

Evaluation of the Physicochemical Properties of a Novel Antimalarial Drug Lead, Cyclen Bisquinoline

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Received date: Apr 21, 2016; Accepted date: May 11, 2016; Published date: May 13, 2016

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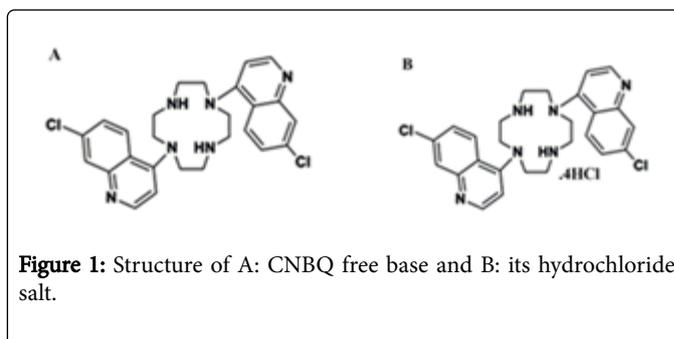
Abstract

The purpose of this study was to evaluate physicochemical properties of an antimalarial drug lead, 4,10-bis(7-chloroquinoline)-1,4,7,10-tetraazacyclododecane (Cyclenbisquinoline; CNBQ) and its hydrochloride salt. The free base (FB) of CNBQ is a white polymorphic crystalline powder and the salt is off-white powder. The application of standard experimental protocol including differential scanning calorimetric (DSC) analyses revealed that the FB has at least four different crystalline polymorphs melting at 166°C, 178°C, 195°C and 234°C, respectively, and the salt showed a broad endotherm, suggesting it to be amorphous in nature. Equilibrium solubility and stability of both FB and salt were carried out in different mediums, and samples were analyzed using reverse phase-high performance liquid chromatography (RP-HPLC). The compound is highly hydrophobic; however, salt formation improved its water solubility by approximately 370-fold. Both FB and salt forms were stable in a wide range of conditions (acid, base, water, light and heat), except oxidation. All these properties, in addition to previously determined and published log P and pKa values would be useful in implementing the modern quality by design (QbD) approaches for further development of the drug lead.

Keywords: Physicochemical properties; Phase transition; Stability; Solubility; pKa; Log P

Introduction

Amongst the numerous life threatening health problems the world is facing today, malaria has been very prominent especially in developing countries of Southeast Asia, South America, and South Saharan Africa [1]. The 4-aminoquinoline based drugs, for instance, chloroquine that has been profoundly used as an anti-malarial agent, has now been proven to be ineffective towards its resistant strains [2]. Even with potent curative effect, artemisinin and its derivatives also have issues like thermal instability [3,4] and high cost [5] which make its access in developing countries very limited. Addressing to all these limitations, taking an advantage of the similarity in structure with heme, and its ability to bind with metal complexes, tetraazamacrocycles (e.g., Cyclen) based antimalarial drugs are being discovered. It is important to note that within the past decades, the cyclen derivatives have become an important structural moiety for variety of diagnostic and therapeutic pharmaceutical agents [6-8]. Cyclen based anti-malarial drugs showed potent *in vitro* and *in vivo* anti-malarial activity. The drug lead, Cyclenbisquinoline (Figure 1) with IC_{50s} of 7.5 and 19.2 nM against chloroquine sensitive and chloroquine resistant strains of *Plasmodium falciparum*, respectively, was proven to have the most potent anti-malarial activity [9].



This lead structure (CNBQ) and the related compounds fulfilled important criteria for promising new antimalarial drug leads and warrant further study: 1) active against chloroquine-resistant as well as multidrug resistant isolates of *P. falciparum*, 2) simple pharmacophore structure that will afford low cost manufacturing, 3) orally effective in non-clinical malaria model, 4) potential to be developed as a single dose antimalarial drug, 5) long half-life and metabolic stability against human liver microsomes as well as CYP2C8, enzymes responsible for chloroquine metabolism [9,10]. Early evaluation of compounds with drug-like properties will be helpful for speculation and recognition of potential challenges during the process of drug discovery and development. Evaluation of physicochemical properties not only saves time but also makes the process economic. These evaluations have significantly decreased the attrition rate in drug discovery and development due to factors like suboptimal physicochemical parameters, pharmacokinetics, and bioavailability. Compounds with suitable biopharmaceutical perspective and optimal physicochemical parameter such as solubility, lipophilicity, ionization constant and stability should be selected for discovery and development [11]. As

physicochemical properties relate to the absorption, distribution, metabolism, and excretion (ADME) process of drugs *in vitro* and *in vivo*, these characterizations will be helpful in selection, optimization, and development of potential drug-like compounds [12]. Occurrence of solid-state phase transformation is very prominent during drug and its formulations development due to processing, or drug-excipient(s) interaction [13-16]. This phase transformation may have a huge effect on factors like dissolution, bioavailability, and stability of the drug. Determination and quantification of phase transition and conformational changes hold significant value in drug discovery and development.

