ORIGINAL ARTICLE

DETERMINATION OF THE PHYSICOCHEMICAL PROPERTIES OF PYRONARIDINE – A NEW ANTIMALARIAL DRUG

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ABSTRACT

The physicochemical properties of pyronaridine, a new antimalarial drug, have been determined for the first time in this study, since these parameters are comprehensively not available in literature.

UV-Vis spectral analysis of both pyronaridine and its tetraphosphate salt were carried out in various solvents, in addition to solubility of the two drugs in these solvents. Partition coefficient was done in *n*-octanol-water mixture using the Leo-Hansch method as well hydrophobicity index determination. pKa determination was carried out on the tetraphosphate.

UV-Vis spectral characteristics showed that both the base and the tetraphosphate salt have significant light absorption in the range 190-380nm. Solubility in different solvents revealed that pyronaridine base is sparingly soluble in chloroform (1.34%) while it is slightly soluble in methanol (0.29%) and ethanol (0.42%) and very slightly soluble in octanol and distilled water. The tetraphosphate salt was sparingly soluble in water (1.46%) while it is only very slightly soluble in other solvents. The higher aqueous solubility of the salt was further revealed by a greater R_m value on extrapolation to 100% water concentration in hydrophobicity index determination. Log P value determination showed that the base (log P of 0.26±0.02) is more liposoluble than the salt {logP of – (1.24±0.21)}.

Four prominent pK_a values were obtained for the tetraphosphate titrated which when extrapolated to the base gave values of 7.08±0.05, 7.39± 0.05, 9.88± 0.05 and 10.30± 0.10.

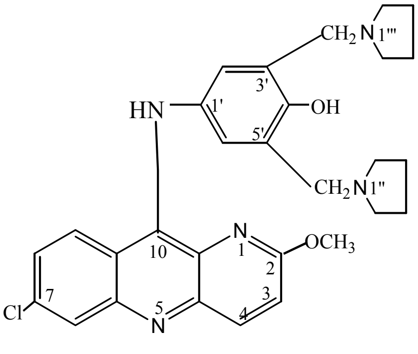
The results should guide in formulation of appropriate dosage forms to improve bioavailability of the drug especially from oral routes.

Keywords: Pyronaridine, physicochemical properties, solubility, pK_a, partition coefficient.

INTRODUCTION

Pyronaridine (as shown in fig. 1) is a relatively new antimalarial agent synthesized in 1970 at the Institute of Parasitic Disease, Chinese Academy of Preventive medicine (Zheng *et al.,* 1982). It is a benzonaphthyridine derivative that has been in use in China for more than 20 years. The pyronaridine nucleus was synthesized from mepacrine (a 9-aminoacridine) and after a lot of selection and screening, the addition of an amodiaquine-like side chain was found with the greatest activity and fewer adverse effects (Chang *et al.,* 1992).

The antimalarial activity of pyronaridine has been attributed to both blood schizontocidal (Renu *et al.,* 2000) and gametocytocidal activities (Charaliteshewinkoon-Petmir *et al.,* 2000).



7-Choro-2-methoxy-10-[3′,5′-bis(pyrolidinyl-1-methyl)-4’-hydroxyanilino]benzo[*b*]-1,5-naphthyridine

Fig. 1: Structure of pyronaridine

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Table 1: UV-VIS spectral characteristics for pyronaridine and tetraphosphate

	Pyronaridine base		Pyronaridine tetraphosphate	
Solvent	λ_{max} (nm)	$A^{(1\%,1cm)}$	λ_{max} (nm)	$A^{(1\%,1cm)}$
Water	260	ND[a]	204	642
	274		260	521
	414		420	152
n-octanol	254	444	252	ND[a]
	280	551	280	
	428	165	420	
0.1M HCl	ND[b]	ND[b]	202	631
			340	145
			424	196
Methanol	206	647	ND[b]	ND[b]
	276	599		
	428	131		
Chloroform	242	2075	ND[b]	ND[b]
	264	1532		
	274	1688		
Ethanol	ND[b]		205	ND[a]
			275	
			425	

ND[a] Not determined because saturated solutions were used
ND[b] Not determined due to solubility limitations

Most strains of *Plasmodium falciparum* are now resistant to chloroquine and resistance to alternative drugs has also been discovered. This has led to a consequent need for the development of new antimalarials, an offspring of which is pyronaridine.

