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*Research Article*

# Physicochemical Characterization of NPC 1161C, A Novel Antimalarial 8-Aminoquinoline, in Solution and Solid State

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***Abstract.*** NPC 1161C is a novel antimalarial drug of interest because of its superior curative and prophylactic activity, and favorable toxicity profile against *in vivo* and *in vitro* models of malaria, *pneumocystis carinii* pneumonia, and leishmaniasis. The preformulation studies performed included determination of $pK_a$s, aqueous and pH solubility, cosolvent solubility, log *P*, pH stability, thermal analysis, and preliminary hygroscopicity studies. The mean $pK_{a1}$, $pK_{a2}$, and $pK_{a3}$ were determined to be 10.12, 4.07, and 1.88, respectively. The aqueous solubility was found to be $2.4\times10^{-4}$ M having a saturated solution pH of 4.3–5.0 and a low intrinsic solubility of $1.6\times10^{-6}$ M. A mathematical model of the pH-solubility profile was derived from pH 2.2 to 8.0. An exponential decrease in solubility was observed with increasing pH. The excess solid phase in equilibrium with the solution in aqueous buffers was determined to be the free-base form of the drug. A significant increase in solubility was observed with all the cosolvents studied, in both unbuffered and buffered systems. Mean log *P* of the salt and the free base were estimated to be 2.18 and 3.70, respectively. The compound had poor stability at pH 7.0 at 37°C, with a $t_{90}$ of 3.58 days. Thermal analysis of the drug using DSC and TGA revealed that the drug is present as a semi-crystalline powder, which transformed into the amorphous state after melting. The drug was also found to sublime at higher temperatures. Determination of physicochemical properties of NPC 1161C provided useful information for the development of a dosage form and preclinical evaluation.

**KEY WORDS:** log *P*; NPC 1161C; $pK_a$; preformulation; solubility; thermal analysis.

## INTRODUCTION

Effective treatment of parasitic diseases affecting the developing world, such as malaria, trypanosomiasis, leishmaniasis, and *pneumocystis carinii* pneumonia, has been severely hindered by the limited availability of safe and efficacious drugs, coupled with the emergence of drug resistant strains. NPC 1161C, (±8-[(4-amino-1-methylbutyl)amino-5-(3,4-dichlorophenoxy)-6-methoxy-4-methylquinoline succinate]; Fig. 1), a novel analog of 8-aminoquinoline, has been found to be more active and less toxic when administered subcutaneously in mice as compared to primaquine (1).

NPC 1161C was found to have prophylactic activity against malaria when mice were treated with a single dose of 2 mg/kg within the period of 2 days preinfection through 2 days post infection. Superior curative antipneumocystic activity at 1.2 mg/kg/day of the compound was observed, as compared to 50/250 mg/kg/day trimethoprim-sulfamethoxazole combination. *In vitro* antileishmanial activity of NPC 1161C against a variety of strains of leishmania was also observed at very low concentrations (1).

The development of any dosage form for a new drug candidate necessitates prior evaluation of the fundamental physical and chemical properties of the drug molecule, providing essential information for the development of a rational pharmaceutical formulation. The two fundamental properties which must be evaluated for a new drug molecule are the intrinsic solubility ($S_0$) and the acid dissociation constant(s) ($pK_a$). Particularly with solid dosage forms, the evaluation of these properties might indicate the need or possibility of making more soluble salts or incorporating solubilizing agents to eliminate solubility-related poor bioavailability (2). A wide variety of information must be generated to develop formulations rationally. However, limited time and resources often dictate that only a focused set of studies can be conducted (3,4).

The objective of the present study was to evaluate selected solution and solid-state physicochemical properties of NPC 1161C, to identify the physical properties of the drug that might yield formulation concerns, and to generate sufficient information so that formulation and preclinical development can be initiated. The current study is driven by the hypothesis that NPC 1161C possesses the desired physicochemical properties warranted for further pharmaceutical and preclinical development. The properties eval-

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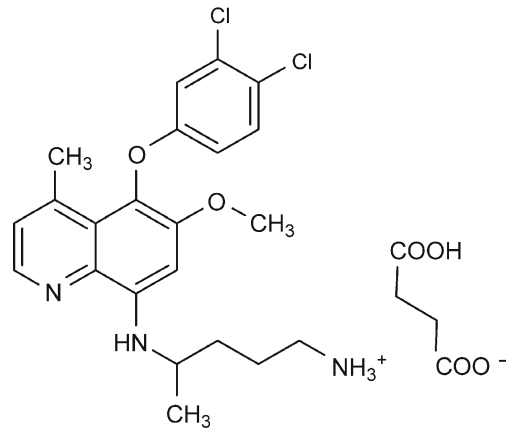


**Fig. 1.** Chemical structure of NPC 1161C ($C_{26}H_{31}Cl_2N_3O_6$)

uated include: $pK_a$(s), aqueous and pH-solubility profile, evaluation of the excess solid phase in aqueous buffers, solubility in cosolvent mixtures in both buffered and unbuffered systems, partition coefficient (log *P*) of the free base and the salt form of the drug, pH stability, thermal analysis and melting point of the solid, and preliminary hygroscopicity studies. A stability-indicating reversed-phase (RP) HPLC method was used for the detection and quantitation of NPC 1161C (5).

## MATERIALS AND METHODS

### Materials

NPC 1161C was obtained from National Center for Natural Products Research (The University of Mississippi, University, MS, USA). Methanol (HPLC grade) and chloroform (ACS reagent grade) were purchased from Fischer Scientific (Fairlawn, NJ, USA), and trifluoroacetic acid (TFA; purity >99%, HPLC grade) was purchased from Aldrich (Milwaukee, WI, USA). $CDCl_3$ and $CD_3OD$ were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). All other chemicals were of ACS reagent grade and purchased from Aldrich (Milwaukee, WI, USA). Double-distilled nanopure water was obtained in-house from a Millipore water purification unit.

### Reversed-Phase HPLC Stability-Indicating Method

The HPLC system consisted of a Waters 2695 HPLC Separations Module equipped with Waters 2696 Photodiode Array detector (Waters Corporation, Milford, MA, USA) and Empower software (©Waters Corporation, Milford, MA, USA) for system control, data collection, and processing. Liquid Chromatography was carried out using Phenomenex Luna (4.6×150 mm), 5 μm C-18 (2) reversed-phase column. The mobile phase consisted of premixed HPLC grade methanol, nanopure water, and TFA in the ratio of 71:29:0.065, which was vacuum filtered using a 0.2-micron nylon filter in a Millipore vacuum filtration assembly and degassed prior to operating under isocratic conditions at a flow rate of 1.2 ml/min. Sample injection volume was 50 μL, and NPC 1161C was detected at a single wavelength of 254 nm or at a wavelength range of 210–400 nm, with a chromatographic run time of 15 min (5).

### $^1$H-NMR Spectroscopy

$^1$H-NMR experiments were performed at 30°C using 3 NMR spectrometers, Varian AS 400 (Varian Inc., Palo Alto, CA, USA, Model# EUR 0020), Varian AS 600 (Varian Inc., Palo Alto, CA, USA, Model# DLN 3654) and Bruker DRX 500 (Bruker Biospin Corp., Billerica, MA, USA, Model# BH077685). All samples were prepared in a 3-mm sample tube. The Varian AS 400 spectrometer is equipped with a Mercury Plus console, a 54-mm bore, 9.4-T Oxford magnet and a 3-mm broadband probe operating at 400.13 MHz. Data control and processing were done with a Sun Blade computer equipped with the Solaris operating system and VNMR 6.1C software. The Varian AS 600 NMR spectrometer is equipped with Unity Inova console, a 51-mm bore, 14.1-T Oxford magnet and 3-mm broadband probe operating at 599.89 MHz. Data control and processing were done with a Sun Blade computer equipped with the Solaris operating system and VNMR 6.1C software. The Bruker DRX 500 NMR spectrometer is equipped with DRX 500 console, 52-mm bore, 11.7-T Spectral-Spin magnet, and a 5-mm dual probe operating at 500.13 MHz. Data control and processing were done with a personal computer equipped with the Windows operating system, and XWinNMR software. The magnetic field of all the instruments remained stable with the solvent ($CD_3OD$) field lock for all the experiments, confirming negligible change in the signal frequency before and after each experiment.

### Acid Dissociation Constants ($pK_a$s)

An estimation of the $pK_a$s of the drug, based on the presence of three ionizable amino groups in the molecule, was first obtained using ACD/Chemsketch software (ACD Labs, Toronto, Canada). A proposed ionization scheme for NPC 1161C is shown in Fig. 2. The $pK_{a1}$ of the drug was determined by potentiometric titration (6). Approximately 5 mg of NPC 1161C was dissolved in 50 ml of nanopure water previously boiled and cooled to remove dissolved carbon dioxide and oxygen that might interfere with the assay. The drug solution was titrated with 0.1N NaOH in 0.05 ml increments with adequate stirring, and the pH of the solution was measured after each addition. All pH measurements were done using an Orion pH meter (Model 720A) and an Accumet AB15 pH meter with microprobe (Accumet 13-620-95; Fisher Scientific, Fairlawn, NJ, USA). The titration was carried out until no significant increase in pH was observed with each addition of NaOH. The resulting solution was back-titrated with 0.1N HCl in similar volume increments, and the pH of the solution was recorded after each addition. The $pK_{a1}$ of NPC 1161C was determined from the single point neutralization obtained from the plot of pH as a function of the volume of titrant added, as well as from the mean $pK_a$ values determined after each incremental addition of 0.05 ml of 0.1N HCl (6). The additional $pK_a$s of the compound, $pK_{a2}$ and $pK_{a3}$, were determined using the solubility method (7).