The solubility of drugs is referred as a maximum amount of drug in a solvent to form a homogenous mixture in a specific condition. Besides properties of the drug molecules and the solvent, factors like polarity, ionization potential, size, form, and pKa of the drugs have huge effects on the solubility [11]. Thermodynamically stable crystalline form is usually less soluble compared to its correspondent amorphous form, which is usually less stable and prone to crystallization and degradation [17]. Thus, solubility is an important factor to be evaluated in the process of drug discovery and development, which can create obstacles during the development process [12].

The stability of drug candidates is another indispensable physicochemical property to be considered during drug discovery process. The stability screening in various pharmaceutical conditions helps to identify impending hurdles during the development process [11]. ICH guidelines requires the parent drugs to be tested under stress conditions such as the effect of pH, temperature, humidity, light, and oxidizing agents [18]. Stability of the compounds in solution of different pHs is required to formulate the solution dosage forms. Oxidation is the most common degradation pathways for organic compounds, and thus analysis of oxidative stability is one of the crucial steps in the drug discovery and development. Photostability, and thus its screening are important for functions like handling, packaging, and labeling of the compounds [19].

Two most important physicochemical parameters for drug development, such as determination of *in vitro* metabolic stability, lipophilicity (*log P*), and pKa values have already been reported for the drug lead CNBQ [10,20,21]. This paper reports the detail DSC analyses, solubility and stability studies of the free base (FB) and the salt form of antimalarial drug lead CNBQ.

Materials and Methods

Materials

All the solvents used in these studies (both HPLC/LC-MS and reagent grades), sodium hydroxide, hydrochloric acid, hydrogen peroxide, triethylamine, dibasic anhydrous sodium phosphate and phosphoric acid were purchased from Fisher Scientific. Deuterated chloroform and formic acid were purchased from Sigma-Aldrich. Chloroquine diphosphate was purchased from Pfaltz and Bauer. The drug lead, CNBQ (Figure 1) was synthesized in the laboratory.

Experimental methods

Drug lead and its salt synthesis: Both FB and salt form of the CNBQ were synthesized in the laboratory according to the literature methods [9,22]. The FB was recrystallized using ethanol and acetonitrile. Hydrochloride salt of the FB was prepared treating 0.1 N hydrochloric

acid at 80°C and was crystallized from methanol: water (50:50). The elemental analysis of hydrochloride salts of CNBQ was conducted by PerkinElmer (Series II) Autosampler Carousel on the 2400 CHN Elemental Analyzer. Infrared spectrum of the compound was obtained using Thermo Scientific Nicolet 380 FT-IR Spectrometer. NMR spectra were recorded on Bruker Avance III HD- Ascend 400 MHz NMR spectrometer in deuterated chloroform for FB and deuterated oxide for hydrochloride salt. Mass of the CNBQ was taken using Agilent 6490 triple quadrupole LC/MS system with iFunnel technology ESI ion source in positive mode.

Thermal analysis by DSC: The energy of phase transition, conformational changes, and melting point for both FB and salt of CNBQ was determined using differential scanning calorimeter (DSCQ2000). For the DSC analysis, 1-2 mg of drug sample was taken in a tzero pan-lid and, sealed using a compressor. Thermal events were quantified using Universal Analysis 2000 version 3.9A software. The compounds were either heated or subjected to heat-cool-heat cycle. In the first cycle (Cycle 1), the compounds were heated at a constant rate of 5°C/min above their melting points but below their degradation temperatures, and kept at this condition for one minute. In the second cycle (Cycle 2) the compounds were cooled at 5°C/min up to 25°C, and reheated again at 5°C/min in the third cycle up to 250°C (Cycle 3) (Tables 1 and 2). The thermal events such as glass to rubber transition, recrystallization and melting were detected and quantified.

Sample Purge flow	50 ml/min
Flange Temperature	25°C
Heater Temperature	25°C
Heater Temperature	25°C, Isothermal for 1 min
Ramp (Cycle 1, Heat)	5°C/min to 250°C
Ramp (Cycle 2, Cool)	5°C/min to 25°C
Ramp (Cycle 3, Heat)	5°C/min to 250°C

Table 1: Method for DSC analysis.

