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Nortriptyline Hydrochloride Solubility-pH Profiles in a Saline Phosphate Buffer: Drug-Phosphate Complexes and Multiple pH_{max} Domains with a Gibbs Phase Rule "Soft" Constraints

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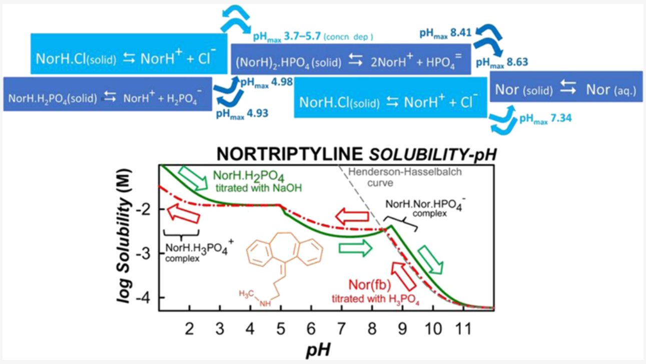
ABSTRACT: The solubility of a model basic drug, nortriptyline (Nor), was investigated as a function of pH in phosphate and/or a chloride-containing aqueous suspension using experimental practices recommended in the previously published "white paper" (Avdeef et al., 2016). The pH-Ramp Shake-Flask (pH-RSF) method, introduced in our earlier work (Marković et al., 2019), was applied. An improved and more detailed experimental design of the Nor solubility measurement allowed us to exploit the full capacity of the pH-RSF method. Complex equilibria in the aqueous phase (cationic and anionic complex formation between Nor and the phosphate) and solid-phase transformations (Nor free base, 1:1 Nor hydrochloride salt, 1:1 and 1:2 Nor phosphate salts) were characterized by a detailed analysis of the solubility measurements using the computer program *p*DISOL-X. The solid phases were characterized by thermogravimetric analysis, differential scanning calorimetry, powder X-ray diffraction, and elemental analyses. The results of the present investigation illustrate the influence of competing counterions, such as buffering agents, complexing agents, salt coformers, tonicity adjusters, and so forth, on the aqueous solubility of drugs and interconversion of salts. Careful attention given to these factors can be helpful in the formulation of drug products.



KEYWORDS: *nortriptyline, nortriptyline hydrochloride, phosphate salts, complexes, solubility, solubility product, pH and buffer effect*

INTRODUCTION

Nortriptyline (Nor) (Figure 1a) belongs to a class of drugs called tricyclic antidepressants (TCAs). It is a dibenzocycloheptadiene having a chain containing a secondary amine attached to the fused rings. As a free base, Nor is practically insoluble in water, but, as the amine group undergoes protonation with the lowering of pH, the compound exhibits amphiphilic or surface-active properties with a higher solubility. It can produce supersaturated solutions near the pH_{max} due to the formation of sub-micellar or micellar aggregates, a phenomenon well described in literature studies.[1−7] Nor has certain structural similarities with desipramine, another TCA which we studied previously in our laboratory,[8] where the tricyclic moiety is a dibenzazepine instead of dibenzocycloheptadiene (Figure 1b). Many basic drugs exhibit supersaturation during the phase transition from free basic forms to salts or vice versa.[9−16] A special property of these amphiphilic drugs is that they may separate out as oil under alkaline pH conditions, exhibiting elevated solubility, thus potentially confounding solubility determination and interpretation. The primary objective in the present investigation was to determine whether Nor would also behave in a similar manner.

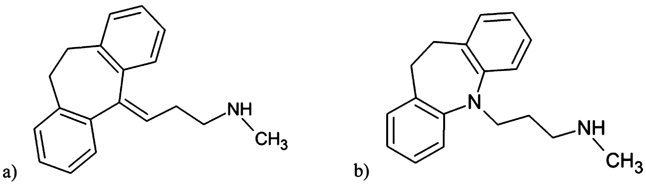


Figure 1. Structures of (a) Nor and (b) desipramine.

Various methods for solubility determination of Nor have been reported in the literature. In a comparative study, Box et al.[17] determined the intrinsic solubility of Nor at 25 °C using a saturation shake-flask (SSF) and the CheqSol potentiometric titration methods. The Britton-Robinson buffer was used in the

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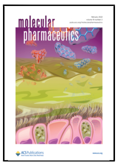

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SSF method to determine the solubility at pH 11.5, where 95% of Nor was in the unionized form. The resultant intrinsic solubility was reported as $\log S_0 = -3.73 \pm 0.01$ (log molarity units). In the CheqSol method, a lower intrinsic solubility was determined in 0.15 M KCl: $\log S_0 = -3.99 \pm 0.02$. Nor behaved as a "CheqSol non-chaser," which did not form supersaturated solutions. As part of a solubility prediction study, Llinàs et al.[18] used the CheqSol method to determine the solubility of Nor in 0.15 M KCl at 25 °C as −4.02 (intrinsic) and −3.99 ("kinetic"). Melero et al.[19] determined the solubility of Nor in phosphate buffer at pH 5.5 at 32 °C ($\log S = -0.98$) and at pH 7.4 ($\log S = -2.56$) using the SSF method as part of a Nor skin penetration study. Also, the dissolution template titration method was used to determine $\log S_0 = -4.18$.[20] A solid-state analysis was not reported in the above studies.

As pointed out by Llinàs et al.,[18] most solubility measurements in the literature are reported with no reference to the solid state of the materials in equilibrium with solutions, which could be crystalline, amorphous, or both. There could even be a change in the chemical form of the equilibrium solid depending on the buffers used (e.g., change from one salt form to another).[8,21] The impacts of the buffers on the drug-buffer precipitation and complexation were not explored in the early studies.

The newly described pH-Ramp Shake-Flask (pH-RSF) method was applied to a related amphiphilic tricyclic base, desipramine (Figure 1b).[8] Solubility-pH simulations using the computer program *p*DISOL-X (in-ADME Research) guided the assay design. In a systematic way, titrations were performed from high-to-low and low-to-high pH directions using both the chloride salt and the free base forms of the drug. Solid-state characterization of precipitates isolated at various pH points guided the equilibrium model construction.

In the present study, the above method was directed to interpret the complex aqueous solubility-pH behavior of Nor hydrochloride (NorHCl) in a saline phosphate buffer. Equilibrium solubility data were acquired as described in the previously published "white paper".[22] Multiple pH_{max} domains were found. The intrinsic solubility (S_0) and the solubility products (K_{sp}) of three Nor salts were determined: 1:1 hydrochloride salt plus 1:1 and 2:1 phosphate salts. Constraints arising out of the Gibbs phase rule were evident in certain multiphasic-pH/titrant profiles. The analysis of phase distributions was supported by solid-state characterizations of the various solid species isolated at various pH points.