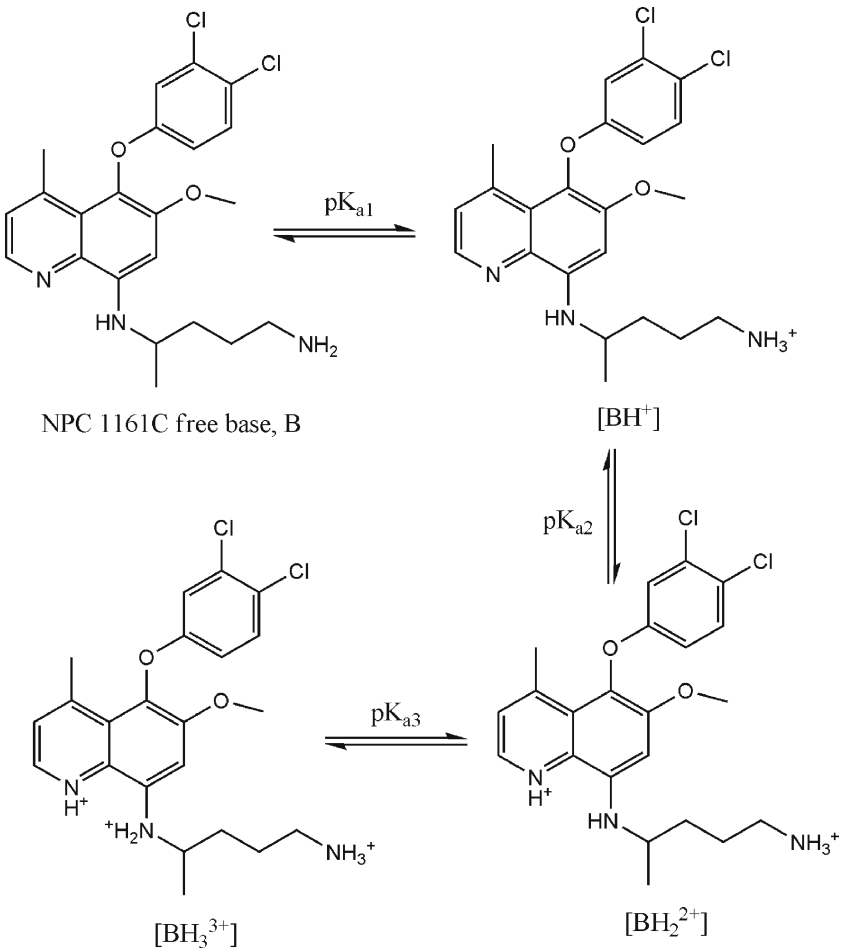


**Fig. 2.** Proposed ionization scheme of NPC 1161C

## Solubility Measurements

The equilibrium solubility of NPC 1161C in various solvents was determined by adding an excess of solid drug to 5 ml of the solvent in 2-dram (approximately 8 mL) Teflon-lined screw-capped glass scintillation vials. It was determined from preliminary experiments that equilibrium was achieved in 7 days in a shaker bath at 25°C with 50 shakes per min (spm). After equilibration, samples were withdrawn and centrifuged at 13,000 rpm, under controlled temperature of 25°C, and the supernatant of the dissolved drug was analyzed using HPLC. All measurements were done in duplicate vials for the above studies. The pH of all the samples were measured using an Orion pH meter (Model 720A) and an Accumet AB15 pH meter with microprobe (Accumet 13-620-95; Fisher Scientific, Fairlawn, NJ, USA).

### *Solubility of NPC 1161C in Water*

The apparent equilibrium solubility of NPC 1161C, a succinate salt, was evaluated in unbuffered nanopure water and the $K_{sp}$ (solubility product) of the salt was determined using Eq. 1 (8).

$$K_{sp} = [B^+][C^-] \tag{1}$$

For the salt of a weak acid and weak base, like NPC 1161C, both participating reactants moderate the overall pH of the stoichiometric product, *i* the salt, and is expressed by Eq. 2 (8):

$$\mathrm{pH} = \frac{1}{2}(\mathrm{pK_a(acid)} + \mathrm{pK_a(base)}) \tag{2}$$

### *pH Solubility of NPC 1161C in Selected Buffers*

The drug solubility in buffer was determined in a pH range of 2.2–8.0 McIlvaine citric acid–phosphate buffer systems (9,10). The buffers were not adjusted to constant ionic strength because no significant increase in solubility of the drug was found from preliminary experiments at pH 4.2 and 7.0 McIlvaine citric acid–phosphate buffers of different concentrations and ionic strengths. This might be because of the extremely low solubility of the drug and the conversion of the excess solid phase in equilibrium with the solution in aqueous buffers to the free-base form. A pH-solubility model was developed according to the Henderson–Hasselbalch equation for the total solubility of a weak base as given by Eq. 3 (11). Non-linear least square regression fitting of the plot of pH as a function of total solubility of NPC 1161C was done according to Eq. 4

(11) to obtain $pK_{a2}$ and $pK_{a3}$ of the drug using Statistica 6.0.

$$\text{Total Solubility, } C_{tot} = C_{BH^+} + C_{BH_2^{2+}} + C_{BH_3^{3+}} \quad (3)$$

$$C_{tot} = C_{BH^+} \times \left[1 + 10^{(pK_{a2} - pH)} + 10^{(pK_{a2} + pK_{a3} - 2pH)}\right] \quad (4)$$

*Isolation of the Free Base from the Succinate Salt, NPC 1161C*

The salt was converted into free base by adding 5% NaOH, and the free base was extracted in chloroform. The solvent was evaporated, using a Buchi Rotavapor assembly, and the free amine was dried under vacuum. The free base was analyzed by HPLC and NMR spectroscopy (Varian AS 400 NMR spectrometer) for structural confirmation. The succinate salt form of the drug was analyzed by dissolving approximately 5 mg of the drug in $CD_3OD$ and the NMR spectrum was obtained using a Varian AS 600 NMR spectrometer.

*Evaluation of the Excess Solid Drug in Equilibrium with Solution in Aqueous Buffers*

The excess solid drug in equilibrium with the solution in aqueous buffers was separated by filtration. The residue obtained (the solid drug) was dried at room temperature and dissolved in chloroform. The chloroform solution was then filtered and the chloroform in the filtrate was evaporated under vacuum using a speed-vac assembly. The solid residue obtained after evaporation of the solvent ($CHCl_3$) was redissolved in $CDCl_3$ for NMR-spectroscopic analysis. The $^1$H-NMR spectrum of the solid phase was obtained using a Bruker DRX 500 NMR spectrometer, and compared with that of the free base to evaluate the nature of the excess solid phase in equilibrium with the solution in aqueous buffers during pH-solubility measurements.

*Solubility of NPC 1161C in Unbuffered and Buffered Aqueous-Cosolvent Systems*

The solubility of NPC 1161C was evaluated in both unbuffered aqueous-cosolvent mixtures and cosolvent mixtures buffered at pH 7.0 using phosphate buffers (12), with varying cosolvent concentrations (10–40%). The cosolvents used were ethanol (EtOH), polyethylene glycol 400 (PEG 400), dimethyl sulfoxide (DMSO), and propylene glycol (PG). The solubility data for the drug was fitted to the cosolvent solubility models, as given by Eqs. 5 and 6 for cosolvent-water mixtures and cosolvent-buffer mixtures (at pH 7.0), respectively, to determine the solubilization power, σ (or solubilization slope), for all the cosolvents studied in unbuffered aqueous medium and in aqueous medium buffered at pH 7.0.

The solubility of NPC 1161C in cosolvent-water mixtures can be expressed by Eq. 5 (11):

$$\log S_{mix} = \log S_w + \sigma_{water} f_c \quad (5)$$

where $S_{mix}$ is the solubility of the drug in cosolvent-water mixture; volume fraction $f_c$; $S_w$ is the water solubility of the drug; and $\sigma_{water}$ is the solubilization power of the cosolvent in water. A plot of the logarithm of the solubility of the drug in the mixed solvent composition, as a function of cosolvent volume fraction, gives an intercept equal to the aqueous solubility of the drug and the slope, $\sigma_{water}$, which is independent of the aqueous solubility of the drug but related to the difference between the drug's octanol-water and octanol-cosolvent partition coefficients (11).

