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RESEARCH ARTICLE

The pH-solubility profiles of levofloxacin hemihydrate and ciprofloxacin lactate

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Keywords
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Abstract

Background: Levofloxacin and ciprofloxacin are popular fluoroquinolone antibiotics that offer diverse therapeutic potentials due to their broad-spectrum activity. Levofloxacin and ciprofloxacin, commonly used as active pharmaceutical ingredients (API) in parenteral form, are amphoteric compounds with pH-sensitive solubility. This characteristic may affect the safety and efficacy of levofloxacin and ciprofloxacin, especially in the parenteral dosage forms. **Objective:** This study aims to assess the solubility of levofloxacin hemihydrate and ciprofloxacin lactate in buffer solutions with pH ranging from 3.0 to 8.0. **Method**: The solubility test was conducted in a 0.02 M buffer pH solution featuring an ionic strength (μ) of 0.2. After weighing several API samples, ultrasonication was carried out for 15 minutes. The solution was agitated at 150 rpm and 30 ± 0.5°C until it reached equilibrium (four hours). The API concentration was observed using a UV-Vis spectrophotometer. **Result**: The solubility ranges of levofloxacin hemihydrate and ciprofloxacin lactate were 44.39 to 70.66 mg/mL and 0.23 to 243.00 mg/mL, respectively, within the experimental pH range. **Conclusion**: This study concluded that levofloxacin hemihydrate and ciprofloxacin lactate solubility were increased due to decreasing pH medium.

Introduction

Ciprofloxacin and levofloxacin are members of the fluoroquinolone antibiotic class, renowned for their broad-spectrum activity against various infections (Ezelarab *et al.*, 2018). Additionally, intravascular administration of pharmaceutical preparations remains a primary intervention in emergency cases involving patients with acute medical conditions, severe infections, as well as unresponsiveness due to nausea or unconsciousness among elderly individuals (Nagarsenkar & Dhawan, 2020). This approach assures 100% bioavailability and facilitates rapid physiological responses (Aulton & Taylor, 2018).

Parenteral preparations administered intravenously are generally in the form of aqueous carrier solutions. Consequently, the solubility of active pharmaceutical ingredients (API) in water emerges as a crucial physicochemical attribute, fundamentally influencing preparation formulation. Water-soluble drugs mix more easily with body fluids post-injection, expediting their interaction with physiological systems. This acceleration translates into a quicker onset of therapeutic effects than oil-based carrier solutions. However, only API with greater solubility than the required dosage and good water stability can be produced in solution forms.

Levofloxacin, an amphoteric compound, exhibits dual characteristics as a weak base and weak acid with a pKa_1 value of 5.83 and pKa_2 of 8.75 (Czyrski, 2022). Furthermore, its solubility is affected by pH, spanning from 32 to 91 mg/mL within the pH range of 1.2 – 6.8 (Sarisaltik & Texin, 2007). Levofloxacin, as a levorotatory isomer of D, L ofloxacin, possesses one chiral carbon, three polymorphic forms (anhydrous α, β, and γ), hemihydrate and monohydrate pseudo polymorphs, and six solvate variations (A, B, C, F, G, and

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H). The stability of the hemihydrate crystalline form in storage and under moisture exposure renders it a widely used active ingredient in pharmaceutical preparations (Koeppe *et al.*, 2011). Similarly, ciprofloxacin, characterised by amphoteric traits with a pKa_1 value of 5.76 and pKa_2 of 8.68 (Jiang, 2012; Vidyavathi & Srividya, 2018), shows maximum solubility (> 40 mg/mL) at a pH range of 4.0 – 5.0 (Parwe, 2013). The ciprofloxacin lactate salt form, extensively employed in pharmaceutical preparations, features a water solubility exceeding 100 mg/ml (Yeon, 1996).

The pH of the medium often influences the solubility of weakly acidic or basic compounds. Weak acid compounds manifest enhanced solubility in alkaline environments, while weak bases dissolve more easily in acidic surroundings (Isadiartuti *et al.*, 2022). The pH selection for API preparations affects their solubility, stability, and acceptability upon usage. To minimise tissue irritation, parenteral preparations usually adopt pH ranges of 3.5-9.0 (Roethlisberger *et al.*, 2017). The Henderson-Hasselbalch equation, combined with knowledge of media pH and compound pKa values, facilitates predicting the number of ionised and unionised molecules in the solution. A higher percentage of ionised molecules correlates with greater compound solubility (Florence & Attwood, 2016; Isadiartuti *et al.*, 2021).