The present investigation of Nor validated and further fine-tuned the assay design approach introduced in the earlier desipramine study, called pH-RSF. The core feature of the method is a complete simulation of the expected multi-phasic equilibrium reactions as a function of pH based on *in silico* predicted solubility constants. The results of the simulation contribute to an optimized design of an actual assay—the simulation guides the selection of optimal reactant concentrations and the critical pH regions for identifying the stoichiometries of the solids and the dissolved species which form. The research also highlights the role of counterions and buffering agents in the solubility of ionizable drugs. Different counterions or buffering agents may be used for the determination of solubility in the gastrointestinal pH range. In some cases, the solubility may be determined in one aqueous medium or buffer, while the composition of the formulation could be different, which may have a major influence on the physical stability of the product. For this reason, the solubility in the present investigation was determined in chloride-containing media and phosphate-containing media to ascertain what effects the composition of media may have on the solubility of Nor.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents. Nor hydrochloride was purchased from Tokyo Chemical Industry Co., Ltd. and used without further purification. Other chemicals used in the study were purchased from the following companies: sodium dihydrogen phosphate dihydrate (Sigma-Aldrich, analytical reagent grade), disodium hydrogen phosphate dihydrate (Alkaloid AD Skopje), hydrochloric acid and sodium hydroxide (Merck, Titrisol ampoules), and phosphoric acid (Fisher Chemical, analytical reagent grade). Deionized (DI) water was used for the preparation of all aqueous solutions. Nominal 1 M HCl and NaOH titrants were standardized to 0.9397 and 0.9065 M, respectively.

2.2. pH Measurement and Conversion to the p[H$^+$] Scale. The Crison pH-Burette 24 2S equipped with the Hach 52 09 micro combination electrode was used to measure the pH (operational activity scale). First, the electrode was calibrated with Hach standard buffer solutions (pH 4.01, 7.00, and 9.21). Since the reported equilibrium constants are based on the concentration scale, that is, the "constant ionic medium" thermodynamic standard state,[20] the operational pH-meter values were converted by means of a "HCl−NaOH blank titration" to those based on the concentration scale, $\text{p[H}^+] (= -\log[\text{H}^+])$ using the relationship[23]

$$\text{pH} = \alpha + k_{\text{S}}\text{p[H}^+] + j_{\text{H}}[\text{H}^+] + j_{\text{OH}}K_{\text{w}}/[\text{H}^+] \qquad (1)$$

where α corresponds to the negative logarithm of the activity coefficient of H$^+$ at a working temperature and ionic strength; the k_{s} term denotes the ratio between the actual slope and the Nernst slope; K_{w} is the ionization constant of water, taken as a function of temperature and ionic strength.[24] The j_{H} term corrects the pH readings for the nonlinearity due to the liquid junction and the glass asymmetry potentials in highly acidic solutions (pH < 1.5), whereas the j_{OH} term corrects for high pH (pH > 11.5) nonideal behavior.

2.3. HPLC Concentration Determination. The concentration of the drug in the supernatant solutions was determined using a HPLC-UV/Vis system (Agilent Technologies 1260 Infinity LC System), incorporating the Hypersil Gold 50 × 3 mm column packed with 5 μm particles. Chromatographic separation was conducted at a column temperature of 25 °C using gradient elution: from 70% A + 30% B to 100% B for 5 min, 100% B for 1 min, and back to 70% A + 30% B for 1 min (solvent A: water with 1% acetic acid; solvent B: acetonitrile). The flow rate was 0.5 mL/min, and the detection wavelength was 250 nm.

2.4. Differential Scanning Calorimetric Analysis. Differential scanning calorimetric (DSC) scans of solids isolated from suspensions during the determination of solubility as a function of pH and then air-dried for 3−4 days were obtained using a Q200 differential scanning calorimeter (TA Instruments, DE, USA). Accurately weighed samples (5−10 mg each) were sealed in a Tzero pan with a pinhole for the escape of any volatile material. The samples were heated to 250 °C at a ramp of 5 °C/min with a modulation of ±1.0 °C every min. The results were analyzed using the Universal Analysis software version 2000 (TA Instruments).

2.5. Thermogravimetric Analysis. The thermogravimetric analysis (TGA) Q50 thermogravimetric analyzer (TA Instruments, DE, USA) was used for the determination of any weight loss as a function of temperature. The samples were heated from ∼25 °C up to 400 °C at a heating rate of 10 °C/min in a nitrogen environment.

2.6. Powder X-ray Diffraction Analysis. The powder X-ray diffraction (PXRD) patterns were generated at room temperature using a powder X-ray diffractometer (Shimadzu 6000, Kyoto, Japan) having a monochromatic CuKα radiation source that was operated at 40 kV and 30 mA. The test materials were placed as thin layers in glass sample holders. The scanning rate of 2°/min was used over the 2θ range of 10−60°.

2.7. Elemental Analysis. The elemental analysis was accomplished by combustion analysis on a Vario EL III C,H,N,S/O Elemental Analyzer (Elementar Analysensysteme GmbH, Hanau-Germany). The samples were air-dried for 3 days before the analysis.

2.8. Solubility Determination Using the pH-RSF Titration Method. Titrations were conducted in two directions: low-to-high pH (titration sets 1−4) and high-to-low pH (titration sets 5−7). Furthermore, phosphate-free titrations (titration sets 8 and 9) and chloride-free titrations (titration sets 10 and 11) were also performed. The detailed stock solution preparation, titration, and solubility data are summarized in the Supporting Information (Tables S1−S14).

2.8.1. Titration Sets 1−4 (Low-to-High pH Titrations). Acidified stock suspensions (pH 1.66−2.17) were prepared by adding the solution of 0.15 M NaH_2PO_4 and 1 M HCl to a vessel containing weighed NorHCl. While the suspension was vigorously stirred, 1 mL aliquots were drawn from it and placed into each of 3−10 sample vials. This produced 3−10 nearly identical suspensions. The initial pH in the vials was measured. Then, different volumes of standardized 1 M NaOH were added to each vial. After equilibration (6 h of stirring and 18 h of sedimentation), the phases were separated by centrifugation.

Titration set 1, with three repeats, was the trial experiment to validate the predicted titrant volume additions in the pH-RSF setup. Titration sets 2 and 3 were replicate experiments, done in the pH range 2.12−11.35. Titration set 4 was prepared to complete the pH range in the alkaline region (three repeats in the pH range 11.33−12.41 and two control repeats with the pH value in the acidic region, pH 2.99 and 6.81).

2.8.2. Titration Sets 5−7 (High-to-Low pH Titrations). Alkaline stock suspensions (pH 11.24−12.57) in titration sets 5 and 6 were prepared by the addition of 10.00 mL of a solution of 0.15 M NaH_2PO_4 and 1 M NaOH to a vessel containing weighed NorHCl. Well-mixed stock suspensions were divided equally into 10 vials. The initial pH in 1 mL aliquots was measured and then adjusted by the addition of 1 M HCl. The equilibration time and phase separation were the same as in the low-to-high pH titrations.

