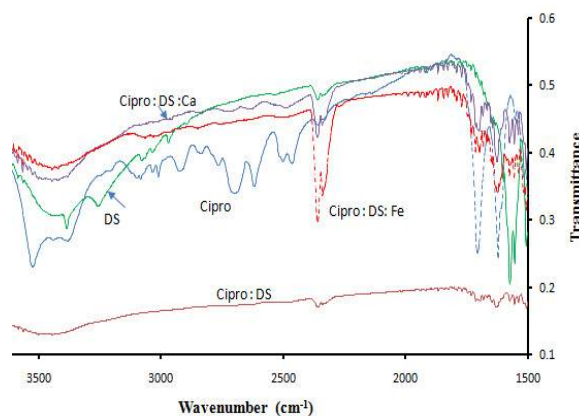
**Figure 2:** NMR spectra for Cipro (A), DS (B), Cipro:Ca<sup>2+</sup>:DS (C), Cipro:DS (r= 1:5, D), Cipro:DS (ratio = 5:1, E)

### FTIR spectra

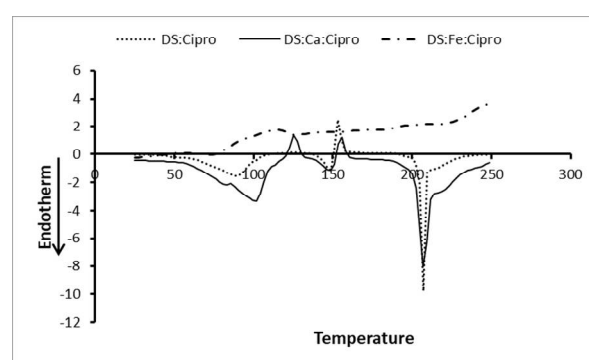
FTIR spectra for parent compounds and the potential ion pair products/complexes are shown in Figure 3. The most obvious changes were those of the absorption bands corresponding to the carbonyl groups of Cipro and DS, where they were significantly diminished in intensity or shifted in frequency as a result of the complex formation. The other area of significant change was that of the exchangeable protons (3300 - 3550 cm<sup>-1</sup>), where it also decreased significantly for complexes as compared to the parent compounds.

### DSC thermograms

The DSC profiles for the complexes are shown in Figure 4. The most prominent observation in the obtained DSC thermograms was the clearly different thermograms for the obtained products as compared to free Cipro.



**Figure 3:** FTIR spectra for Cipro, DS, Cipro:DS and Cipro:Fe<sup>2+</sup>:DS



**Figure 4:** DSC thermograms of Cipro:metal complexes

### Solubility and partition coefficient

A summary of the solubility and partition coefficients (log *P*) for the prepared complexes is presented in Table 2. Only one product (Cipro:DS) with a ratio of 1:2 exhibited a higher solubility than native Cipro, while other prepared complexes showed obviously lower solubility values.

**Table 2:** Solubility (in phosphate buffer pH 6.8) and octanol/buffer apparent partition coefficient (log *P*) of Cipro and the complexes

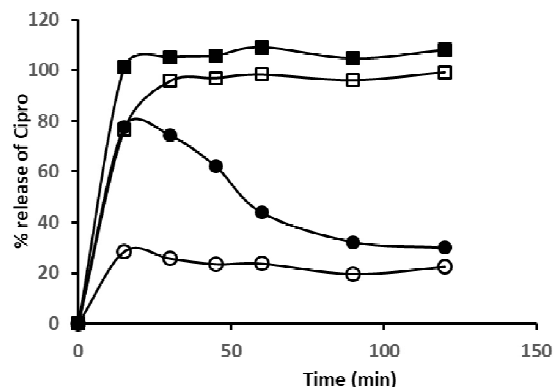
Compound	Solubility (µg/ml)	log <i>P</i>
Cipro	90.5	0.8
Cipro:DS (1:1)	55.6	6.4
Cipro:DS (1:2)	146.5	0.77
Cipro:Ca <sup>2+</sup> :DS	57.8	6.2
Cipro:Fe <sup>2+</sup> :DS	22.5	7.4

### Dissolution

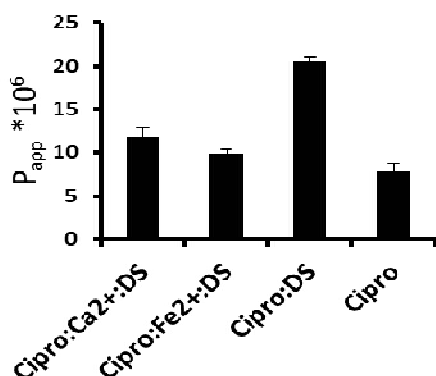
The dissolution behavior of Cipro in the absence and presence of DS was studied in distilled water and in the phosphate buffer pH 6.8. The results are presented in Figure. 5.

## Permeation through Caco-2 cells

The obtained apparent permeability coefficients for the diffusion of Cipro through a Caco-2 monolayer in the presence and absence of DS and/or test metal ions are presented in Figure 6.