This study aims to assess the solubility of levofloxacin hemihydrate and ciprofloxacin lactate in buffer solutions with pH values of 3.0 – 8.0, a range necessary for parenteral preparation acceptability. There is no previous investigation encompassing the solubility of both antibiotics within this specific pH range. The solubility assessment will yield the pH-solubility profiles for levofloxacin hemihydrate and ciprofloxacin lactate, serving as essential criteria in the pre-formulation exploration of parenteral preparation development.

Methods

Materials

The materials employed in this study were injection-grade levofloxacin hemihydrate (Shangyu Jingxin Pharmaceutical Co., Ltd), ciprofloxacin lactate (Zheijian Guobang Pharmaceutical Co., Ltd), water for injection (PT. Satoria Aneka Industri), and buffer ingredients with a pro-analytical quality, including $C_6H_8O_7.H_2O$, $C_6H_5O_7Na_3.2H_2O$, $NaH_2PO_4.H_2O$, and Na_2HPO_4 (Merck).

Determination of maximum wavelength and standard curve equation of levofloxacin hemihydrate and ciprofloxacin lactate in various pH

The maximum wavelength of levofloxacin hemihydrate and ciprofloxacin lactate was determined through UV-Vis spectrophotometry (Hitachi UH 5300). This analysis involved measuring two concentrations of the working standard solution within different pH buffer solutions (3.0, 4.0, 5.0, 6.0, 7.0, and 8.0). The wavelength scan was conducted in the 200 – 400 nm range, identifying the wavelength that exhibited the highest absorbance for each pH using a UV-Vis spectrophotometer. Subsequently, a standard curve regression equation was formulated, along with calculating the correlation coefficient (*r* count), and V_{x0} value.

Determination of the solubility of levofloxacin hemihydrate and ciprofloxacin lactate in various pHb buffer solutions

Excess amounts of levofloxacin hemihydrate and ciprofloxacin lactate were introduced into vials containing 5 mL buffer solutions with varying pH levels (ranging from 3.0 – 8.0) at a 0.02 M concentration and an ionic strength of 0.2. Subsequently, the solution was ultrasonicated (Ultrasonic Cleaner 020S) for 15 minutes and agitated in a water bath shaker (Memmert WBB14) at 150 rpm at 30 ± 0.5°C until saturation. The sample was filtrated through a 10 μm filter, followed by a 0.22 μm millipore filter paper. The resulting filtrate was diluted using a suitable buffer solution and examined for absorbance at the maximum wavelength of the active ingredient in the respective pH buffer solution. This process was repeated three times for each pH, and the acquired data were analysed with One-way Analysis of variance (ANOVA), followed by the Post Hoc Tukey Honestly Significant Difference (HSD) test at a significance level of $\alpha = 0.05$.

Results

Maximum wavelength and standard curve equation of levofloxacin hemihydrate and ciprofloxacin lactate in various pH buffer solutions

The maximum wavelengths and standard curve equations for levofloxacin hemihydrate and ciprofloxacin lactate across diverse pH buffer solutions are presented in Tables I and II, respectively. In each standard curve, the correlation coefficient (*r* count) exceeded the *r* table, and the V_{x0} value was ≤ 5%, indicating a linear relationship between concentration increase and absorbance.

Table I: Maximum wavelength and standard curve regression equation of levofloxacin hemihydrate at various pH levels

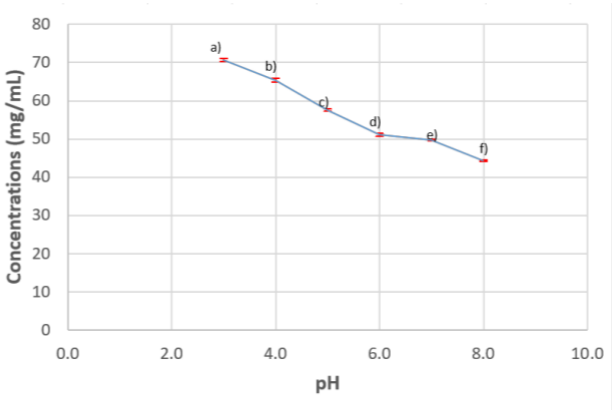
Buffer solution	pH	Λmax (nm)	Regression equation	r_{count}	r_{table}	V_{x0}
Citrate	3.0	293.5	y = 0.0733x + 0,0080	0.9995	0.8783	1.72%
Citrate	4.0	293.5	y = 0.0970x – 0.0675	0.9994	0.8783	1.47%
Citrate	5.0	293.5	y = 0.0983x + 0.0001	0.9993	0.8783	1.70%
Citrate	6.0	290.0	y = 0.0717x – 0.0266	0.9995	0.8783	1.27%
Phosphate	7.0	287.2	y = 0.0737x – 0.0478	0.9998	0.8783	0.66%
Phosphate	8.0	286.8	y = 0.0700x + 0.0179	0.9994	0.8783	1.83%