Since in titration sets 5 and 6, the phosphate buffer was considerably diluted with NaOH to reach pH > 11, titration set 7 was designed to minimize the phosphate buffer dilution: an alkaline stock suspension (pH 11.12) was prepared by the addition of 0.15 M Na_2HPO_4 and 1 M NaOH to a vessel containing weighed NorHCl. The last steps were the same as for titration sets 5 and 6.

2.8.3. Titration Sets 8 and 9 (Phosphate-Free Titrations). Two sets without the phosphate buffer were designed to determine the drug-hydrochloride solubility product in the phosphate-free suspensions. Stock suspensions of NorHCl were prepared by the addition of 0.15 M NaCl to a vessel containing weighed NorHCl. To each of the 10 vials, a 1 mL aliquot of vigorously stirred stock suspension was added, and the pH was adjusted (1.80−12.37) using 1 M NaOH/HCl. The last steps were similar to the previously described ones.

2.8.4. Titration Sets 10 and 11 (Chloride-Free Titrations). Two sets without chlorides were designed to determine the drug-phosphate solubility products in chloride-free suspensions.

2.8.4.1. Titration Set 10.

(a) free base preparation: 1.800 mL aliquot of 1 M NaOH was added to a vessel containing 0.60050 g of NorHCl. The suspension was stirred for 10 min (pH 12.64). The stirring was stopped, and the suspension was allowed to settle, after which the solution portion was decanted. DI water (0.500 mL) was added to the residual oil and the mixture was gently agitated. The aqueous solution was decanted after centrifugation. DI water (0.200 mL) was added to the oil, and the vial was vortexed. The phases were separated by centrifugation. This procedure was repeated twice.

(b) Sample stock suspension preparation: 10.00 mL of DI water was added to a vial containing 0.50105 g of Nor (oil isolated in the previous step) and 0.65020 g of $NaH_2PO_4 \cdot 2H_2O$ (pH 4.93).

(c) Sample preparation: A 1 mL aliquot of vigorously stirred sample stock suspension was added to each of the 10 vials. The pH (1.82−6.07) was adjusted using 2.00 M H_3PO_4 and 1 M NaOH.

2.8.4.2. Titration Set 11.

(a) Free base preparation: 2.400 mL of 1 M NaOH was added to a vial containing 0.60075 g of NorHCl and mixed for 10 min (pH 12.48). The aqueous solution was decanted, and 0.200 mL of DI water was added to the isolated oil, vortexed, and centrifuged. The supernatant aqueous solution was discarded. This procedure was repeated three times.

(b) Sample suspension preparation and titration: 1.000 mL of DI water was added to a vial containing 0.05020−0.05250 g of Nor (oil) and 0.06435−0.06685 g of $NaH_2PO_4 \cdot 2H_2O$. The pH (1.82−5.93) was adjusted with 2.00 M H_3PO_4.

2.9. Preparation of the Samples for the Solid-State Characterization. The following procedures were designed to isolate enough solid precipitates from various Nor suspensions for solid-state characterization. The equilibration time (6 + 18 h, stirring + sedimentation time) at 25 °C was the same for all samples, except for Solid Sample 3 as noted below where the equilibration was continued up to 72 + 18 h (stirring + sedimentation time). The separated solid samples below were air-dried for 3−4 days, except as noted. Solid Samples 1−3 were obtained from the phosphate-free suspensions, while Solid Samples 4−9 had phosphate ions in equilibrium with solids.

Solid Sample 1: 2.000 mL of 0.15 M NaCl in DI water was added to a vial containing 0.3 g of NorHCl (pH 5.3). Then,

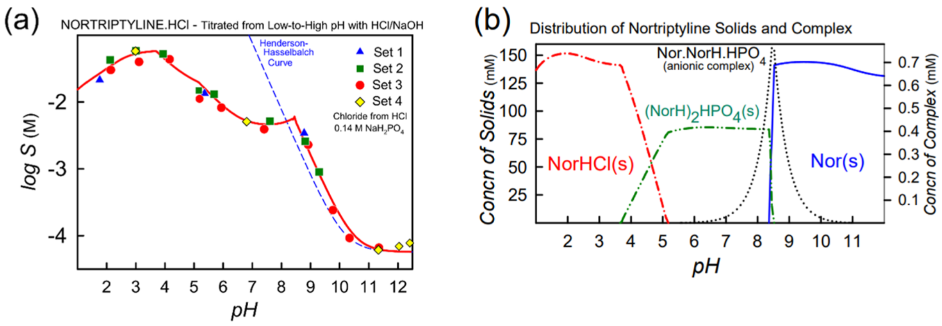

Figure 2. Titration sets 1−4: (a) log *S*−pH diagram of NorHCl in the presence of 0.14 M phosphate, titrated with NaOH from low-to-high pH; (b) distribution of solid phases and complex species ("concn of solids" (mM) means the number of millimoles of solid precipitate per liter of solution). The anionic complex occurs only in solution. Two pH_{max} points are evident: 3.71 (chloride to 2:1 phosphate transformation) and 8.36 (2:1 phosphate to free base transformation).

0.100 mL of 1 M HCl was added to the suspension (pH 2.3). After equilibration, the phases were separated by filtration.

Solid Sample 2: 2.000 mL of DI water was added to a vial containing 0.13090 g of NorHCl and 0.01785 g of NaCl (initial pH 6.28). After sedimentation, the pH value was 6.33. The phases were separated using centrifugation.

Solid Sample 3: 2.000 mL of water was added to a vial containing 0.3 g of NorHCl. Then, 2.000 mL of 1 M NaOH was added to the suspension (pH ∼13). The suspensions were stirred for 72 h and allowed to settle for an additional 18 h. The solid phase was then separated by filtration and dried in a vacuum oven at room temperature for ∼12 h by using the laboratory vacuum line.

Solid Sample 4 (two-step procedure): (1) 5.000 mL of water was added to a vial containing 0.3 g of NorHCl (pH 5.79). Then, 2.700 mL of 1 M NaOH was added to the suspension (pH 12). The phases were separated and the isolated oil washed with water. (2) 4.000 mL of 0.15 M NaH_2PO_4 was added to oil (pH 7.15) and the pH was adjusted using 2 M H_3PO_4 (pH 2.0). After equilibration time, the phases were separated by filtration.

Solid Sample 5 (two-step procedure): (1) 2.000 mL of DI water was added to a microtube containing 0.22750 g of NorHCl followed by the addition of 0.790 mL of 1 M NaOH (pH = 12.25). The phases were separated by centrifugation. The supernatant was discarded; 0.200 mL of DI water was added to the isolated oil, the suspension was vortexed and centrifuged. The process was repeated twice. (2) 1.500 mL of DI water was added to the isolated oil. The pH value was adjusted with 2.00 M H_3PO_4 (pH 4.46 after 6 + 18 h). The phases were separated by centrifugation.