At pH 7.0, where the drug is primarily present in $BH^+$ form, the solubilization curve of NPC 1161C in cosolvent-buffer mixtures can be expressed by Eq. 6 (11):

$$S_{tol} = S_{BH^+} \times 10^{\sigma_{BH^+} f_c} \quad (6)$$

where, $S_{tol}$ is the total solubility of the solute in the cosolvent-buffer mixture, $S_{BH^+}$ is the solubility of the $BH^+$ species of the drug at pH 7.0, $\sigma_{BH^+}$ is the solubilization power of the cosolvent for the $BH^+$ species at pH 7.0, and $f_c$ is the volume fraction of the cosolvent. A plot of logarithm of the total solubility of the drug as a function of volume fraction of the cosolvent gives the intercept equal to the solubility of the $BH^+$ species of the drug and the slope of $\sigma_{BH^+}$.

**Partition Coefficient of NPC 1161C**

*Octanol-Water Partition Coefficient (log $P_{o/w}$) of NPC 1161C Using the Shake-Flask Method*

Octanol-water, in the ratio of 1:9 (*v*/*v*), was mixed and allowed to presaturate for 48 h at room temperature. An accurately weighed aliquot of the drug was added to the presaturated octanol-water mixture and allowed to equilibrate for 48 h at room temperature with shaking at 75 spm. After equilibration, samples were carefully drawn from the aqueous phase and analyzed by HPLC. The concentration of the drug in the octanol phase was calculated assuming all the drug partitioned into either the water or octanol phase after equilibration. Log $P_{o/w}$ was calculated to be the logarithm of the ratio of the concentration of the drug in the octanol and aqueous phase according to Eq. 7 (13).

$$\text{LOG } P_{o/w} = \text{LOG}\left[\frac{C_{octanol}}{C_{water}}\right] \quad (7)$$

where, $C_{octanol}$ and $C_{water}$ are the equilibrium concentrations of the drug in the octanol and water phases, respectively.

*Octanol-Water Partition Coefficient (log $P_{o/w}$) of the Free Base Using HPLC Method*

Various standards with known log *P* values, in the range of 1.26–5.75, were chosen to determine the log $P_{o/w}$ of NPC 1161C. The standards used were: acetanilide [log *P*, 1.26], acetophenone [log *P*, 1.66], *p*-nitrophenol [log *P*, 1.73], anisole [log *P*, 2.08], 4-iodophenol [log *P*, 2.92], diphenylamine [log *P*, 3.47], phenothiazine [log *P*, 4.15], and hexachlorobenzene [log *P*, 5.75] (14,15). These standards were chosen so that the log *P* value of the drug would likely lie within the range of the log *P* values of the

standards. Log *P* and LOG *k′* (capacity factor) bear a linear relationship as expressed by Eq. 8 (16,17):

$$\mathrm{LOG}P_{\mathrm{o/w}} = a\mathrm{LOG}k' + b \tag{8}$$

where, *a* and *b* are empirical constants which characterize the solvent system used. The drug with the unknown log $P_{\mathrm{o/w}}$ and various standards of known log $P_{\mathrm{o/w}}$ values within the desired range were analyzed by the RP-HPLC method to determine the *k′* (16,17).

*Moriguchi log P (mlog P) of NPC 1161C by Molecular Modeling Method*

Molecular modeling was done using a Silicon Graphics workstation equipped with IRIX 6.5 operating system. mlog *P* of NPC 1161C was determined, using the method developed by Moriguchi *et. al* (18,19). The molecular structure of NPC 1161C free base was built, using the SKETCH module of SYBYL 6.9. The structure was minimized, using the Powel's method of optimization, with 1,000 iterations, and a convergence gradient of 0.001. The *mlog P.spl* script was run in SYBYL 6.9 to obtain the mlog *P* of NPC 1161C free base.

**pH Stability of NPC 1161C**

Solution stability of NPC 1161C at an intended range of pH 3.0 to 7.0 was evaluated at 37°C for a period of 180 days. The buffers used for solution stability studies included pH 3.1 potassium biphthalate-HCl buffer, 0.07 M; pH 4.9 potassium biphthalate-NaOH buffer, 0.07 M; and pH 6.9 phosphate buffer, 0.07 M (12). An accurately weighed amount of the drug was dissolved in freshly prepared buffer solutions at the appropriate pH in a 20 ml sealed screw-capped Teflon-lined scintillation vial and stored at 37°C. Initial drug concentration was determined at time, $t=0$, for all the samples using HPLC. Samples were drawn at time intervals of 75, 110, and 180 days and the amount of drug remaining in the samples was analyzed using HPLC. A plot of the natural logarithm of concentration as a function of time (*t*) was used to determine the degradation rate constant and $t_{90}$ of the drug, i.e. the time required by the drug to degrade by 10% of the initial concentration, in each buffer. All determinations were done in duplicate (two vials for the same solution) for the study.

**Thermal Analysis of NPC 1161C**

*Differential Scanning Calorimetry*

Differential scanning calorimetry (DSC) of NPC 1161C was carried out using Pyris I DSC (Perkin Elmer, Norwalk, CT, USA). Accurately weighed aliquots of the solid drug (2–5 mg) were placed in standard aluminum DSC sample pans, which were sealed by crimping, with a lid. Pans containing the samples were placed in the sample cell of the DSC, and similarly sealed empty pans were placed in the reference cell of the DSC. DSC thermograms were obtained by heating the samples from 25°C to 450°C at a linear scanning rate of 10°C/min under a constant nitrogen purge of 40 ml/min. Step-scan DSC (20,21) and Hyper-DSC experiments (20,22) were performed for appropriate characterization of the thermal events obtained during standard DSC runs. Step-scan DSC of the solid drug was conducted from 0°C to 250°C at a heating rate of 5°C/min over 1°C incremental temperature steps and an isothermal hold step time of 1 min. Samples for hyper-DSC experiments were wrapped in aluminum foil of predetermined weight, and an empty aluminum foil wrap of equal weight was placed in the reference cell of the DSC. Samples were then heated from −20°C to 215°C at a linear scanning rate of 450°C/min, under a constant helium purge. Aluminum foil was used instead of standard DSC pans, to facilitate effective heat transfer during such high scanning rates.

*Thermogravimetric Analysis*

Thermogravimetric Analysis (TGA) of NPC 1161C was carried out using Pyris I TGA (Perkin Elmer, Norwalk, CT, USA). TGA thermograms were obtained by heating the samples from 25°C to 450°C at a linear heating rate of 10°C/min, under constant nitrogen purge of 40 ml/min. Sample weight loss was measured from the temperature at which the thermogram departed from the baseline to the temperature at which the new baseline was achieved.

*Determination of Melting Point of NPC 1161C*

The melting point of NPC 1161C was determined using SRS Optimelt Automated Melting point system (MPA 100, Stanford Research Systems, Inc., Sunnyvale, CA). The compound was filled to a height of 2–3 mm in a capillary tube and heated from 35°C to 200°C at a heating rate of 2°C/min. The melting point was determined by visual observation of the noticeable changes that occurred in the sample in the capillary as well as from the melt curve of the intensity of response as a function of temperature, as generated by the apparatus. The results obtained were correlated with those from DSC to confirm the thermal events occurring during heating.

**Hygroscopicity Studies**

The solid drug was exposed to various controlled humidity chambers prepared using saturated salt solutions. The saturated salt solutions used to attain relative humidities (RH) of 32%, 56%, 76%, and 89% were: $MgCl_2{\cdot}6H_2O$, $Mg(NO_3)_2{\cdot}6H_2O$, NaCl, and $BaCl_2{\cdot}2H_2O$, respectively (23). Approximately, 3–5-mg aliquots of accurately weighed drug were placed in weigh boats, exposed to the appropriate environment for 24 h, and the amount of moisture adsorbed was quantitated using TGA. TGA thermograms were obtained by holding the temperature at 70°C for 30 min for samples exposed at 32% and 56% RH, and for 60 min for samples exposed at 76% and 89% RH, under constant nitrogen purge. Weight loss was measured from the temperature at which the thermogram departed from the baseline to the temperature at which the new baseline was achieved. The loss in weight of the samples on heating was taken as the measure of adsorbed water by the samples. However, since the equilibrium moisture content at various relative humidities was not determined in this experiment, this study provided important preliminary hygroscopicity information for handling drug samples at various relative humidities.

## RESULTS

### Acid Dissociation Constants

The pH at half-neutralization of the weak base ($pK_{a1}$) was determined as the pH at half the volume required for single point neutralization during the acid titration from the titration curve, and was determined to be 9.92±0.08 (average of duplicate experiments). The average $pK_{a1}$ calculated after incremental addition of the acid was found to be 10.08±0.12 (6). $pK_{a2}$ and $pK_{a3}$ of NPC 1161C, as determined by the solubility method, were found to be 4.08± 0.05 and 2.14±0.11, respectively, as discussed below. $pK_{a1}$, $pK_{a2}$, and $pK_{a3}$, as determined by ACD/Chemsketch, were 10.37±0.10, 4.06±0.45, and 1.62±0.45, respectively, and correlated very well with the experimentally determined values. The mean $pK_{a1}$, $pK_{a2}$, and $pK_{a3}$ as determined by potentiometric titration, solubility method, and ACD/ Chemsketch were calculated to be 10.12±0.10, 4.07±0.25, and 1.88±0.28, respectively.