Table II: Maximum wavelength and standard curve regression equation of ciprofloxacin lactate in various pH conditions

Buffer solution	pH	Λmax (nm)	Regression equation	r_{count}	r_{table}	V_{x0}
Citrate	3.0	277.6	y = 0.0448x + 0.1524	0.9995	0.8783	1.47%
Citrate	4.0	277.4	y = 0.0996x – 0.0048	0.9998	0.8783	0.80%
Citrate	5.0	277.8	y = 0.0722x – 0.0622	0.9998	0.8783	0.95%
Citrate	6.0	274.8	y = 0.0540x + 0.0034	0.9993	0.8783	1.73%
Phosphate	7.0	271.4	y = 0.1023x + 0.0060	0.9995	0.8783	1.40%
Phosphate	8.0	271.0	y = 0.0706x + 0.0687	0.9998	0.8783	0.68%

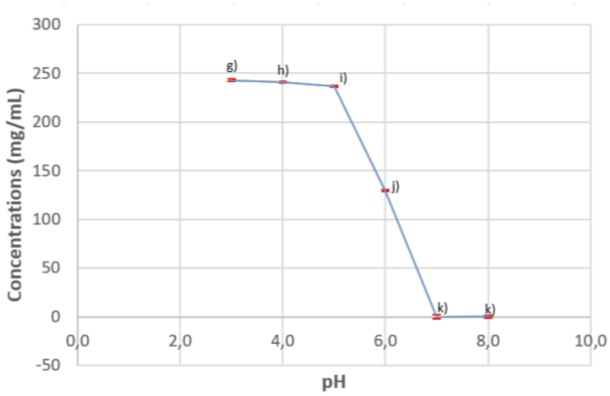
Solubility of levofloxacin hemihydrate and ciprofloxacin lactate in various pH buffer solutions

The solubility of levofloxacin hemihydrate and ciprofloxacin lactate in different pH buffer solutions was assessed until saturation solubility was attained, which occurred after four hours of agitation in a water bath shaker at 30 ± 0.5°C. The attained constant concentration levels were determined through one-way ANOVA testing. The solubility test results for both antibiotics across various pH conditions can be seen in Figures 1 and 2.



a) b) c) d) e) f) The different superscript letters showed significant differences between groups (α = 0.05; n=3)

Figure 1: The pH-solubility profiles of levofloxacin hemihydrate at 30 ± 0.5 °C



g) h) i) j) k) The different superscript letters showed significant differences between groups (α = 0.05; n=3)

Figure 2: The pH-solubility profiles of ciprofloxacin lactate at 30 ± 0.5 °C

Discussion

Effect of pH on the wavelength of levofloxacin hemihydrate and ciprofloxacin lactate

The maximum wavelength of levofloxacin hemihydrate and ciprofloxacin lactate across the pH range of 3.0 - 8.0 showed a shift towards shorter wavelengths. Both antibiotics exhibited identical wavelengths within pH 3.0 - 5.0 due to their cationic form. The maximum wavelength of levofloxacin hemihydrate at pH 3.0 – 5.0 reached its highest value because of an ionisation event that led to the formation of cations. The formation of cations during ionisation appears to give rise to additional chromophore groups, causing longer wavelengths in the UV region (Pavia 2015). Furthermore, the presence of the auxochrome -NCH_3 group in levofloxacin contributed to the chromophore system widening, prompting a shift towards longer wavelengths (Wisudyaningsih *et al.*, 2014; Pavia, 2015). At a higher pH of 6.0 – 8.0, levofloxacin hemihydrate and ciprofloxacin lactate transitioned into the zwitterion forms with distinct ionisation levels. The difference in the degree of ionisation causes protonation of the carboxyl group of both compounds, disrupting the conjugate bond and causing a shift to shorter or hypochromic wavelengths (Pavia *et al.*, 2015).