The melting point was also determined by using DigiMelt MPA 160. The drug substance was packed in the capillary tubes and placed into the DigiMelt oven to record the melting point. The melting point analysis was carried out within the temperature range of 25°C-260°C at the rate of 5°C/min.

Solubility study by RP-HPLC: Solubility study for FB was carried out using various solvents (DMSO, ethanol, isopropyl alcohol, tween 80, propylene glycol, PEG 400, water, pH 1, pH 4.5, pH 7.4, pH 9.0, pH 12.0). The solubility of the salt was determined in water, propylene glycol, DMSO, ethanol, and PEG 400. Substrate was added to both FB and salt solutions till saturation and were sonicated for 5 minutes and kept in shaker for 24 hours at 25°C. To quantify the saturation level of the drug in the above mediums, a standard solution of FB (0.0067 mg/ml) and salt (0.0067 mg/ml) were prepared and analyzed by RP-HPLC.

Chromatographic conditions: Chromatographic separation of CNBQ was obtained from Waters X-Bridge C-18 column (4.6 mm × 250 mm, 5.0 μm particle size, Part no. 186003117) purchased from Waters Corporation. An isocratic separation mode with mobile phase consisting of 70% of 0.1% triethylamine in methanol and 30% of 0.02 M dibasic sodium phosphate (anhydrous) was used and pH 3.5 was

adjusted with Phosphoric Acid. Mixture of acetonitrile and water (50:50) was used as diluent. The instrumental set up included the flow rate which was maintained at 1.0 ml/min, and temperature of the column oven at 40°C. The injection volume was set to 5 µl and the run time to 10 minutes. After the run time, effluent was detected at 325 nm.

Stability study by RP-HPLC: Stability study for both FB and salt were carried out using different mediums (0.3% H₂O₂, 0.1 M HCl, 0.1 NaOH, water, and buffer: pH 1.0-12.0) for 48 hours. Also, the samples were treated with sunlight and heat (60°C) for seven days. Stock solution (0.08 mg/ml) of FB and salt were prepared to check the solution stability in the above mediums.

Medium	Sample ID	Substrate Added (ml)	Medium Added (ml)	Final Volume (ml)	Final Concentration (mg/ml)
0.3% H ₂ O ₂	Blank	N/A	0.1 ml	10 ml	N/A
	Test Sample	1 ml	0.1 ml	10 ml	0.008
0.1 M HCl	Blank	N/A	1 ml	10 ml	N/A
	Test Sample	1 ml	1 ml	10 ml	0.008
0.1 M NaOH	Blank	N/A	1 ml	10 ml	N/A
	Test Sample	1 ml	1 ml	10 ml	0.008
H ₂ O	Blank	N/A	1 ml	10 ml	N/A
	Test Sample	1 ml	1 ml	10 ml	0.008
pH (1.0-12.0)	Blank	N/A	1 ml	10 ml	N/A
	Test Sample	1 ml	1 ml	10 ml	0.008

Table 2: Sample preparation details for stability study.

After adding substrate to the medium, the samples were kept in water bath (80°C) for 30 minutes and were left at room temperature for next 48 hours. The samples were then dissolved in acetonitrile and water (50:50) to obtain a concentration of 0.008 mg/ml that were analyzed using RP-HPLC. For solid-state stability, FB and salt were kept in the sunlight and in oven (60°C) for seven days. These samples (0.008 mg/ml) were then analyzed using similar chromatographic conditions.

Chromatographic conditions: Chromatographic separation of CNBQ, and its degradants were estimated by Waters X-Bridge C-18 column (4.6 mm × 250 mm, 5.0 µm particle size, Part no. 186003117), purchased from Waters Corporation. A gradient separation mode (Table 3) with mobile phase consisting of 0.1% of triethylamine in methanol as organic phase and 0.02 M dibasic sodium phosphate (anhydrous) at pH 3.5 adjusted with phosphoric acid as buffer.

Time (min)/solvent	Buffer (0.02 M Na ₂ PO ₄) (%)	Organic Phase (0.1% TEA in Methanol) (%)
0	48	52
6	48	52
15	10	90
20	48	52
25	48	52

The instrumental set up included the flow rate which was maintained at 1.0 ml/min, and temperature of the column oven at 40°C. The injection volume was set to 10 µl and the run time for 25 minutes. After the run time, effluent was detected at 325 nm (based on

the λ of isoquinoline). The peak purity of the sample chromatograms were obtained using photodiode array detector (PDA, G1315D-Agilent). The method was found linear over the concentration range of 0.2 µg/ml (limit of detection) to 9.6 µg/ml (R²=0.9998) for drug lead CNBQ.