Solid Sample 6 (two-step procedure): (1) 2.000 mL of DI water was added to a microtube containing 0.15780 g of NorHCl followed by the addition of 0.790 mL of 1 M NaOH (pH = 12.45). The phases were separated by centrifugation. The supernatant was discarded; 0.200 mL of DI water was added to the oil, the suspension was first vortexed and then centrifuged. The process was repeated twice. (2) 1.500 mL of DI water was added to the isolated oil. The pH value was adjusted with 2.00 M H_3PO_4 (pH 8.23 after 6 + 18 h). The phases were separated using centrifugation.

Solid Sample 7: 4.700 mL of 0.15 M NaH_2PO_4 was added to a vial containing 0.3 g of NorHCl (pH 4.7). Then, 0.300 mL of 1 M HCl was added to the vial (pH 2.9). After equilibration time, the phases were separated by filtration. The solid was dried under vacuum.

Solid Sample 8: 4.700 mL of 0.15 M NaH_2PO_4 was added to a vial containing 0.3 g of NorHCl (pH 4.7). After the equilibration time, the phases were separated by filtration.

Solid Sample 9: 4.700 mL of 0.15 M NaH_2PO_4 was added to a vial containing 0.3 g of NorHCl (pH 4.7). Then, 0.200 mL of 1 M NaOH was added to the vial (pH 6.38). After the equilibration time, the phases were separated by filtration.

3. RESULTS AND DISCUSSION

3.1. Solubility Analysis. The pH-dependent solubility profiles of NorHCl and distribution of the Nor solid and complex species, calculated using *p*DISOL-X, are shown in Figures 2−5 (detailed solubility and titration data are summarized in the Supporting Information, Tables S1−S14). The profiles are obtained according to solubility measurements performed in the phosphate buffer in the presence of a physiologically relevant chloride concentration (Figure 2 with low-to-high pH and Figure 3 with high-to-low pH), in a phosphate-free aqueous medium (Figure 4), and in a chloride-

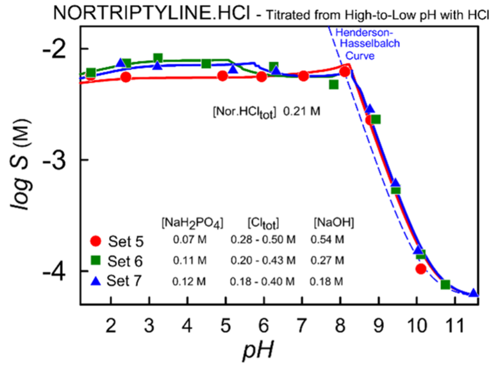

Figure 3. Titration sets 5−7 log *S*−pH diagram. Initially, NorHCl was added to the phosphate solutions, after which the suspensions were made alkaline (pH > 10) with NaOH. These were then incrementally titrated with 1 M HCl. In the excess free-base region from pH 12 to pH 8.3 (pH_{max}), the drug solubility was notably elevated, compared to values expected from the HH equation (dashed curve), which is consistent with the formation of an anionic Nor−phosphate complex (cf., the dotted curve in Figure 2b). Below pH 8.3, different Nor salts formed in the three sets. In the case of titration set 5, there was not enough phosphate in the solution to satisfy the solubility product of the phosphate salt, so only the 1:1 Nor−chloride salt formed. For the other two sets, 2:1 phosphate and 1:1 chloride salts formed with slightly different pH_{max} values near pH 5.0 and 5.7, indicated by the solubility maxima in the green and blue curves, respectively.

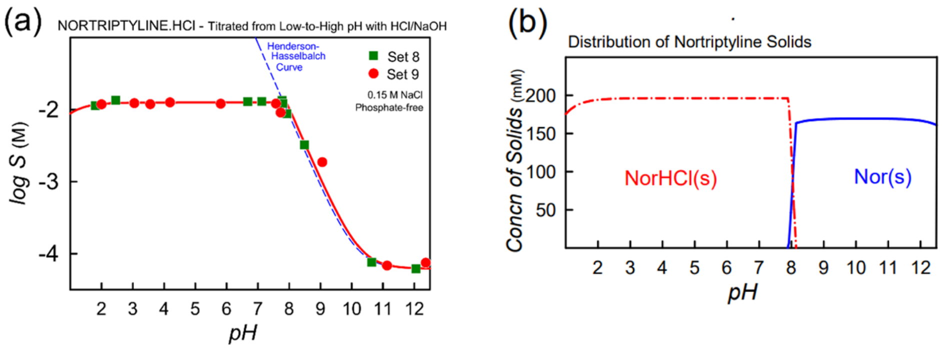

Figure 4. Titration sets 8 and 9 (0.15 M NaCl; phosphate free): (a) log *S*−pH diagram of NorHCl; (b) distribution of Nor solid phases [“concn of solids” (mM) means the number of millimoles of solid precipitate per liter of solution].

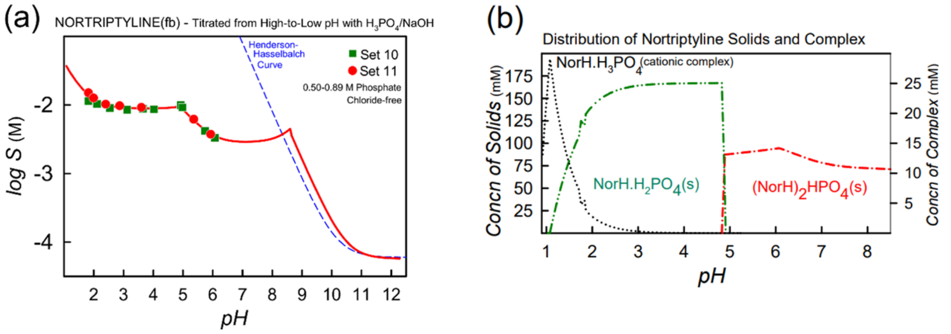

Figure 5. Titration sets 10 and 11 (0.50−0.89 M phosphate; chloride free): (a) log *S*−pH diagram of NorHCl; (b) distribution of Nor solid phases and complex species [“concn of solids” (mM) means the number of millimoles of solid precipitate per liter of solution].

free medium (Figure 5). The refined constant values are summarized in Table 1.

The pK_a values of Nor reported in the literature are 10.13 ± 0.06 (26 °C, $I = 0.15$ M, extrapolation from MeOH−water)[25] and 10.10 ± 0.02 (25 °C, $I = 0.15$ M, extrapolation from DMSO−water).[20] The pK_a value 10.13 was used in the present study.

The solubility data points from titration sets 1−4 (low-to-high titrations, Figure 2) were combined for the regression analysis since the total reactant concentrations in the different sets were nearly identical and since nearly the same equilibrium constant values were obtained when the sets were treated separately.