Solubility of levofloxacin hemihydrate in various pH buffer solutions

As presented in Figure 1, the results of the levofloxacin hemihydrate solubility test showed dissolution levels of 70.66 ± 0.43, 65.40 ± 0.56, 57.55 ± 0.32, 51.07 ± 0.44, 49.66 ± 0.17, and 44.39 ± 0.18 mg/mL at pH 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0, respectively. The solubility of this antibiotic increased with decreasing pH value, particularly evident within the pH range of 3.0 – 5.0, where ionisation and cation formation occurred. Protonation of the piperazinyl molecular group instigated hydrogen bonding between levofloxacin hemihydrate and water molecules, leading to enhanced solubility (Blokhina *et al.*, 2016). The highest solubility was observed at pH 3.0, where the pH was approximately two units less than the pKa_1 value. Within these conditions, levofloxacin hemihydrate became completely ionised, promoting enhanced interaction with water molecules and increased solubility (Florence & Attwood, 2016). At pH 6.0 – 8.0, the unionised hemihydrate form equilibrated with the zwitterion molecule, usually difficult to dissolve. Consequently, the solubility obtained at pH 6.0 – 8.0 was lower than at pH 3.0 – 5.0 (Wisudyaningsih *et al.*, 2014; Blokhina *et al.*, 2016). The one-way ANOVA and the HSD test conducted at α 0.05 showed significant differences in solubility achieved at pH 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0.

Solubility of ciprofloxacin lactate in various pH buffer solutions

Based on Figure 2, the results of the ciprofloxacin lactate test showed dissolution levels of 243.08 ± 1.12, 240.72 ± 0.92, 236.91 ± 0.69, 129.75 ± 1.16, 0.182 ± 0.00, and 0.23 ± 0.00 mg/mL in buffer solutions with pH values of 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0 respectively. The solubility of this antibiotic increased with decreasing pH, particularly at a pH below the pKa_1 value of 5.76. The event was driven by the presence of ionic base groups in the cationic form and protonation of the amine groups within the piperazine molecule. Lactate ions binding to the carboxylic group further boosted solubility by alkalising ciprofloxacin. Theoretically, the number of ionised and unionised molecules of a substance at various pH levels was predicted using the Henderson-Hasselbalch equation (Sinko, 2017). At two pH units below the pKa value, 99.99% of ciprofloxacin was ionised, while at two units above the pKa value, the ionised form was only 0.01% (Florence & Attwood, 2016). Furthermore, the solubility decline observed at a pH close to 7.0 stemmed from the equilibrium between the protonation of the amine group and deprotonation of the carboxylic acid group, producing zwitterions (Jiang, 2012). The Zwitterions, which reached their peak concentration around pH 7.5, were formed due to the balance of charges between cations (amine groups) and anions (carboxylic acids). The isoelectric point was the state of balance of the total charge for both ions, where the lowest solubility was attained (Jalil *et al.*, 2015). One-way ANOVA followed by the HSD test at a confidence level of α 0.05 revealed significant differences in the solubility of the examined antibiotic at each pH, excluding pH 7.0 and 8.0.

The results collected from the solubility assessments were employed in preparing the pH-solubility profiles for each active compound, as depicted in Figures 1 and 2. The gradual decrease in the solubility of levofloxacin hemihydrate with increasing pH was evident. However, the pH-solubility profiles of ciprofloxacin lactate exhibited a significant decline at pH 7.0 and 8.0. These profiles offered valuable insights for pH determination when designing aqueous dosage forms for both antibiotics. The formulation of solution-based preparations would become feasible by conducting pH adjustments to ensure greater solubility than the preparation dosage.

Conclusion

In conclusion, levofloxacin hemihydrate and ciprofloxacin lactate were amphoteric compounds with solubility levels often affected by their pKa value and solution medium pH. The pH-solubility profiles deduced from the experimental results indicated that the solubility of these two antibiotics increased with decreasing pH values. This phenomenal increase was due to ionisation which caused an elevation in the number of ionised molecules. The ionised form of a compound would be more soluble in water than the unionised counterpart.

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