Results and Discussion

Chemistry

The FB is a white polymorphic crystalline powder whereas the salt is off-white powder. The conversion of the FB to its HCl salt improved the water solubility by up to 370-fold. The peak at 3294.82 cm⁻¹ in FT-IR spectrum that was observed in FB disappeared with the salt formation, but a new broader peak observed at higher wavelength (Figure 2). The elemental analysis results for hydrochloride salts of CNBQ are within the acceptable limit (Calc. for C₂₆H₂₈Cl₂N₆.4HCl.H₂O: C, 47.36; H, 5.20; N, 12.75; Found: C, 47.22; H, 5.54; N, 12.51). The ¹H NMR and Mass spectra are shown in Figure 3. ¹H NMR (CNBQ FB in CDCl₃): δ 3.0 (t, 8H, CH₂), δ 3.5 (t, 8H, CH₂), δ 7.0 (d, 2H, Ar-H), δ 7.3 (q, 2H, Ar-H), δ 8.0 (d, 2H, Ar-H), δ 8.6 (d, 2H, Ar-H), δ 8.7 (d, 2H, Ar-H). ¹H NMR (CNBQ salt in D₂O): δ 3.5 (t, 8H, CH₂), δ 4.2 (t, 8H, CH₂), δ 7.1 (d, 2H, Ar-H), δ 7.4 (q, 2H, Ar-H), δ 7.9 (d, 2H, Ar-H), δ 8.0 (d, 2H, Ar-H), δ 8.5 (d, 2H, Ar-H). ¹H NMR data of hydrochloride salt shows that the protons (CH₂) present in cyclen ring shifted downfield with respect to FB, due to the deshielding effect of neighboring electron deficient 2NH⁺ atoms. The protons (CH) present in quinoline ring has shown negligible shifting as the NH⁺ formed is in only one ring containing two protons. In the ¹H NMR of CNBQ FB, chemical shift at δ 3.8 (t) and δ 1.2 (q) are from residual solvent (ethanol) of crystallization. In the previous studies, mass and NMR data of CNBQ were also published [22].

closer to the one observed in cycle 3, in the previous experiment (Figure 4B). This suggests that the three crystalline forms seen in cycle 1, amorphoused on melting. However, some portion of the amorphous form recrystallized in cycle 2. The mixture of amorphous and crystalline forms when heated melted at 229°C. It also suggests that the polymorph obtained at 229°C is the stable polymorph. Even though three different polymorphs were identified in cycle 1, but it recrystallized to the most stable form on cooling. The shift in melting endotherms in cycle 3 for Figure 4B and 4C could be due to presence of different ratios of amorphous to crystalline compound present when reheated in cycle 3.

In Figure 4D, the HCl salt of the FB was heated up to 300°C. A broad endotherm was observed at 100°C. This suggests the loss of residual solvent from the compound evaporated on heating. A broad endotherm was also observed at 260°C. The DSC results indicate that the salt form is amorphous in nature due to the absence of any crystalline melting endotherm. The compound started to degrade when heated above 270°C. The DSC data is summarized in Table 4 below.

Compound	Cycle 1, Heat		Cycle 2, Cool		Cycle 3, Reheat	
	Melting Point (°C)	Enthalpy of Fusion ΔH(J/g)	^a Recrys Temp. (°C)	Enthalpy of Recrys ΔH (J/g)	Melting Point (°C)	Enthalpy of Fusion ΔH(J/g)
CNBQ (Crystallized from acetonitrile)	235.88	90.09	-	-	-	-
CNBQ (Crystallized from acetonitrile)	234.90	81.85	127.12	39.58	230.72	70.05
CNBQ (Crystallized from ethanol)	164.66, 175.79, 193.50	31.33, 28.36, 74.63	116.49	-	229.92	55.86

Table 4: Summary of Thermal Events of CNBQ. ^aRecrys: Recrystallization.