A higher solubility in the pH 8.5−11 interval in Figure 2a than that predicted by the Henderson−Hasselbalch (HH) equation can be rationalized by the formation of anionic complex species (dotted curve in Figure 2b)

$$\text{Nor} + \text{NorH}^+ + \text{HPO}_4^{\;2-} \leftrightarrows \text{Nor·NorH·HPO}_4^{\;-} \qquad (2)$$

Figure 2 shows that under the experimental conditions in the acidic solution, the sole excess solid in the suspension up to pH 3.71 (first pH$_{max}$) is the chloride salt, NorH·Cl(s). On further titration, a salt−salt mixture forms in the pH domain 3.71−5.19 as the chloride salt concomitantly transforms into the 2:1 phosphate salt, (NorH)$_2$·HPO$_4$(s). As suggested by Bogardus and Blackwood (1979),[9] the system is generally not thermodynamically invariant at pH$_{max}$ by having a fixed pH and a definite solubility since a phase separation occurs around pH$_{max}$. Therefore, as shown in Figure 2, the mixture spans from a maximum solubility of Nor at pH$_{max}$ to a minimum solubility at pH$_{min}$ in the mixture domain,[26,27] encompassing 1.49 pH units. The corresponding solubility product equilibria are defined by eqs 3 and 4

$$\text{NorH·Cl(s)} \leftrightarrows \text{NorH}^+ + \text{Cl}^- \qquad (3)$$

$$(\text{NorH})_2\text{·HPO}_4(\text{s}) \leftrightarrows 2\text{NorH}^+ + \text{HPO}_4^{\;2-} \qquad (4)$$

The low-to-high pH transformation of the chloride to phosphate salts, which begins at pH 3.71 and ends at pH 5.19, can be called a process driven by a “soft” constraint, since the phase transformation spans a substantial range of pH and solubility. By contrast, when the pH is lowered with a salt-forming acid in a saturated solution of the pure free base (cf., Figure 3), pH$_{max}$ indicates a sharp phase transformation near pH 8.5, driven by the “hard” constraint as originally implied by Bogardus and Blackwood (pH$_{max}$ = pH$_{min}$, with fixed solubility during the phase transformation).

Between pH 5.19 and 8.36, the sole excess solid is consistently interpreted as the 2:1 phosphate salt. A second pH$_{max}$ is encountered at pH 8.36, which marks the beginning of the mixture domain comprising the 2:1 phosphate salt and the free base. The span of the second domain is relatively narrow: 8.36 (pH$_{max}$) to 8.47 (pH$_{min}$), as indicated in Figure 2b by the dashed−dotted−dotted and the solid curves undergoing steep changes. The anionic complex (dotted curve in Figure 2b) is predicted to reach its highest concentration of 0.88 mM in the pH$_{max}$−pH$_{min}$ domain. For pH > pH$_{min}$, the sole excess solid is the free base.

Figure 3 shows the solubility profiles of titration sets 5−7, each titrated from high-to-low pH. The inset in the figure summarized the critical concentrations in the three sets. Each set was analyzed separately since the total phosphate and chloride concentrations are substantially different from set to set. Although the appearance of the log *S*−pH profile in Figure

Table 1. Nor Solubility−Refinement Results[a]

titration sets	initial form	titrant	pH range	log S_0	SD	log $K_{sp}^{BH.Cl}$	SD	log $K_{sp}^{BH.H_2PO_4}$	SD	log $K_{sp}^{(BH)_2.HPO_4}$	SD	log K_{221}	SD	log K_{141}	SD	I_{avg}	$[B]_{tot}$	$[PO_4]_{tot}$	$[Cl]_{tot}$	$[NaOH]_{init}$
1−4	BHCl + HCl	NaOH	1.8 → 12.4	−4.24	0.03	−2.52	0.03	[b]	[c]	−6.50	0.03	5.20	0.07	[b]	[c]	0.33	0.20	0.14	0.13−0.27	[f]
5	BHCl + NaOH	HCl	12.5 → 1.5	−4.24	0.08	−2.80	0.03	[b]	[c]	[a]	[b]	5.14	0.41	[b]	[c]	0.50	0.20	0.07	0.28−0.50	0.54
6	BHCl + NaOH	HCl	10.8 → 1.5	−4.23	0.09	−2.83	0.04	[b]	[c]	−7.22	0.06	5.07	0.54	[b]	[c]	0.40	0.23	0.11	0.20−0.43	0.27
7	BHCl + NaOH	HCl	11.5 → 2.3	−4.24	0.06	−2.84	0.04	[b]	[c]	−6.67	0.07	5.11	0.31	[b]	[c]	0.47	0.20	0.12	0.18−0.40	0.18
8, 9[d]	B + HCl	NaOH	1.8 → 12.4	−4.23	0.02	−2.72	0.01	[b]	[c]	[b]	[c]	[b]	[c]	[b]	[c]	0.21	0.21	[d]	0.17−0.28	[f]
10, 11[e]	B	H_3PO_4	6.1 → 1.8	[b]	[c]	[b]	[c]	−2.95	0.01	−6.64	0.01	[b]	[c]	0.69	0.08	0.46	0.19	0.33−0.84	[e]	[f]
			wt mean	**−4.24**		**−2.72**		**−2.95**		**−6.63**		**5.19**		**0.69**						
			SD	**0.02**		**0.01**		**0.01**		**0.01**		**0.07**		**0.08**						

[a] 25 °C, ref. ionic str. = 0.15 M. pK_a = 10.13 in all cases. "B" refers to a Nor-free base. The entries in the "Initial Form" column refer to the starting form of the drug, with the initial pH adjusted with HCl or NaOH, or otherwise not adjusted. The following column identifies the titrant used to adjust the pH of the suspensions in the assay. Complexes 221 and 141 are BH·B·HPO_4 (anion) and BH·H_3PO_4(cation), resp. pH electrode standardized using a blank titration: $pH_{meter} = 0.048 + 1.0040\ p[H^+] -5.1\ [H^+] -3.3\ [OH^-]$. Standardized titrants: HCl 0.9397 M, NaOH 0.9065 M, H_3PO_4 2.00 M. The concentrations in the last five columns are in molarity units. [b] Not detected. [c] Not applicable. [d] Phosphate-free. [e] Chloride-free. [f] Initial pH not adjusted with NaOH.

3 is different from that of Figure 2, the individual-set refined constants were practically the same (cf., Table 1). The variable shapes of log S−pH profiles depend on the relative total concentrations of the reactants (cf., Figures 2−5).

Titration set 5 has the lowest amount of phosphate. There was not enough of it (0.07 M) to satisfy the solubility product of either of the phosphate salts. However, the anionic drug−phosphate complex is predicted to form, with a maximum concentration of 0.58 mM at pH 8.22. The hydrochloride salt forms for pH < 8.27 (pH_{min}). The free base forms for pH ≥ 8.21 (pH_{max}). Therefore, a mixture of NorH·Cl(s) and Nor(s) occupies a very narrow pH domain.

Titration sets 6 and 7 had enough phosphate (0.11−0.12 M) to form the 2:1 salt. Also, there was enough chloride to form an excess solid chloride salt in the pH 5−8 interval. Furthermore, the anionic complex was predicted to form. However, the small total concentration differences between titration sets 6 and 7 were enough to bring out subtle differences in the mixture domain structures.