**Figure 5:** Dissolution profiles of Cipro in distilled water (□), Cipro:DS in distilled water (■), Cipro in phosphate buffer pH 6.8 (○), and Cipro:DS in phosphate buffer pH 6.8 (●)



**Figure 6:** Calculated apparent permeability coefficient ( $P_{app}$ ) for Cipro, Cipro:DS, Cipro:Fe<sup>2+</sup>:DS, and Cipro:Ca<sup>2+</sup>:DS

## Antimicrobial activity

The MIC values for Cipro and Cipro:DS against *E. coli* were 0.004  $\mu\text{g/ml}$  and 0.026  $\mu\text{g/ml}$ , respectively, but against *S. aureus* 0.258  $\mu\text{g/ml}$  and 0.406  $\mu\text{g/ml}$ . Ternary complexes involving metal ions against *E. coli* exhibited MIC values of 0.016  $\mu\text{g/ml}$  and 0.032  $\mu\text{g/ml}$  for calcium and iron metals, respectively. However, against *S. aureus* they exhibited values of 0.5  $\mu\text{g/ml}$  and 1  $\mu\text{g/ml}$ , respectively.

## DISCUSSION

The ability of Cipro to associate with DS to form an ion pair has been shown using different techniques including titrations, DSC, FTIR and

NMR spectroscopy. Cipro appeared to form complexes with DS at ratios of 1:2 and/or 1:1. Interestingly, the presence of metal ions in the titration medium appeared to modify the ratios and perhaps the affinity of Cipro binding to DS with the apparent formation of ternary complexes. In the case of iron, the occurrence of a ternary complex was even evident visually because the obtained precipitate was of a dark orange color, while DS:Fe<sup>2+</sup>, Cipro:Fe<sup>2+</sup> and Cipro:DS showed no such color.

The composition of Cipro:DS and the ternary complexes (Cipro:Metal:DS) were assessed by HPLC using a condition shown to separate Cipro from DS. Accordingly, a percentage of Cipro, DS and the metal component could be estimated with the aid of properly constructed calibration curves. The association between Cipro and DS was found to be 1:1 when the two compounds were mixed at equimolar ratio. However, the ratio was influenced by the added ratio of the two drugs so that 1:1 and 1:2 ratios could be obtained.

FTIR provided evidence for the presence of the two compounds in the obtained products through the existence of their characteristic absorption bands, thus confirming the occurrence of the ion pair. Moreover, in presence of the metals, the obtained products exhibited dramatically different FTIR spectra but still kept the absorption bands characteristic for the carbonyl stretching in both DS and Cipro.

Further powerful evidence came from NMR spectra which clearly showed the presence of the characteristic protons for both DS and Cipro in the obtained product. However, the frequency of some aromatic protons of DS, in particular, were clearly shifted downfield, i.e., die-shielded. Similar to what was observed by HPLC analysis, the products obtained by the addition of Cipro to DS at different ratios appeared to have different ratios for the integrated peak areas of selected protons in Cipro to those of DS, e.g., the peak of Cipro aromatic proton at 8.7 compared to that of DS at 6.3. When Cipro was in excess, only a 1:1 product was obtained, but when DS was in excess, a product with a ratio of 1:2 (Cipro:DS) was obtained.

It is noteworthy that the NMR spectrum of the product at a 1:2 ratio appeared similar to that of 1:1 in terms of number and frequency for the signals of protons, but these signals were clearly broader. NMR spectra also provided evidence for the ternary complex formation between Cipro, DS and Ca<sup>2+</sup>. The spectrum for the ternary product of Cipro:Ca<sup>2+</sup>:DS exhibited frequencies

for aromatic protons that were intermediate between those of free and the complexed Cipro, with the emergence of a new broad signal at about 8 ppm. This signal was mostly related to the NH protons of either Cipro or DS. Calculations of the ratios for the integrated peak areas for proton 1 and 4 on Cipro and DS, respectively, provided a stoichiometry of 1:1 for the two compounds in the ternary complexes.

It is obvious that the different products obtained had significantly different DSC thermograms compared to each other and to free Cipro. While all the examined complexes did not show the characteristic peak for the thermogram of Cipro alone at about 150 °C, all of the complexes that involved DS with Cipro exhibited a sharp endotherm at about 210 °C. The complex Cipro:Fe<sup>2+</sup>:DS was obviously different because it showed absolutely no endothermic peaks in the entire range of temperatures examined.

Cipro exhibited a solubility of 90.5 µg/ml, which was close to previously reported values [14]. The ternary complexes of Cipro:Ca<sup>2+</sup>:DS and Cipro:Fe<sup>2+</sup>:DS exhibited obviously lower solubility values. It was quite interesting to observe that the product of 1:2 Cipro:DS complexes exhibited a significantly higher water solubility than the 1:1 product and even higher than that of Cipro. The obvious explanation is that the 1:2 complex exhibits a higher net negative charge (due to having 2 molecules of the acidic DS) at the employed pH. To a large extent, that was reflected in the values of the obtained apparent partition coefficient log *P*, where Cipro:DS (1:1) showed an almost 10 times higher log *P* than free Cipro, while the 1:2 complex had an almost similar log *P* to free Cipro.