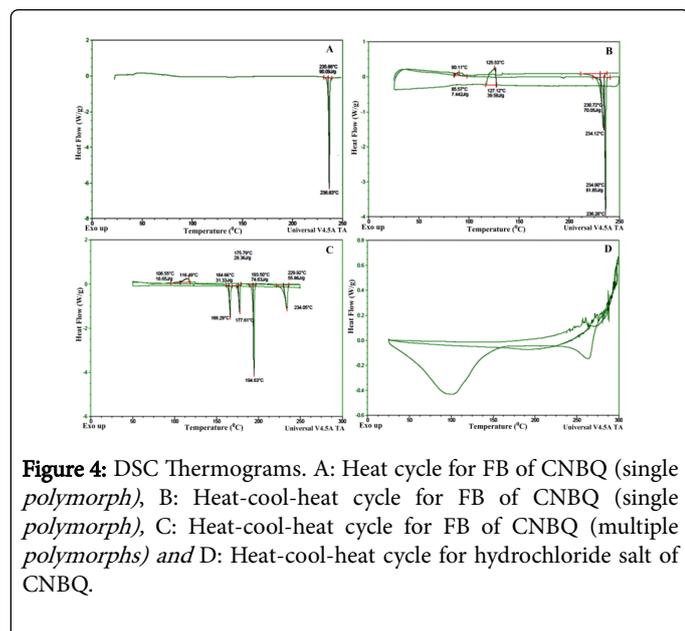


Figure 4: DSC Thermograms. A: Heat cycle for FB of CNBQ (single polymorph), B: Heat-cool-heat cycle for FB of CNBQ (single polymorph), C: Heat-cool-heat cycle for FB of CNBQ (multiple polymorphs) and D: Heat-cool-heat cycle for hydrochloride salt of CNBQ.

Overall, it is observed that the FB is a white polymorphic crystalline powder; the polymorphs melt at 166°, 178°, 195°, and 234°C, respectively. The salt is off-white powder that showed a broad endotherm in DSC analysis suggesting it to be amorphous in nature.

Solubility study

Figure 5 shows the HPLC chromatograms obtained during solubility studies of FB and its salt in water. To quantify the saturation level of the drug in water, a known concentration of standard solution of FB (Figure 5A, 0.0067 mg/ml) and salt (Figure 5C, 0.0067 mg/ml)

were prepared using acetonitrile and water (50:50) as diluent and analyzed by RP-HPLC. AUC of the both standard solutions were used to calculate the unknown amount of drug substance dissolved at their saturation level using their AUC (Figure 5B for FB; saturation level 0.03 mg/ml) (Figure 5B for FB and Figure 5D for salt; saturation level 8.581 mg/ml).

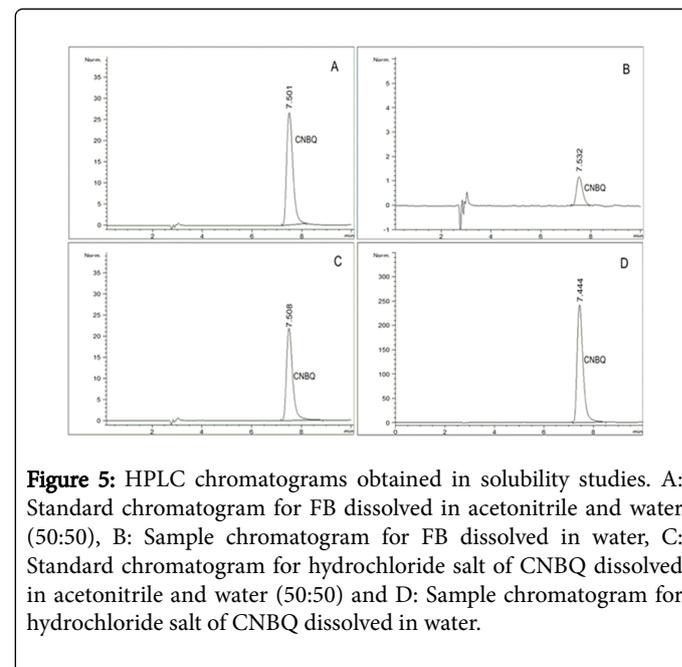


Figure 5: HPLC chromatograms obtained in solubility studies. A: Standard chromatogram for FB dissolved in acetonitrile and water (50:50), B: Sample chromatogram for FB dissolved in water, C: Standard chromatogram for hydrochloride salt of CNBQ dissolved in acetonitrile and water (50:50) and D: Sample chromatogram for hydrochloride salt of CNBQ dissolved in water.

Under the provided experimental conditions carried out on various mediums, it was observed that the equilibrium solubility order of the FB is: DMSO>ethanol>pH 1>isopropyl alcohol>tween 80>propylene

glycol>PEG 400>water>pH 4.5>pH 7.4>pH 9.0>pH 12.0; and that of the salt is: water>propylene glycol>DMSO>ethanol>PEG 400. It was previously shown that CNBQ is highly lipophilic with a log P value of 5.14, signifying very low water solubility and high lipid solubility [20]. The observed solubility data of the FB is in consistent with its log P value. It was also shown that the compound is a weak base with pKa

values of 5.9, 6.6 and 8.7 [21]. As expected of any base, the salt formation improved its water solubility considerably. According to the solubility ratios of salt and FB, the salt formation improved its water solubility by approximately 370-fold (Table 5). The solubility of FB and its HCl salt in different mediums and pharmaceutical excipients are shown in Table 5.