Titration sets 8 and 9 (phosphate-free, Figure 4) were designed for the intrinsic solubility determination of Nor and the solubility product of NorH·Cl salt; data points from two sets were combined for the analysis. As anticipated, there is no deviation from the HH curve for pH > 8 since no drug−phosphate complexes are possible. The excess solid-phase diagram is shown in Figure 4b. The mixture domain is narrow in pH.

Chloride-free assays (titration sets 10 and 11, Figure 5) were performed to determine the solubility products of both 1:1 and 2:1 possible drug−phosphate salts.

Based on the analysis of data points from titration sets 10 and 11, the pH 2−5 domain can be rationalized with the formation of a 1:1 salt and the pH 5−9 domain with the formation of a 2:1 salt (eqs 4 and 5)

$$NorH{\cdot}H_2PO_4(s) \leftrightarrows NorH^+ + H_2PO_4^- \tag{5}$$

In the acidic region, pH < 2, the solubility is elevated, which is consistent with the formation of a drug−phosphoric acid cationic complex:

$$NorH^+ + H_3PO_4 \leftrightarrows NorH{\cdot}H_3PO_4^+ \tag{6}$$

3.2. Nor Solid-State Characterization by Elemental Analysis.

The detailed results of the elemental analyses are listed in Tables S15−S18 in the Supporting InformationSection.

Solid precipitates from titration sets 3, 6, 7, and 9 are analyzed by elemental analysis. Theoretically calculated values of % C, H, and N in NorHCl are 76.11% C, 7.40% H, and 4.67% N.

Results of the elemental analysis of solid precipitates isolated from titration set 9 (phosphate-free suspension) in the pH range 2.00−7.58 are shown in Table S15. Data suggest that isolated solids are possible hydrated NorH·Cl(s), which agrees with the equilibrium analysis (*p*DISOL-X).

Elemental analyses of solids isolated from titration set 3 (low-to-high titration) in the pH range 2.14−7.41, titration set 6 (high-to-low titration) in the pH range 1.48−7.83, and titration set 7 (high-to-low titration) in the pH range 2.25−6.32 are shown in Tables S16−S18. Data from low-to-high titration (Table S16) indicate the phase conversion from hydrated NorH·Cl(s) at pH 2.14−3.11 to hydrated 2:1 Nor−phosphate salt at pH 5.19−7.41, which confirms the combined

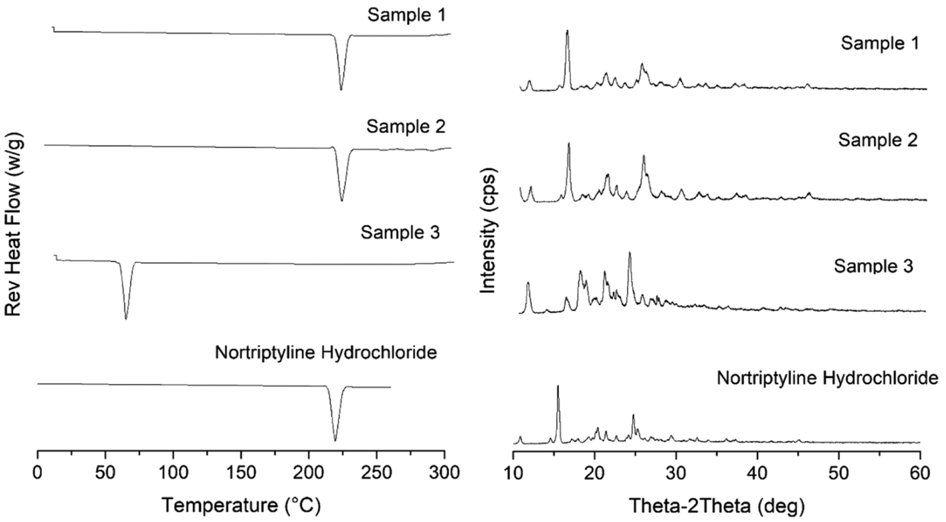

Figure 6. DSC scans (left-hand side) and PXRD patterns (right-hand side) of Solid Samples 1−3 isolated as Nor precipitates in equilibrium with solutions containing chloride only and no phosphates: Solid Sample 1 isolated at pH 2.3, Solid Sample 2 isolated at pH 6.33, and Solid Sample 3 isolated at pH ∼ 13 (vacuum-dried). DSC scan and PXRD pattern of Nor HCl are given for reference.

equilibrium analysis of titration sets 2−4 and Solid Sample 5. Data from high-to-low titrations (Tables S17 and S18) are also compatible with the phase transition from hydrated NorH·Cl(s) to hydrated 2:1 Nor−phosphate salt.

3.3. Nor Solid-State Characterization for Crystalline Properties and Weight Loss.

To perform DSC, PXRD, and TGAs, larger amounts of solid precipitates were needed, so nine additional samples were prepared. To facilitate the interpretation of the solubility profiles, the following Nor suspensions were prepared: chloride-free, phosphate-free, and those containing phosphate and chloride. DSC, PXRD, and TGA scans of nine isolated precipitates are presented in Figures 6−9. The results for the NorHCl salt "reference" are also given for comparison.

Solid Samples 1−3 were isolated from chloride-containing suspensions of Nor (phosphate-free), whose DSC scans and PXRD patterns as well as that of NorHCl as the reference are shown in Figure 6. A comparison of DSC and PXRD of Solid Samples 1 and 2 with that of NorHCl shows that all three materials have similar melting endotherms (onset of endotherms: 215 °C) and similar PXRD patterns. Thus, only the chloride salt was formed at the lower pH range of 2.3 to 6.3, which agrees with the solubility versus pH profile given in Figure 4 and indicates that only the chloride salt would be present as the solid phase at pH < pH_{max} when no phosphate is present in the solution. As shown by the TGA scans in Figure 7, Solid Samples 1 and 2, like NorHCl, were also anhydrous as there was no weight loss up to 200 °C due to any possible dehydration.

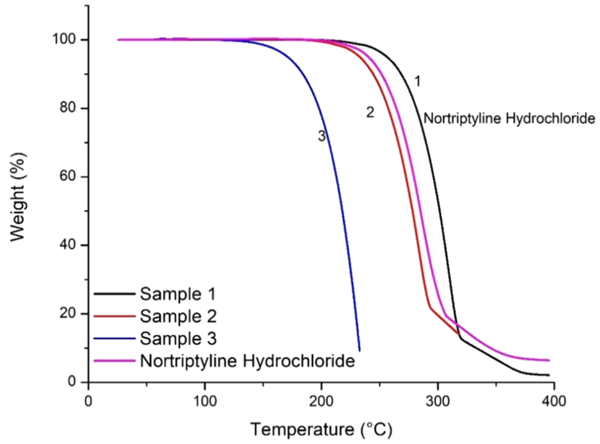

Figure 7. TGA scans of Solid Samples 1−3 isolated as Nor precipitates in equilibrium with solutions containing chloride only and no phosphates: Solid Sample 1 isolated at pH 2.3, Solid Sample 2 isolated at pH 6.33, and Solid Sample 3 isolated at pH ∼ 13 (vacuum-dried).