### Solubility Measurements

#### *Aqueous and pH Solubility*

The aqueous solubility of NPC 1161C was found to be $2.4\times10^{-4}$ M with a final pH varying from 4.3 to 5.0 for various aqueous samples of the drug used in the study. The calculated pH of a saturated solution of the succinate salt using Eq. 2 is 4.15. The $K_{sp}$ of the salt was determined to be $5.76\times10^{-8}$ according to Eq. 1.

Figure 3 shows the solubility of the drug as a function of pH and the non-linear fitting of the solubility data according to the pH-solubility model described by Eq. 4. The intrinsic solubility of the drug (solubility at pH 8.0) was found to be $1.6\times10^{-6}$ M. A non-linear regression fit of the solubility of the drug (micromolar) as a function of pH was evaluated according to the pH-solubility model, using $pK_{a2}$ and $pK_{a3}$ as unknowns. An excellent correlation ($R$=0.993) was obtained and $pK_{a2}$ and $pK_{a3}$ were determined to be 4.08 and 2.14, respectively.

#### *Isolation of the Free Base from the Succinate Salt, NPC 1161C*

The purity of the extracted free base was verified using HPLC, PDA spectrum and the corresponding extracted UV spectrum (Fig. 4), the free base having the same retention time as that of NPC 1161C. Figure 5 shows the segment of $^1$H-NMR spectrum of NPC 1161C, the succinate salt, dissolved in $CD_3OD$, where a singlet is observed for the 2–$CH_2$ groups of succinic acid at a chemical shift of 2.486 ppm. The corresponding $^1$H-NMR segment of the free–base form of NPC 1161C dissolved in $CDCl_3$ is shown in Fig. 6. Conversion of the salt to the free base is confirmed by the absence of the methylene protons in the sample.

#### *Evaluation of the Excess Solid Phase in Equilibrium with the Solution in Aqueous Buffers*

Figure 7 shows the $^1$H-NMR spectrum of the excess solid phase of the drug (*i.e.* the $[B_{(solid)}]$ species of NPC 1161C, see Fig. 8b) in equilibrium with the solution in aqueous buffers. Comparing the segment of $^1$H-NMR spectrum of NPC 1161C salt, where the methylene protons appear at a chemical shift of 2.486 ppm, with that of the free base and the excess solid phase, no protons of the two methylene groups of succinic acid were observed in the $[B_{(solid)}]$ species. This indicates that the succinate moiety is not present in the excess solid phase. A multiplet was observed from 2.313 to 2.435 ppm but considering the multiplicity of the $-CH_2$ peak of the succinic acid (a singlet for the 2–$CH_2$ groups) the multiplet could be assumed to be from other impurities. This confirmed that the excess solid phase in equilibrium with the ions in solution in aqueous buffers is the free base of the drug.

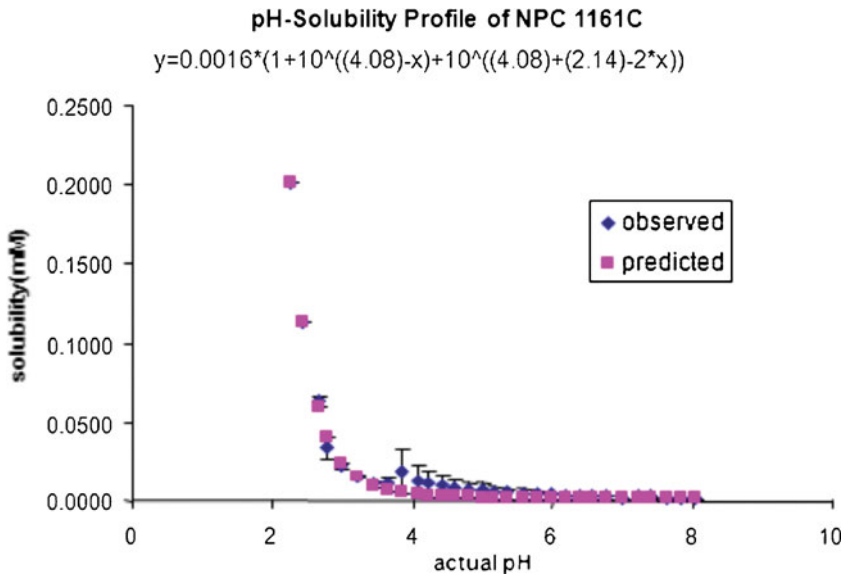


**Fig. 3.** pH-Solubility profile of NPC 1161C at a pH range of 2.2–8 using citric acid–phosphate buffers

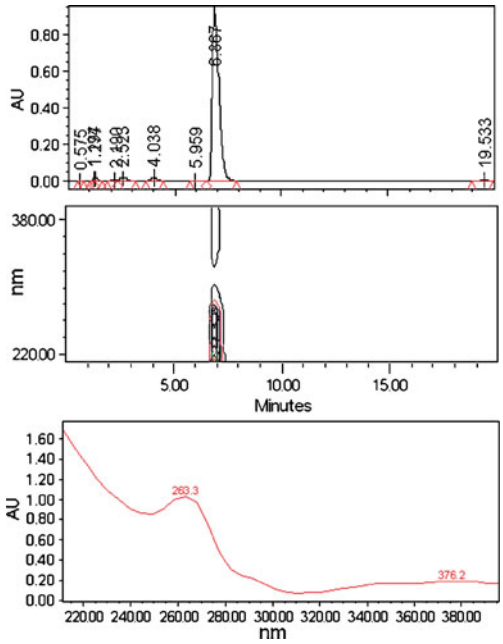


**Fig. 4.** HPLC chromatogram, PDA contour, and extracted UV spectrum of the free base of NPC 1161C. The chromatographic conditions were: column: Phenomenex Luna (4.6×150 mm), 5 μm C-18(2); mobile phase: methanol:water:TFA in the ratio of 71:29:0.065 (volume units); flow rate 1.2 ml/min; detector wavelength 254 nm; run time 20 min

*Cosolvency in Unbuffered and Buffered Aqueous Systems*

The solubility of NPC 1161C in all the cosolvent systems was found to increase exponentially with an increasing concentration of the cosolvent. Figure 9 summarizes the LOG molar solubility of the drug with increasing concentration of cosolvents in water and at pH 7.0. The solubilization power of the cosolvents in unbuffered and buffered systems was determined from the slope of the log-linear plot of the total solubility of the drug as a function of volume fraction of the cosolvent (Fig. 9), according to Eqs. 5 and 6, respectively. The solubilization power of the cosolvents in water ($\sigma_{water}$) was found to be 5.65, 5.31, 4.28, and 4.21 for EtOH, PEG 400, DMSO, and PG, respectively. The solubilization slopes of the cosolvents at pH 7.0 ($\sigma_{BH^+}$) were found to be 9.05, 8.42, 7.04, and 6.68 for EtOH, PEG 400, DMSO, and PG, respectively.

## Partition Coefficient

The use of HPLC methods for the determination of log $P_{o/w}$ has been successfully demonstrated in various studies (24,25) for a wide range of compounds (17). The determination of log $P_{o/w}$ by RP-HPLC method is highly dependent on the retention of the solutes and hence the capacity factor ($k'$).

Log $P_{o/w}$ of NPC 1161C succinate salt was found to be 2.18±0.01 (average of duplicate measurements) from the shake-flask method. Table I. summarizes the parameters of the calibration standards calculated for the determination of log $P_{o/w}$ of NPC 1161C free base by the RP-HPLC method. The retention time ($t_r$) of NPC 1161C free base, as obtained from the RP-HPLC method, was 7.624, and the LOG $k'$ was calculated to be 0.6780 (Table I). Log $P_{o/w}$ of NPC 1161C free base was calculated from the regression line of the standard plot of log $P_{o/w}$ as a function of LOG $k'$ (Fig. 10), and was found to be 3.58. mlog $P$ of the free-base form of NPC 1161C, as determined by molecular modeling method, was found to be 3.82. The mean log $P_{o/w}$ of NPC 1161C free base, as determined by RP-HPLC method and Molecular modeling, was calculated to be 3.70.