Solubility (mg/ml) of the FB and Salt in Different Media				Solubility (mg/ml) of FB in different Buffer Solutions	
Media	FB	HCl salt	Solubility Ratio (Salt/FB)	pH	Solubility
PEG 400	0.183	0.234	1.7	pH 1	0.555
PG	0.204	1.080	6.9	pH 4.5	0.012
Water	0.030	8.581	370.2	pH 6.8	0.003
Ethanol	0.868	0.455	0.7	pH 7.4	0.008
DMSO	3.323	0.887	0.4	pH 9.0	0.008
Tween 80 (1%)	0.274	---	---	pH 12	0.000
Isopropyl alcohol	0.359	---	---	---	---

Table 5: Solubility of FB and hydrochloride salt of CNBQ in different solvents and excipients.

To increase the water solubility, and thus dissolution in the stomach, basic drugs are generally administered in their salt forms. As the experimental outcome shows that the aqueous solubility of CNBQ has been increased by approx. 370-folds in its HCl salts form, it will be very effective for future development as per FDA requirements [23] and formulation efforts of the compound. Further solubility studies on excipients and solvents were carried out to expedite the dosage form design for clinical studies of the CNBQ.

Stability study

The stressed samples were compared to the unstressed sample (control). Samples were analyzed by stability indicating RP-HPLC method equipped with a peak purity analyzer (photodiode array). The method is found to be highly sensitive (0.2 µg/ml, limit of detection) and linear ($R^2=0.9998$) over a range of limit of detection to 120% of the target concentration level (Figures 6 and 7). Degradation peaks were well resolved from the main peak of CNBQ. The peak purity of the principle peak was greater than 0.999 in all stress conditions suggesting that there was no interference of degradants with the principle peak.

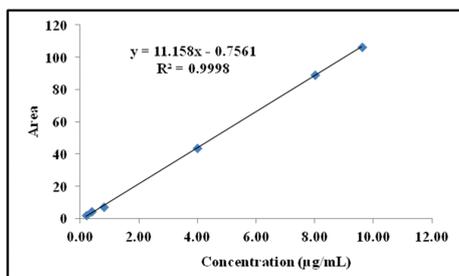


Figure 6: Linear curve (0.2 µg/ml to 9.6 µg/ml).

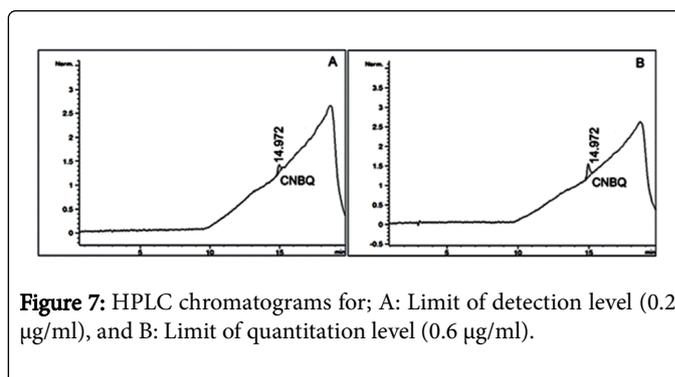


Figure 7: HPLC chromatograms for; A: Limit of detection level (0.2 µg/ml), and B: Limit of quantitation level (0.6 µg/ml).

Figures 8 and 9 represents the HPLC Chromatograms obtained in forced degradation studies of FB of CNBQ and its HCl salt. From the blank chromatograms (Figure 8A) it is clearly seen that diluent and mobile phase has no interference in the studies because there is no co-eluting peak at the retention time of CNBQ (~ 14.75 min) in the blank chromatograms. FB has one process related impurity at the retention time of about 16.75 minute, which completely disappears in the salt form. Both FB and salt are found to be stable in all stress conditions except oxidation, as there were no degradants observed in the HPLC chromatograms (Figures 8C and 9B). Ten and seven degradants are observed during oxidative degradation of FB and its salt, respectively. Thus, both FB and salt are susceptible to oxidative degradation; the FB is more susceptible to oxidative degradation compared to the salt form.