Solid Sample 3 in Figures 6 and 7 was prepared by raising the pH of NorHCl suspensions from low to high levels (pH ∼ 13) by the addition of NaOH. It was observed that when the pH of the NorHCl suspensions was raised above 9, the suspended solid converted to an oily material and remained as such up to pH 13. Since, as mentioned earlier, the pK_a of Nor is 10.13, we raised the pH of the suspension to 12.5−13 to ensure full conversion of NorHCl to the free base. It was, however, observed that, upon continued equilibration, the free base began changing its form from an oil to a solid. When the separated phase was isolated after 6 h of stirring followed by 18 h of sedimentation, like other samples, and the material was air-dried for solid-state characterization, it appeared to be a waxy solid, possibly because all the oily free base did not convert to crystalline solid. DSC and PXRD scans of the material changed depending on the equilibration time, and the TGA scan indicated the presence of moisture in the sample (data not shown). For these reasons, Solid Sample 3 was prepared after 72 h of shaking plus 18 h of equilibration following the pH value adjustment of a NorHCl suspension to 13 and then vacuum-drying the isolated solid in an oven at room temperature using the laboratory vacuum line. It may be observed that the crystalline free base thus prepared (Solid Sample 3) does not show any weight loss up to ∼150 °C in the TGA scan (Figure 7), gives a sharp melting endotherm with the onset temperature of 54.8 °C in the DSC scan, and has distinct peaks in the PXRD pattern (Figure 6), indicating that an anhydrous crystalline free base of Nor exists, and the oily free base initially formed may convert fully to the free base. It may also be mentioned here that a Nor-free base may also exist as a hydrate since DSC and PXRD scans prior to its vacuum-

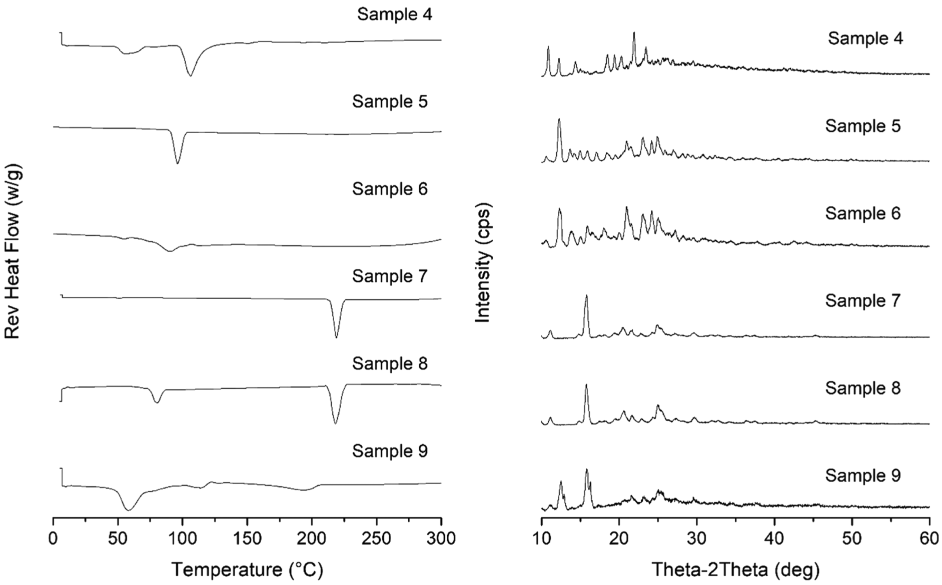

Figure 8. DSC scans (left-hand side) and PXRD patterns (right-hand side) of Solid Samples 4−9 isolated as precipitates from suspensions where NorHCl was used as the starting material and then equilibrated with media containing phosphates. Solid Sample 4: NorHCl was first converted to an oily free base and then pH was lowered to 2.0 using phosphate salt and an acid; Solid Sample 5: it was prepared similar to the above and pH was lowered to 4.46; Solid Sample 6: it was prepared similar to above and pH was adjusted to 8.23; Solid Sample 7: NaH_2PO_4 was added to a suspension of NorHCl (pH 4.7) and then the pH was further adjusted to 2.9 using HCl solution; Solid Sample 8: similar to Solid Sample 7, but using HCl solution, no pH adjustment was made (final pH 4.7); and Solid Sample 9: it was prepared similar to Solid Sample 7, but the pH was adjusted higher to 6.38 using NaOH solution.

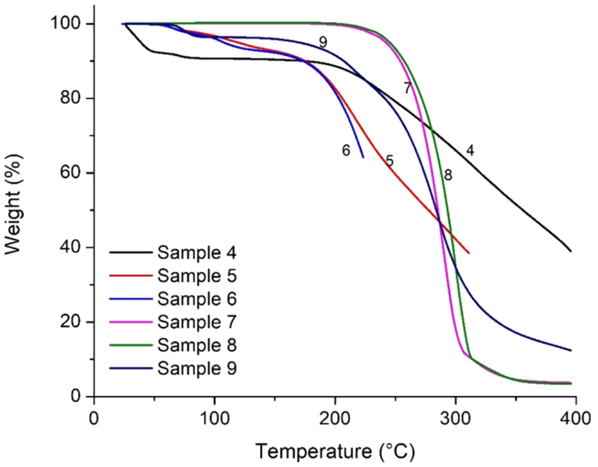

Figure 9. TGA scans of Solid Samples 4−9. Sample description is given in the legend of Figure 8.

drying differed from that of the vacuum-dried sample, and the TGA scan showed a weight loss (data not shown). Based on the above considerations, it was concluded that a Nor-free base exists in the oily liquid form as well as anhydrous and hydrate forms (data not shown). During the determination of aqueous solubility at pH > pH_{max}, the excess free base could be either the oily material or the solid hydrate depending on how long the suspensions are equilibrated; the anhydrous free base is formed only after drying under vacuum.

Figure 8 shows the DSC scans and PXRD patterns of Solid Samples 4−9. The results of the TGA are given in Figure 9. Solid Samples 4, 5, and 6 were isolated from phosphate suspensions (chloride-free) at pH 2.0, 4.46, and 8.23, respectively. Solid Sample 4 was isolated at a pH slightly above that of the phosphoric acid pK_{a1} (1.73), and therefore, it could be a Nor−phosphoric acid 1:1 salt.