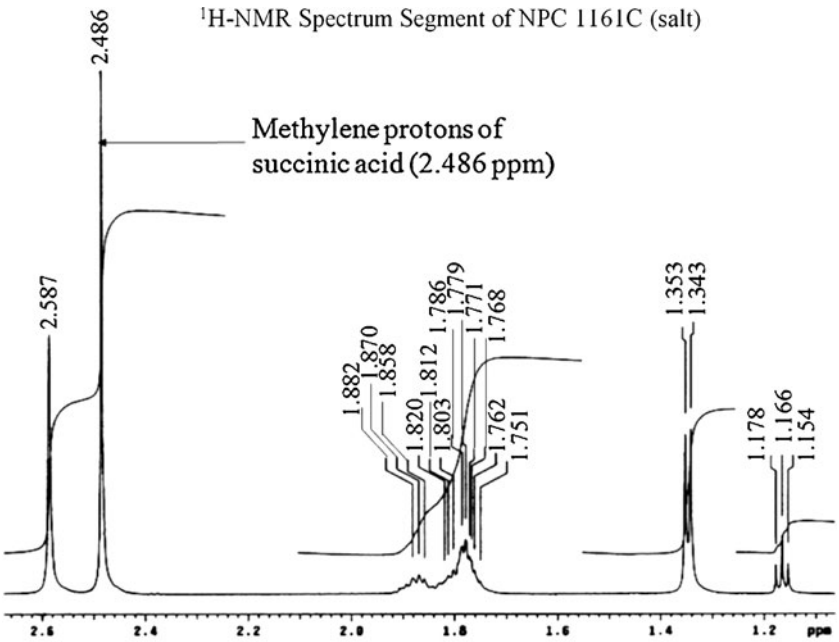


**Fig. 5.** A segment of $^1$H-NMR spectrum of NPC 1161C using Varian AS 600 NMR spectrometer

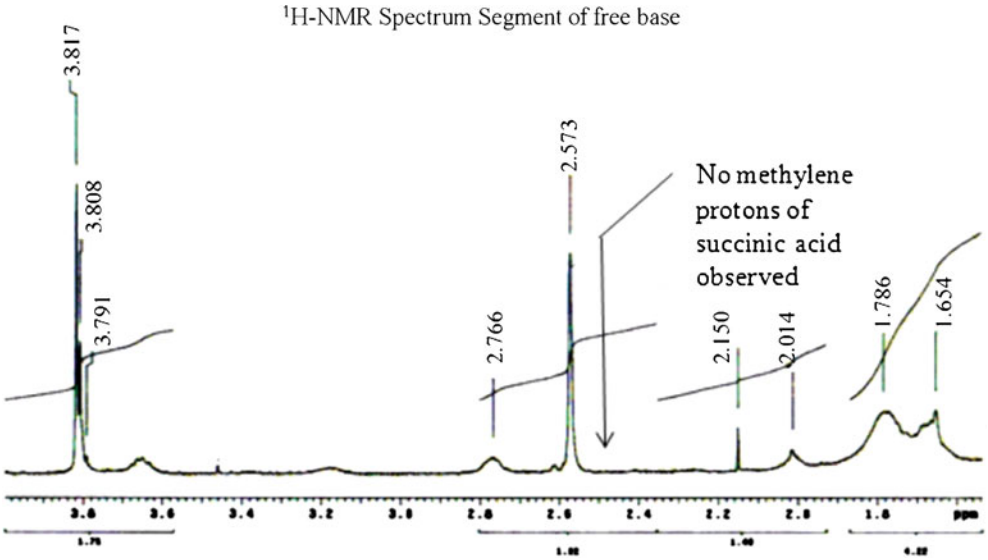


**Fig. 6.** A segment of $^1$H-NMR spectrum of the free base of NPC 1161C using Varian AS 400 NMR spectrometer

## pH Stability

The decomposition of the compound at 37°C was found to follow first-order kinetics in the pH range tested. The first-order decomposition rate constants ($k_1$) for the drug at pH 3.1, 4.9, and 6.9 were determined from the slope of the plot of natural logarithm of drug concentration as a function of time (Fig. 11), and the $t_{90}$ of the drug was determined from Eq. 9.

$$t_{90} = \frac{0.105}{k_1} \tag{9}$$

The drug concentrations in all the pH solutions after 180 days of storage were below the lower limit of quantitation, *i.e.* 0.3125 μg/mL, using the HPLC method (5). Figure 12 shows the pH kinetic profile, $k_1$ and $t_{90}$ of NPC 1161C at pH 3.1, 4.9, and 6.9 at 37°C, showing a higher degradation rate and lesser stability at pH 6.9 as compared to pH 3.1 and 4.9.

## Thermal Analysis

### *Differential Scanning Calorimetry and Thermogravimetric Analysis*

DSC thermograms revealed two endothermic events, one with a peak at 81.9°C (onset temperature at 76.0°C; end temperature at 86.1°C) and the other with a peak at 184.6°C (onset temperature at 164.9°C; end temperature at 189.1°C; Fig. 13). The first endothermic peak showed no corresponding weight loss by TGA, where as the second broad endothermic peak showed a corresponding 8.7% weight loss by TGA. An exothermic event, observed at a peak of 329.8°C (onset temperature at 297.9°C; end temperature at 368.6°C), corre-

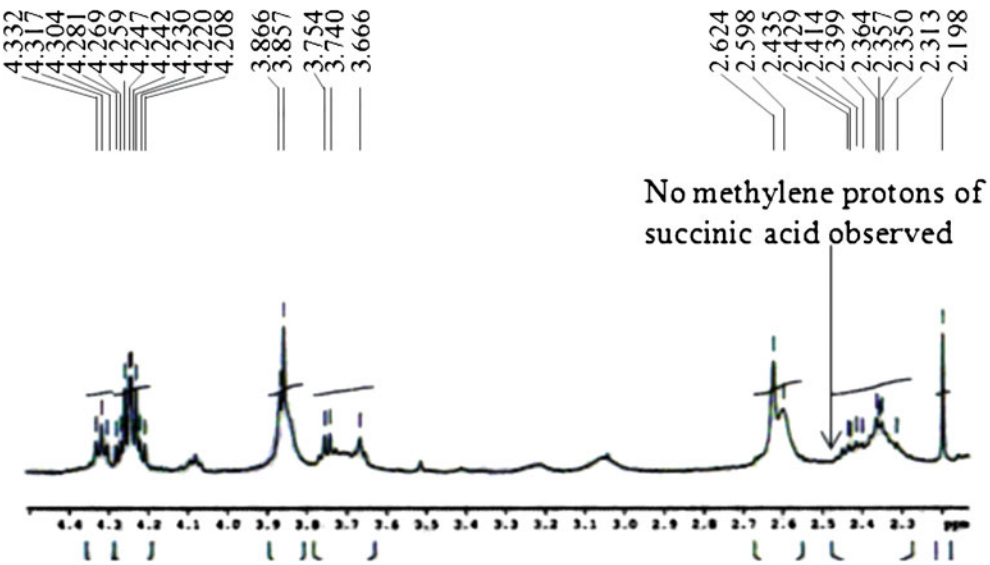


**Fig. 7.** $^1$H-NMR segment of the solid phase of NPC 1161C in equilibrium with the solution in aqueous buffers using Bruker DRX 500 NMR spectrometer

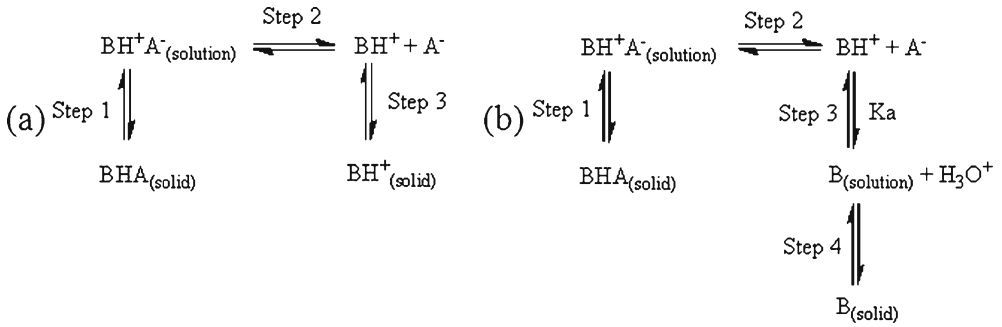


**Fig. 8.** Species of NPC 1161C in equilibrium **a** in unbuffered water **b** in pH controlled media

sponded to 74.7% of weight loss by TGA. Further investigation of the nature of the first endothermic event was done by heating the sample from 25°C to 100°C, followed by subsequent cooling to 0°C, and then reheating to 365°C, at a linear scanning rate of 10°C/min (Fig. 14). During the cooling and reheating cycle, neither a recrystallization peak nor the melting endothermic peak was observed, indicating a probable transformation from crystalline to amorphous form of the drug. A determination of the glass transition temperature (Tg) of the amorphous form of the drug was attempted using StepScan DSC and high-speed DSC experiments. The accurate determination of Tg was not possible using StepScan DSC experiments, owing to the poor baseline of the heat capacity (Cp) curve, however, an estimate of the Tg around 40°C was determined using StepScan DSC. In hyper-DSC experiments, the first endothermic event was more prominent than in normal DSC experiments (Fig. 15). An appropriate determination of the temperature of this endothermic event with a peak at 78.2°C (onset temperature at 43.7°C; end temperature at 93.4°C) was accomplished by smoothing the hyper-DSC thermogram, using the standard smoothing algorithm. When the solid sample was heated from −20°C to 115°C, followed by subsequent cooling to −20°C at a linear scanning rate of 10°C/min, and then reheated from −20°C to 215°C at 450°C/min, a clear shift in baseline was observed, with an onset at 47.7°C (end temperature at 63.4°C; Fig. 15), which was considered to be the onset of Tg of NPC 1161C.

*Melting Point Determination*

On heating, a shriveling of the compound was observed in the capillary beginning at 64.0°C with a decrease in intensity in the melt curve with increasing temperature. The compound gradually turned reddish-yellow in color with an onset of melting at 74.6–76.7°C. Coexistence of unmelted solid phase along with the melt was observed up to 136.5°C, after which the solid phase started rising up the capillary, a portion of which was eventually lost probably due to sublimation or melting. On cooling, the melt did not convert to the solid powder form, but remained in a glassy state, which indicates that the sample might have converted completely from the crystalline to the amorphous state after melting.