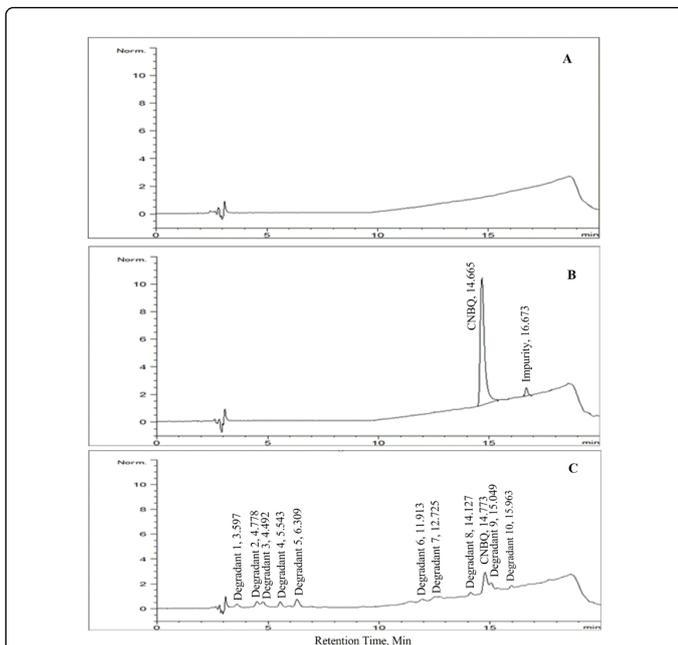


Figure 8: HPLC chromatograms obtained in forced degradation studies of CNBQ FB; A: Blank, B: Acid hydrolysis, C: Oxidation.

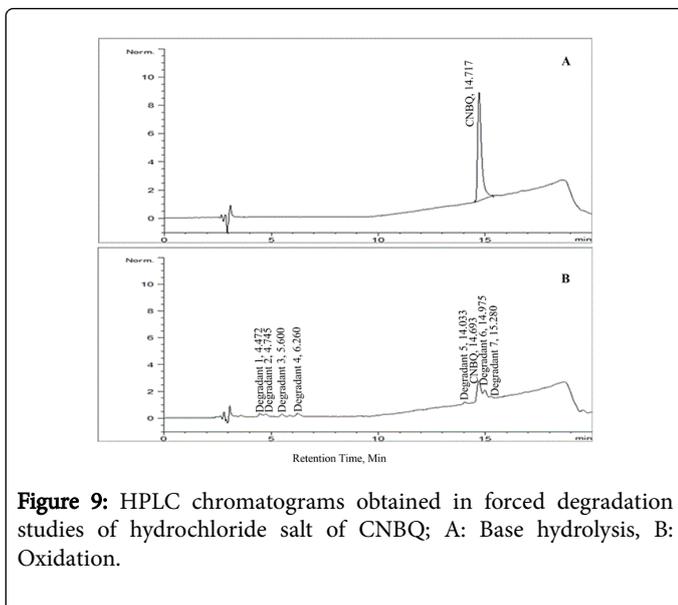


Figure 9: HPLC chromatograms obtained in forced degradation studies of hydrochloride salt of CNBQ; A: Base hydrolysis, B: Oxidation.

Stability testing not only provides prophecy about the quality of a drug substance with time due to variation in environmental factors such as temperature, humidity, and light, but it also helps to establish the recommended storage conditions and shelf life of the drug substance [18]. The preliminary studies showing that CNBQ can be stored at room temperature. Forced degradation studies have been proven to be less time consuming as compared to stability studies in predicting the possible degradants [24,25]. To confirm the storage conditions and shelf-life need long term, intermediate, and accelerated stability studies [26]. Conducting degradation studies earlier in the drug development process will allow adequate time to collect information about the stability of the molecule. The stability indicating

method developed during forced degradation studies can be used for the analysis of samples generated from accelerated and long term stability studies. These studies are also helpful in implementing the quality risk management by QbD approach [27] for formulation development and manufacturing process design, and in determining the storage conditions of the finish product.

Conclusion

Physicochemical properties of the compounds are the fundamental parameters that should be evaluated before any drug development from its discovery stage. They assist in evaluating appropriate dosage form that would be required to resolve any complications during the process. According to the results obtained, an assessment is made for further studies of the compound. All the methods performed for determining each physicochemical property are to find a good balance among time, cost and accuracy. Methods developed to determine the physicochemical properties in this study can be used for the similar class of drug leads. The physicochemical properties of the antimalarial drug lead, CNBQ can be implemented in the modern quality by design (QbD) approaches for better progress of the drug development process.

Acknowledgement

This project was supported by the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 8P20GM103447 (MOFK).