Ternary complexes involving metal ions exhibited similar or slightly higher log *P* values than the 1:1 complexes, which accords with our findings that these complexes form predominantly as 1:1. The hydrophilicity of native Cipro with its consequent poor bioavailability is well known and some approaches have been attempted to overcome that hurdle [15]. Therefore, the observed increase in lipophilicity of Cipro:DS (1:1) compared to Cipro might be an obvious explanation for the previously reported *in vivo* enhancement of Cipro bioavailability [11].

In the phosphate buffer pH 6.8, Cipro exhibited a much lower dissolution (maximum at about 25 %) than that in distilled water which was in accordance with previous reports [16]; Cipro being a zwitterion with its minimum net charge in about the neutral pH range. The presence of DS appeared to significantly enhance the initial rate

of dissolution of Cipro in a phosphate buffer (a maximum of 80 %), but the percentage dissolved was later decreased to reach its expected percentage (~ 20 %) towards the end of the test period. A plausible explanation would be the formation of a 1:2 ion pair at the early stages of dissolution when the amount of Cipro could be low and DS high, but 1:1 as more Cipro is released into the solution. Since 1:2 complexes were shown to be more soluble than native Cipro, an increase in the initial rate of dissolution was observed. Towards the end of the dissolution test, the chances would be higher for 1:1 complexes to form and consequently the extent of the dissolution returned to a lower value.

Cipro exhibited a Papp value of 7.9 x 10<sup>-6</sup> cm/s which was close to previously reported literature values [14,17,18]. Furthermore, in this study it was the lowest value in comparison to any other case where DS was present. In the presence of DS, Cipro exhibited an almost three times increase in Papp (20.6 x 10<sup>-6</sup> cm/s). Even in the presence of metals (calcium and iron), the measured Papp for Cipro was obviously higher than that of Cipro alone (or at least similar). In all cases the obtained Papp values in the presence of DS were shown to be statistically different than those for Cipro alone; a summary of the statistical comparison of the different cases is shown in Table 3.

**Table 3:** Summary of statistical comparison (t-test) of the Papp values calculated for the obtained complexes compared to Cipro alone (*p* < 0.05)

Ion pair/complex	t-value	P-value
Cipro:Ca <sup>2+</sup> :DS	4.074	0.01517
Cipro:Fe <sup>2+</sup> :DS	3.5367	0.0241
Cipro:DS	14.601	0.00013

Thus, it can be stated that the previously reported increase in bioavailability of Cipro when co-administered with DS is as a result of an ion pair complex formation [11]. The different forms that have been shown to form (1:1 and 1:2) are expected to contribute to the overall positive effect on the bioavailability of Cipro through a combination of improved dissolution (1:2), on one hand, and lipophilicity (1:1), on the other. It was particularly interesting to observe that the presence of DS even counteracted the well known negative effect of metal ions on the bioavailability of Cipro.

For gram-positive bacteria, the obtained MIC value for Cipro was fairly comparable to those previously reported [19]. In all cases, MIC values were increased for the Cipro:DS product and even more so for the products with metals. The

effect seemed to be more dramatic in cases of *E. coli* than *S. aureus*. Particularly, MIC for Cipro:DS was ~ 6 times and 2 times higher than that of Cipro against *E. coli* and *S. aureus*, respectively. Such findings accord with the products being more lipophilic and bulkier than free Cipro, thus more difficult to permeate through the inherently tight hydrophilic polysaccharide cell wall of *E. coli* [20]. For *S. aureus*, however, when the theoretical percentage of Cipro in the prepared products is considered, the increase in MIC might be explained simply by the idea that the products contain significantly lower percentages of Cipro (30 - 50 %w/w). Calcium and iron ions appeared to have differential effects on the MIC values; iron clearly decreased antimicrobial activity, while calcium improved it against *S. aureus*.

## CONCLUSION

It has been demonstrated in the present study that complexation between Cipro and DS does occur, with either 1:1 or 1:2 stoichiometry. The complexes have different physicochemical properties from Cipro, including solubility and apparent partition coefficient, which might be the major reason for their greater bioavailability than free Cipro. The improved permeation of the investigated complexes through Caco-2 cells (2-3 times) suggests that the absorption stage is most critical in the interaction between DS and Cipro. These findings may facilitate the design of new ion pairs with Cipro that might be safer (than DS) and more bioavailable.

## DECLARATIONS

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### Conflict of interest

The authors declare that no conflict of interest is associated with this work.

### Contribution of authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Imad I. Hamdan conceived and designed the study. All authors contributed to the laboratory work. The

manuscript was proof-read by all the authors and approved for publication.

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