As shown earlier in Figures 2 and 3, two pH_{max} in solubility versus pH profiles of Nor in the presence of phosphates are evident, one around pH 8.5−9 and the other around pH 4.0−4.5, where a 2:1 Nor phosphate salt would form below pH 8.5, which would, in turn, convert to a 1:1 salt below pH 4. The solids from Solid Samples 5 and 6 were isolated at pH 4.46 and 8.23, respectively, which fall in between the two pH_{max} regions. The PXRD scans of Solid Samples 5 and 6 in Figure 8 exhibit similar patterns, indicating that both samples are indeed similar. The DSC scans of the two samples are also similar, except that the endotherm in Solid Sample 6 is smaller as a less amount of sample was used for DSC analysis due to the shortage of material available. Based on these considerations, both the samples were attributed to the 2:1 Nor phosphate salt. In contrast, Solid Sample 4 that was isolated at pH 2.0 after the adjustment of pH by the addition of phosphoric acid exhibited different DSC scan and PXRD patterns from those of the 2:1 salt (Solid Samples 5 and 6). Therefore, based on the solubility and pH_{max} considerations described earlier, Sample 4 was attributed to the 1:1 salt phosphate salt.

The TGA scans in Figure 9 show that Solid Samples 5 and 6 have similar patterns with ∼2.5% weight in the range of 90−100 °C, which also correspond to their endothermic peaks. Considering that the molecular weight of $(NorH)_2HPO_4$ (2:1 salt) would be 624.76, the weight loss indicates the formation of a monohydrate of the 2:1 Nor phosphate salt. Unlike Solid Samples 5 and 6, the DSC scan of Solid Sample 4 in Figure 8 shows an endotherm at ∼55 °C, its PXRD patterns are different from those of the other two samples, and its TGA in Figure 9 exhibits a much higher weight loss (9.1%) at a relatively low temperature (100 °C). By considering the molecular weight of $NorH{\cdot}H_2PO_4$ to be 361.38, it was postulated that Solid Sample 4 was a dihydrate form of the 1:1 salt.

Solid Samples 7–9 were isolated from suspensions at pH 2.9, 4.7, and 6.38, respectively, which contained both chloride and phosphate ions. DSC scans and PXRD patterns confirm a previous equilibrium analysis based on the solubility profiles in low-to-high titrations. Solid Sample 7 is mostly a hydrochloride salt, and there was practically no phosphate salt present, demonstrating that when HCl is the predominant species and pH is relatively low, only the hydrochloride salt would be present as the equilibrium species in the system despite NaH_2PO_4 being present in the solution. Solid Sample 8 shows that as the pH increases, the HCl salt may convert to the phosphate salt due the presence of phosphate (NaH_2PO_4) in the system since the solid phase was found to be a mixture of phosphate and chloride salts of Nor. It may be noted that although Solid Sample 8 shows a DSC endotherm and the presence of phosphate salt, PXRD scans of Solid Samples 7 and 8 appear similar, possibly because the amount of the phosphate salt in the latter was relatively small. Solid Sample 9 isolated at pH 6.38 was predominantly a phosphate salt; however, it is apparent from the DSC scan and PXRD patterns that the HCl salt could also be present as there were indications of a DSC endotherm at higher temperatures, and the PXRD scan appears to show peaks for both phosphate and HCl salts. TGA (Figure 9) shows that these samples were hydrates, except from Solid Samples 7 and 8, which were either completely or mostly HCl salt. These results demonstrate that during the determination of aqueous solubility in the presence of different counterions, the equilibrium solid phase may change and, consequently, the solubility may vary.

4. SUMMARY AND CONCLUSIONS

Solubility plays critical roles in the development of oral and parenteral dosage forms of drugs, especially when the drugs are poorly soluble in aqueous media and are acidic or basic, exhibiting pH-dependent solubility. Although there are many reports in the literature on the solubility of drugs as a function of pH, only one counterion is generally used to adjust the pH during the determination of a pH versus solubility profile. For example, the pH may be adjusted by HCl or NaOH for determining the solubility of a HCl salt as a function of pH. On the contrary, the situation is more complex in practice during the development of pharmaceutical dosage forms, where multiple counterions may be present in a drug solution. For example, a phosphate or other non-HCl pH adjustors may be used for buffering a HCl salt solution, or a saline (NaCl) solution may be used to adjust the tonicity of a phosphate or other non-HCl salts, which may change the equilibrium species present in solutions and in the solid state and thus the aqueous solubility. To investigate the effects of multiple and possibly competing ions on the solubility of a model basic drug, Nor, we have conducted a systematic investigation of the solubility of NorHCl as a function of pH when both chloride and phosphate ions were present in the same solutions as well as in chloride-free and phosphate-free solutions. When the pH of a NorHCl suspension was adjusted to a pH above 9, it formed an oily and metastable free base that ultimately converts to the solid crystalline form upon prolonged equilibration (>72 h). When the pH of a suspension of the oily free base in an aqueous medium was lowered by the addition of H_3PO_4 in chloride-free solutions, a 2:1 Nor phosphate salt $[(NorH)_2HPO_4]$ was formed in the pH range of 8.4 and 5.0, where pH 8.4 and 5.0 approximately depict, respectively, a high pH_{max} (pH_{max2}) and a low pH_{max} (pH_{max1}) in the pH versus solubility profile. Upon further lowering the pH by the addition of H_3PO_4, the 1:1 salt ($NorH \cdot H_2PO_4$) having a higher solubility acted as the equilibrium species at pH < 5.0, and there was also the indication of a complex formation between Nor and H_3PO_4 at a pH below 2. In separate studies, it was observed that the presence of different concentrations of phosphates (0.07–0.12 M NaH_2PO_4) could also influence the pH versus solubility profiles determined by the addition of HCl to the suspension of the Nor free base. For example, the value of pH_{max1} (between chloride and phosphate salts) ranged from 3.7 to 5.7. In contrast to the pH versus solubility profile of Nor in phosphate solutions, only the monohydrochloride salt (NorHCl) was formed below the pH_{max} of 7.3 when the pH of the aqueous suspension of the free base was lowered by the addition of HCl in a phosphate-free solution. Since salt solubility depends on the product of two concentrations, had the above aqueous suspension contained 0.15 M added NaCl at the start, the resultant pH_{max} would be 7.9. Thus, between the two counterions used for salt formation, Nor can exist in two phosphate salt forms (2:1 and 1:1) and one HCl salt form, in addition to the free base form at pH > 9. The solid-state characterization of solid phases in equilibria with solutions by DSC and PXRD confirmed the existence of the three distinct salt forms, while TGA indicated that while NorHCl is anhydrous, the 2:1 and 1:1 phosphate salts were, respectively, monohydrate and dihydrate. Depending on the buffering agents and other counterion species present in solutions, different salts existed as equilibrium species during the determination of the solubility of Nor as a function of pH. Thus, the results of the present investigation illustrate the influence of different counterions, such as buffering agents, tonicity adjusters, and so on, on the aqueous solubility and the interconversion of salts, and careful attention must be given to these factors in the formulation of drug products.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.molpharmaceut.1c00919.

Additional experimental details; preparation of stock suspensions for low-to-high pH and high-to-low pH titrations; titration and solubility data for titration sets 1–11; elemental analysis data from titration sets 9, 3, 6, and 7; and HPLC UV/VIS analysis—sample chromatograms and calibration diagram (PDF)

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Notes

The authors declare no competing financial interest.

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