References

- Chellan P, Land MK, Shokar A, Aaron A, An SH, et al. (2013) Di- and Trinuclear Ruthenium-, Rhodium-, and Iridium-Functionalized Pyridyl Aromatic Ethers: A New Class of Antiparasitic Agents. *Organometallics* 32: 4793-4804.
- Le Bras J, Musset L, Clain J (2006) Antimalarial drug resistance. *Med Mal Infect* 36: 401-405.
- Tang Y, Dong Y, Vennerstrom JL (2004) Synthetic peroxides as antimalarials. *Med Res Rev* 24: 425-448.
- Mutabingwa TK (2005) Artemisinin-based combination therapies (ACTs): best hope for malaria treatment but inaccessible to the needy. *Acta Trop* 95: 305-315.
- Yeung S, Van Damme W, Socheat D, White NJ, Mills A (2008) Cost of increasing access to artemisinin combination therapy: the Cambodian experience. *Malar J* 7: 84.
- Bradshaw JS, Krakowiak KE, Izatt RM (1993) *The chemistry of heterocyclic compounds*, Wiley & Sons, Inc., New York, pp: 157-165.
- Caravan P, Ellison JJ, McMurry TJ, Lauffer RB (1999) Gadolinium(III) Chelates as MRI Contrast Agents: Structure, Dynamics, and Applications. *Chem Rev* 99: 2293-2352.
- Liu S, Edwards DS (2001) Bifunctional chelators for therapeutic lanthanide radiopharmaceuticals. *Bioconjug Chem* 12: 7-34.
- Khan MO, Levi MS, Tekwani BL, Khan SI, Kimura E, et al. (2009) Synthesis and antimalarial activities of cyclen 4-aminoquinoline analogs. *Antimicrob Agents Chemother* 53: 1320-1324.
- Rudraraju AV, Hossain MF, Shrestha A, Amoyaw PNA, Tekwani BL, et al. (2014) In vitro Metabolic Stability Study of New Cyclen Based Antimalarial Drug Leads Using RP-HPLC and LC-MS/MS. *Mod Chem Appl* 2: 129.
- Chen XQ, Antman MD, Gesenberg C, Gudmundsson OS (2006) Discovery pharmaceuticals-challenges and opportunities. *AAPS J* 8: E402-408.

12. Liping Z, Jianling W (2008) Physico-chemical Characterization in Drug Discovery. *TBI* 4: 12-18.
13. Vippagunta SR, Brittain HG, Grant DJ (2001) Crystalline solids. *Adv Drug Deliv Rev* 48: 3-26.
14. Morris KR, Griesser UJ, Eckhardt CJ, Stowell JG (2001) Theoretical approaches to physical transformations of active pharmaceutical ingredients during manufacturing processes. *Adv Drug Deliv Rev* 48: 91-114.
15. Zhang GG, Law D, Schmitt EA, Qiu YH (2004) Phase transformation considerations during process development and manufacture of solid oral dosage forms. *Adv Drug Deliv Rev* 56: 371-390.
16. Hanawa T, Ikoma R, Watanabe A, Hidaka M, Sugihara M (1996) Preparation and characterization of sealed heated mixture of ethenzamide and porous calcium silicate. *Chem Pharm Bull (Tokyo)* 44: 1367-1371.
17. Brent R (2006) Investigating Differences in Solubility between Crystalline and Amorphous Forms of Pharmaceuticals. AstraZeneca.
18. ICH (2003) Stability Testing of New Drug Substances and Products.
19. ICH (1996) Stability testing: Photostability testing of New drug substances and products Q1B.
20. Rudraraju AV, Amoyaw PN, Hubin TJ, Khan MO (2014) Determination of log P values of new cyclen based antimalarial drug leads using RP-HPLC. *Pharmazie* 69: 655-662.
21. Hossain MF, Obi C, Shrestha A, Faruk Khan MO (2014) UV-Metric, pH-Metric and RP-HPLC Methods to Evaluate the Multiple pKa Values of a Polyprotic Basic FB Antimalarial Drug Lead, Cyclen Bisquinoline. *Mod Chem Appl* 2: 4.
22. Amoyaw PNA (2014) Synthesis of novel tetraazamacrocyclic bisquinoline derivatives as potential antimalarial agents. *Curr Org Synth* 11: 1-6.
23. Guidance for Industry (2014) Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System. FDA.
24. Blessy M, Patel RD, Prajapati PN, Agrawal YK (2014) Development of forced degradation and stability indicating studies of drugs - A review. *Journal of Pharmaceutical Analysis* 4: 159-165.
25. Hossain MF, Bhadra F, Kumar U, Rouf ASS (2013) The ICH guidance in practice: Stress degradation studies on aceclofenac and development of a validated stability-indicating reversed-phase HPLC assay in tablet dosage form. *Der Pharma Chemica* 5: 131-146.
26. ICH (1996) Stability testing for new dosage forms Q1C.
27. Quality by Design for ANDA (2014) An Example for Immediate-Release Dosage Forms, FDA.