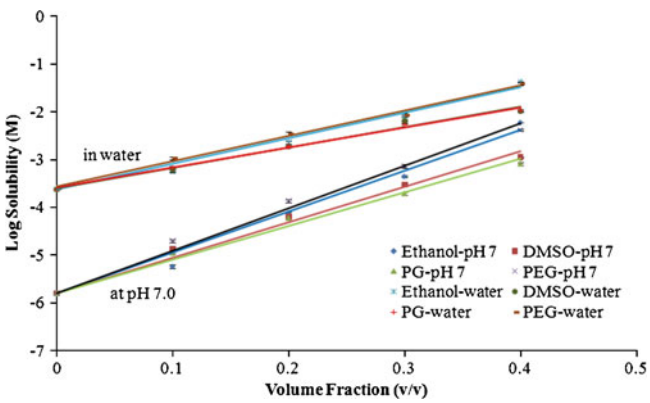


**Fig. 9.** Solubility of NPC 1161C in cosolvent-water mixture and in cosolvent-buffer mixtures at pH 7.0

### Hygroscopicity Studies

Percentage of moisture uptake of NPC 1161C samples exposed at different relative humidity, as measured in terms of percentage of weight loss of the compound using TGA, was observed to be 0.03%, 0.85%, 5.65%, and 10.32% at 32% RH, 56% RH, 76% RH, and 89% RH, respectively.

## DISCUSSION

### Acid Dissociation Constants

The precise determination of $pK_a$(s) of the free base of NPC 1161C was essential as it provided the basis for the subsequent preformulation studies undertaken. At the endpoint of the titration of the aqueous solution of NPC 1161C with NaOH when no significant increase in pH was observed with further addition of NaOH, the salt form of the drug was completely converted into its free base. The free base then ionizes on back-titration with HCl and the pH at single point half-neutralization is calculated as the $pK_{a1}$ of the drug. The $pK_{a1}$ calculated at each incremental acid titration step was found in good correlation with the single point determination at half-neutralization. $pK_{a2}$ (4.08) and $pK_{a3}$ (2.14) of the drug, which represented ionization at the tertiary and secondary amino groups, respectively, were determined using the solubility method. Considering the three $pK_a$s of the drug, it can be said that at pH 7.0 or at physiological pH, $BH^+$ (see Fig. 2) would be the major species of the drug present. At pH 4.0, $BH^+$, and $BH_2^{2+}$ would be the major species present, and $BH_3^{3+}$ will be present in very small quantity and at pH 2.0, $BH_2^{2+}$ and $BH_3^{3+}$ would be the major species of the drug present. Below pH 2.0, $BH_3^{3+}$ would be the major species. This indicates that at the gastric pH (approximately pH 1.6), all the amino groups of the drug will be ionized and $BH_3^{3+}$ would be the major species resulting in significant

**Table I.** Summary of Parameters of Calibration Standards and NPC 1161C Calculated for the Determination of log $P_{o/w}$ of NPC 1161C Free Base by HPLC Method

| Standards used | Void volume ($t_0$), ml | Average retention time (average $t_r$), min | Capacity factor, $k'=(t_r-t_0)/t_0$ | Log $k'$ | Log $P_{o/w}$ (reported) range (average[a]) |
|---|---|---|---|---|---|
| Acetanilide | 1.3225 | 1.976 | 0.4941 | −0.3062 | 1.16–1.36 (1.26) |
| Acetophenone | 1.3225 | 2.370 | 0.7921 | −0.1012 | 1.58–1.73 (1.66) |
| Anisole | 1.3225 | 3.412 | 1.5796 | 0.1985 | 2.04–2.11 (2.08) |
| 4-Iodophenol | 1.3225 | 3.548 | 1.6824 | 0.2259 | 2.92 |
| Diphenylamine | 1.3225 | 5.855 | 3.4268 | 0.5349 | 3.22–3.72 (3.47) |
| p-Nitrophenol | 1.3225 | 2.344 | 0.7724 | −0.1122 | 1.38–2.08 (1.73) |
| Hexachlorobenzene | 1.3225 | 87.258 | 64.9796 | 1.8128 | 5.75 |
| Phenothiazine | 1.3225 | 7.145 | 4.4026 | 0.6437 | 4.15 |
| NPC 1161C | 1.3225 | 7.624 | 4.7648 | 0.6780 | |

[a] Average values of reported log $P$ in parentheses

increase in solubility, however, with little or no absorption. At physiological pH (pH 7.4), $BH^+$ would likely be the major species, and in spite of being ionized on the primary amine, if the drug were sufficiently solubilized, it should be significantly absorbed as a result of its high lipophilicity.

## Solubility Measurements

### *Aqueous and pH Solubility*

NPC 1161C was found to be a sparingly water soluble succinate salt. The calculated pH of the saturated solution of the salt is very near to the experimentally observed pH. The calculated pH was based on $pK_{a1}$ (4.20) of the succinic acid and $pK_{a2}$ (4.08) of the base, assuming the concentrations of all species formed by the addition of more than one proton to a polyacidic base will be negligibly small (8). The solubility of NPC 1161C increased exponentially with decreasing pH (Fig. 3). A good correlation between the experimental and predicted solubility of the drug using the pH-solubility model was obtained.

Based on the $pK_{a1}$ of the drug, the protonated form of the drug at the primary amine group *i.e.* $BH^+$, would be the major starting species for the derivation of the pH-solubility model. The solubility of the drug at pH 7.0, where $BH^+$ is the primary species, was found to be $1.6\times10^{-6}$ M and remained approximately constant till pH 8.0 (Fig. 3). There are two species of the drug present, the free base, B, and $BH^+$, at pH 8.0, however, because of the extremely low solubility of the drug from pH 7 to 8, it is reasonable to assume that the solubility of the free base ($S_0$) is approximately equal to the solubility of the $BH^+$ species of the drug.

### *Isolation of the Free Base of NPC 1161C and Evaluation of the Excess Solid Phase in Equilibrium with the Solution in Aqueous Buffers*

Figure 8a. represents the species of NPC 1161C in equilibrium with each other in unbuffered water, where $BHA_{(solid)}$ represents the salt as the starting solid and $BH^+A^-$ represents the intermediate species of the salt in the solution which is in equilibrium with $BH^+$ (the cationic part of the salt arising from the base) and $A^-$ (the anionic part of the salt arising from the acid). The higher solubility of the salt as compared to that of the free base is due to step 1, when more of the solid phase (BHA species) goes in the solution. The solid phase in equilibrium in unbuffered water is the salt form of the drug and is represented by $BH_{(solid)}^+$ species. It was determined from preliminary studies that the salt form of the drug is not soluble in chloroform and precipitated; whereas the free amine (*i.e.* the free base) is freely soluble in chloroform. On the other hand, the salt form is freely soluble in methanol, and the free base is sparingly soluble in methanol.

The formation of the free base was verified from the structure of the compound using $^1$H-NMR spectroscopy and compared with the structure obtained for NPC 1161C (the salt form of the drug). A singlet for the 2–$CH_2$ groups of succinic acid was observed at 2.486 ppm in the $^1$H-NMR spectrum of NPC 1161C salt. Since no corresponding singlet of the methylene protons of succinic acid was observed in the

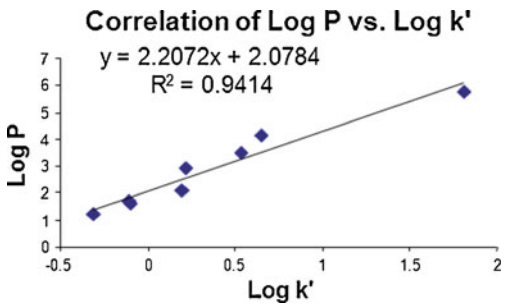


**Fig. 10.** Correlation of log $P_{o/w}$ and Log $k'$ of the calibration standards used for the determination of log $P_{o/w}$ of NPC 1161C free base by HPLC method

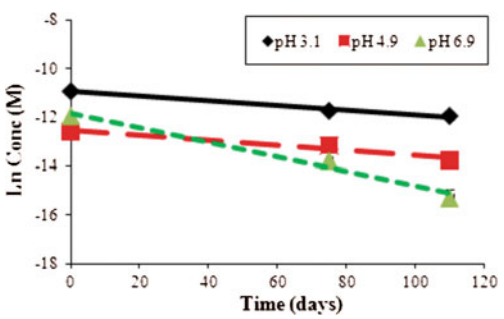


**Fig. 11.** First-order degradation kinetics of NPC 1161C at pH 3.1, 4.9, and 6.9, at 37°C

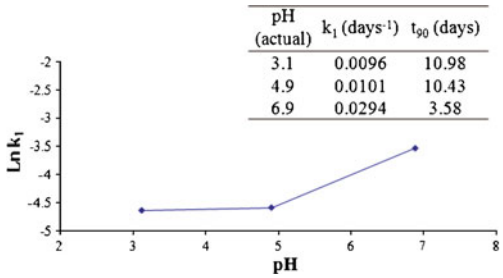


**Fig. 12.** pH kinetic profile and summary of $k_1$ and $t_{90}$ of NPC 1161C evaluated at pH 3.1, 4.9, and 6.9 at 37°C

$^1$H-NMR spectrum of the sample extracted with 5% NaOH, it was confirmed that the salt form of the drug completely converted to the free base following the extraction procedure.

Figure 8b and Eq. 10 (26) represent the equilibrium existing in pH controlled media with buffers and in the presence of other counterions in solution, where $BH^+$ and B are the protonated and the free-base forms of the compound respectively, and $K_a$, the acid dissociation constant of the compound:

$$K_a = \frac{[B][H_3O^+]}{[BH^+]} \tag{10}$$

Under such equilibrium conditions, two situations may arise: either the salt (*i.e.* the $BH^+$ species) is the saturation species, *i.e.* the salt form of the drug exists as the solid phase, or the free-base form (*i.e.* the *B* species) is the saturation species, *i.e.* the free base exists as the solid phase, during the determination of the pH-solubility profile. When the solid phase is the salt, the total solubility ($S_{T,salt}$) is given Eq. 11 (26):

$$S_{T,salt} = [BH^+]_s + [B] = [BH^+]_s\left(1 + \frac{K_a}{[H_3O^+]}\right) \tag{11}$$

where "*S*" represents the saturation species. On the other hand, when the free base is the saturation species, the total solubility ($S_{T,base}$) is given by Eq. 12 (26):

$$S_{T,base} = [BH^+] + [B]_s = [B]_s\left(1 + \frac{[H_3O^+]}{K_a}\right) \tag{12}$$

$pH_{max}$, the pH of maximum solubility of the compound, is defined as the intersecting point of the two independent pH-solubility curves as represented by Eqs. 11 and 12, and $S_{T,salt}$ and $S_{T,base}$ represent the total solubility of the compound below and above $pH_{max}$, respectively. This indicates that when the pH of the suspension is above $pH_{max}$, the salt as the starting material would be converted to free base, and the free base would be converted to the salt form below $pH_{max}$ (26).

The $pH_{max}$ of the free base of NPC 1161C is expected to be below pH 2.2 since the solubility of the drug steadily increased with decreasing pH to pH 2.2. Hence, for NPC 1161C, it could be said that from pH 2.2 to 8, the excess solid phase in equilibrium with the solution in aqueous buffers and in presence of any counterions was the free base of the drug (*i.e. B* species). This was confirmed through preliminary solubility experiments in chloroform as well as using $^1$H-NMR spectroscopy. The excess solid phase in aqueous buffers, at all the pH values tested, dissolved freely in chloroform, an attribute which was similar to the free base of the drug. $^1$H-NMR spectroscopy also revealed the absence of the prominent singlet of the 2–$CH_2$ protons of succinic acid in the solid phase, indicating that the excess solid phase which is in equilibrium with the solution in aqueous buffers and in presence of other counterions is the free-base form of the drug. It was for this reason, the contribution of the succinate counterion of the salt was not considered in the derived pH-solubility model [Eq. 4.], and only the contribution of the free base and/or its protonated

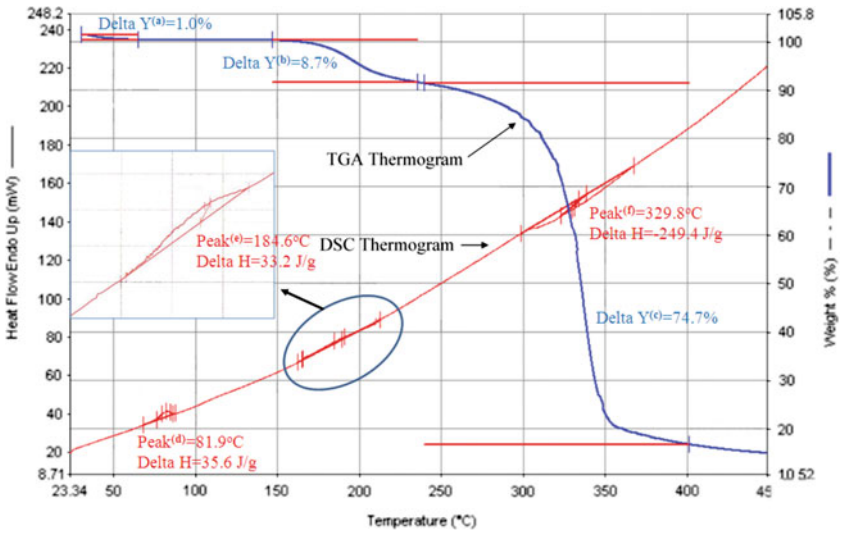


**Fig. 13.** DSC and TGA thermogram of solid NPC 1161C heated from 25°C to 450°C at a linear scanning rate of 10°C/min. Inset: Enlarged DSC thermogram of the second exothermic event with a peak at 184.6°C (onset temperature at 164.9°C; end temperature at 189.1°C). [(a)] X1=30.1°C, Y1=101.4%, *X*2=64.9°C, Y2=100.4%; [(b)] X1=146.6°C, Y1=100.3%, *X*2=235.2°C, Y2=91.6%; [(c)] X1=239.4°C, Y1=91.3%, *X*2=401.0°C, Y2=16.7%; [(d)] onset=76.0°C, end=86.1°C; [(e)] onset=164.9°C, end=189.1°C; [(f)] onset=297.9°C, end=368.6°C

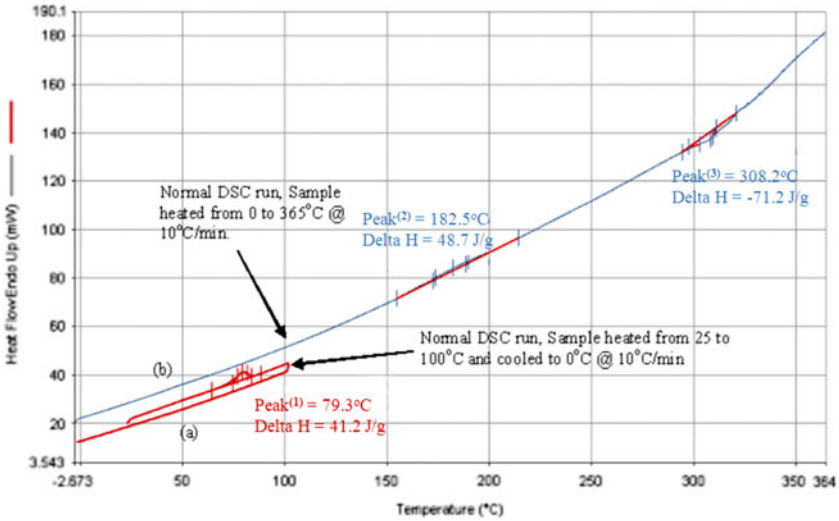


**Fig. 14.** DSC thermogram of solid samples of NPC 1161C: **a** heated from 25°C to 100°C, and subsequently cooled to 0°C, and **b** reheated to 365°C, at a linear scanning rate of 10°C/min. (1) onset=74.6°C, end=84.1°C; (2) onset=172.4°C, end=188.8°C; (3) onset=297.9°C, end=311.3°C

species was considered for drug solubilization in pH controlled buffer media.

*Cosolvency in Unbuffered and Buffered Aqueous Systems*

In cosolvent–water mixtures, ethanol and PEG 400, each produced an approximately 1,800-fold increase in solubility at 40% concentration, and an approximately 4,000-fold increase in the solubility of the drug was observed with 40% ethanol solution buffered at pH 7.0. It was observed that $\sigma_{BH+}$ was always greater than the $\sigma_{water}$ in cosolvent–water mixtures for all the cosolvents studied. It was determined that in unbuffered aqueous solutions, the excess solid phase (saturation species) in equilibrium with the solution is the salt form of the drug and in presence of aqueous buffers (pH 7.0 in this case), the excess solid phase (saturation species) is the free base. Hence, the solubilization power of the cosolvents in solubilizing the free base was found to be greater than that for the salt form of the drug. Ethanol was found to be the most effective cosolvent, having the highest solubilization power, followed by PEG 400, DMSO, and PG, in both water and at pH 7.0.

**Partition Coefficient**

The log $P_{o/w}$ of the salt form of the drug was determined by the shake-flask method, where as log $P_{o/w}$ of the free base was determined using the HPLC method. It was observed that the octanol–water partition coefficient of the free base was higher than that of the salt form. Since solubility is also a measure of log $P_{o/w}$, it was observed that the aqueous solubility of the salt form was higher than the intrinsic solubility of the free base. The mlog $P$ value determined for

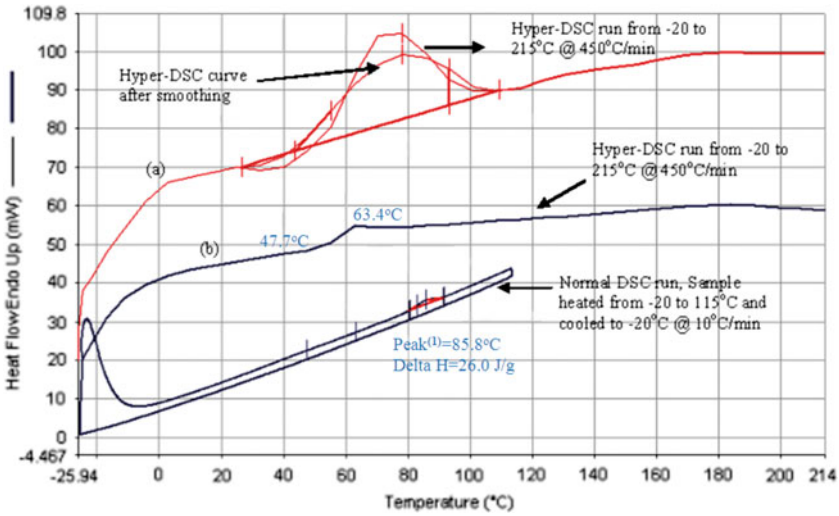


**Fig. 15.** *(a)* High-speed DSC experiments of solid NPC 1161C samples heated from −20°C to 215°C at a linear scanning rate of 450°C/min: onset=43.7°C, peak=78.2°C (delta H=64.1 J/g), end=93.4°C; *(b)* Solid samples of NPC 1161C heated from −20°C to 115°C, and subsequently cooled to −20°C at a linear scanning rate of 10°C/min, and then reheated to 215°C at a linear scanning rate of 450°C/min. (1) onset=80.9°C, end=91.3°C

the free base correlated very well with the log $P_{o/w}$ of the free base determined using the HPLC method. The high partition coefficients obtained for both the salt form and the free base of the drug indicate that both the forms are highly lipophilic and hence have poor aqueous solubility. According to the Lipinski's "rule of 5" (27), a compound having a log *P* over 5 and mlog *P* over 4.15, when formulated can pose a significant absorption and permeation problems. Since the log *P* and mlog *P* of the salt as well as the free base of NPC 1161C was found to be lower than the above-mentioned values, Lipinski's "rule of 5" would predict that drug formulations should have no absorption or permeation issues.

## pH Stability

The three pHs for the solution stability of NPC 1161C were chosen based on the pKas of the drug. At pH 6.9, $BH^+$ would be the major species of the drug present; at pH 4.9, $BH^+$ and $BH_2^{2+}$ would be the major species of the drug; and at pH 3.1, $BH_2^{2+}$ and $BH_3^{3+}$ would be the major species of the drug. Hence, stability information of the individual and combination of the various ionic species of the drug could be obtained at the tested pHs at 37°C. The determination of $t_{90}$ facilitated in describing the stability of the drug under various pH conditions at 37°C. A longer $t_{90}$ indicates higher stability of the drug at the tested pH and temperature condition. The drug was found to be poorly stable at 37°C, especially at pH 6.9, with a $t_{90}$ of 10.98, 10.43, and 3.58 days at pH 3.1, 4.9, and 6.9, respectively. There is no significant difference in the decomposition rate constant or $t_{90}$ of the drug at pH 3.1 and 4.9. Stability issues are mostly encountered with unionized species of weak bases. With increase in pH, more of $BH^+$ is present in the solution, and hence a decrease in stability was observed. Though the drug concentration in the solutions at all the pHs decreased with time, no degradation peaks were observed in the HPLC chromatogram. This might be due to either the absence of chromophores in the degradation products, or the concentration of the degradation products was below their limit of detection by the HPLC assay method. NPC 1161C was found to have poor solubility at the three pHs tested for solution stability. The saturated solubility of the drug at pH 3.1, 4.9 and 6.9 at 25°C, was observed to be 12.4 μg/ml (0.022 mM), 3.85 μg/ml (0.007 mM) and 1.33 μg/ml (0.002 mM), respectively. The drug solutions prepared for stability studies at time, $t$=0 days, were of extremely low concentrations (9.97 μg/ml for pH 3.1, 1.06 μg/ml for pH 4.9 and 1.30 μg/ml for pH 6.9), and hence the concentration of the various degradation products generated would be very low.

The forced degradation studies (acid hydrolysis, base hydrolysis, dry heat, reduction, and oxidation) performed with NPC 1161C revealed that the major degradants of the drug in significant quantities were observed under dry heat conditions, rather than by any other degradation pathway (5). No degradation of the drug was observed during saturated solubility studies performed under various pH conditions and in cosolvent-buffer mixtures at pH 7.0 at 25°C. Hence, temperature might be the most significant factor responsible for major degradation of the drug at the various pHs tested for solution stability. A combination of temperature and pH effects might be responsible for the different degradation profiles (different $t_{90}$) of the drug at various pHs.

## Thermal Analysis

The first endothermic event observed in DSC at a peak of 81.9°C (onset temperature at 76.0°C; end temperature at 86.1°C) might be due to the melting of the crystalline portion of the active ingredient, as no corresponding weight loss was observed in TGA. The melting endotherm observed in DSC had an average ΔH of 34.3 J/g (average of triplicate experiments). Comparing this value with reported heats of fusion of organic compounds (28) having similar melting points, the heat of fusion observed for NPC 1161C was significantly lower, indicating that the compound might be semi-crystalline with a high amorphous content. The second endothermic peak at 184.6°C (onset temperature at 164.9°C; end temperature at 189.1°C) in DSC might be due to sublimation of a part of the drug or its decomposition products at higher temperatures, as visual observation revealed yellow stains on the outside of DSC pan, as well as on the protective glass cover of TGA after experimental runs. The exothermic event observed at the peak of 329.8°C (onset temperature at 297.9°C; end temperature at 368.6°C) in DSC is likely due to the decomposition of the compound. During the heat–cool cycle in DSC, neither the melting endotherm reappeared nor was any recrystallization peak observed (Fig. 14), which indicates that either the crystalline content of the compound converted into the amorphous form, or the recrystallization of the melt did not take place in the same time scale as that of the experimental condition. Hyper-DSC experiments provided enhanced sensitivity in detecting the weak thermal transitions under high-speed experimental conditions. A shift in baseline was observed using hyper-DSC with an onset temperature of 47.7°C (end temperature at 63.4°C), which corresponds to a glass transition (Fig. 15). However, this shift in baseline was not observed in a standard DSC run at a linear scanning rate of 10°C/min (Fig. 14).

The shriveling of the compound as observed with heating in the melting point apparatus might be due to some changes in the crystal state of the solid. This event was, however, not observed in DSC. The first endothermic event observed in DSC was confirmed to be the melting endotherm as this was also observed in the melting point apparatus. It might be also possible that there is a polymorphic transition of the compound starting at 64.0°C, and the endothermic enthalpy of this polymorphic transition is too low to be detected in DSC. Subsequently, the first endothermic event observed in DSC at 81.9°C is due to the melting of the most stable polymorph of the compound. The onset of the melting point was observed at a temperature very close to that observed in DSC. Since the melt did not convert into powder on cooling but remained at a glassy state, it is most likely that the solid converted from the semi-crystalline state to amorphous state on melting. A similar result was obtained with DSC where the melting endotherm did not reappear on reheating the sample, however, a glass transition was observed.

## Hygroscopicity

The compound did not exhibit significant moisture uptake below 56% RH (less than 1%), but can be considered moderately hygroscopic at greater than 60% RH, with 5.7% moisture uptake at 76% RH, and 10.3% moisture uptake at

89% RH. Non-hygroscopicity or moderate hygroscopicity can be directly correlated with the drug's poor aqueous solubility. Limited water adsorption under normal handling conditions suggests that the drug can be easily formulated in a solid dosage form.

## CONCLUSIONS

NPC 1161C was found to be highly lipophillic and sparingly soluble in an aqueous media of uncontrolled pH, but practically insoluble in the pH range of physiological importance. The $pK_a$s of the drug determined, using the potentiometric method and solubility method, provided valuable information about the nature of the ionic species present at a particular pH. The solubility of the drug decreased exponentially with increasing pH and the excess solid phase during pH solubility determination was found to be the free-base form of the drug. Cosolvents, primarily ethanol and PEG 400, were found to significantly increase the solubility of the drug, both at uncontrolled and controlled pH. Based on the hypothesis of this research, NPC 1161C does not possess all the desired physicochemical properties, such as high aqueous solubility and higher stability **(**longer $t_{90}$**)** at 37°C, especially at pH 6.9, which is a major formulation concern for the drug. For further preclinical development of NPC 1161C as an oral solid dosage form, solubilizing and stabilizing agents such as cyclodextrins or using an alternate salt form of the drug having higher intrinsic solubility and better pH stability, may be useful to overcome such challenges. The probable site of absorption for the drug is the small intestine, where at neutral pH and higher, most of the drug will be present as $BH^+$ form which will be readily absorbed because of the high lipophilicity of the drug. Though the drug would be very soluble in the acidic pH of the stomach, it is not likely to be significantly absorbed since it will be present as the protonated species at stomach pH. An enteric-coated and/or pH dependent controlled release solid dosage form might be useful to increase the bioavailability of NPC 1161C. In the solid state, the compound is present as a semi-crystalline powder, which is transformed into the amorphous state after melting. No incidence of polymorphism has been observed. The drug was found to sublime at approximately 184.6°C and was not found to be hygroscopic below 56% RH. The physicochemical characterization of NPC 1161C provided useful information for formulation development and preclinical evaluation of the drug.

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