Pyronaridine was shown in several field studies, involving several thousand cases in China, to have promising therapeutic value in the treatment of malaria infection (Zheng *et al.,* 1982). In clinical trial studies pyronaridine was discovered to be safe and well tolerated by symptomatic children, and is found to be highly efficacious in regions where chloroquine-resistance is well established (Zheng *et al.,* 1982).

Pyronaridine is available as the free base and pyronaridine tetraphosphate. The drug has also been produced as tablets and injectables. It is reported to be soluble in water (tetraphosphate), very sparingly soluble in ethanol and insoluble in chloroform, ether and other organic solvents (Chang *et al.,* 1992).

The pharmacokinetic data of pyronaridine are limited. Oral bioavailability of pyronaridine is low compared to parenteral. Feng *et al* (1986) reported bioavailability of 100% and 19.7% respectively after intramuscular (204 mg) and oral (600 mg as capsule) administration of pyronaridine respectively. The T_{max} and C_{max} were 0.7hr and 525 ng/ml after IM and 14 hr and 225 ng/ml after oral administration. In a recent study with pyronaridine tablets, plasma levels of the drug were so low with C_{max} and T_{max} of 76 ng/ml and 1 hr respectively after administration of 400 mg of the drug (Babalola *et al.,* 2003).

A study of the physicochemical properties of a drug substance is a pre-requisite for product formulation and an aid in understanding the inter-relationship between a drug molecule and drug action (Olaniyi, 2000). Physicochemical properties help to control the processes of drug absorption, distribution, metabolism, excretion and interaction of the drug at the active sites. Despite the acclaimed usefulness and the potential of pyronaridine developing to become drug of choice for malarial chemotherapy, properties of the drug such as partition coefficient, ionization constant, solubility profile, melting point, and others are yet to be established and documented.

This study is therefore aimed at elucidating some of the physicochemical properties of pyronaridine. Proper documentation of these properties will no doubt help to further understand the molecule and aid in maximizing its usefulness by formulation of appropriate dosage forms. It may also help to understand and improve the poor oral bioavailability of pyronaridine while aiding in selection of appropriate solvents for analysis.

Table 2: Some physicochemical properties of pyronaridine base and tetraphosphate

Sample No.	Pyronaridine base					Pyronaridine tetraphosphate
	pKa				Log P	Log P
1	7.0	7.45	9.9	10.2	0.233	-0.933
2	7.1	7.29	9.8	10.2	0.265	-1.286
3	7.1	7.4	9.9	10.2	0.265	-1.394
4	7.1	7.4	9.9	10.4	0.259	-1.337
Mean	7.08	7.39	9.88	10.25	0.26	-1.24
S.D.	0.05	0.07	0.05	0.10	0.02	0.21

R_m of base = 0.773 ; R_m of salt = 0.872.

Table 3: Solubility of pyronaridine base and tetraphosphate in various solvents

Solvent	Mean Solubility (%w/v)	Part of solute per parts of solvent	Inference
Pyronaridine base			
Methanol	0.29±0.06	1 part in 345 parts	Slightly soluble
n-octanol	0.09±0.03	1part in 1111 parts	Very slightly soluble
Ethanol	0.42±0.12	1part in 238 parts	Slightly soluble
Chloroform	1.34± 0.47	1part in 75 parts	Sparingly soluble
Distilled H_2O	0.021± 0.006	1part in 4762 parts	Very slightly soluble
Pyronaridine tetraphosphate			
Methanol	0.027± 0.002	1part in 3703 parts	Very Slightly soluble
n-octanol	0.021± 0.006	1part in 4762 parts	Very Slightly soluble
Ethanol	0.025± 0.011	1part in 4000 parts	Very Slightly soluble
Chloroform	0.021± 0.006	1part in 4762 parts	Very Slightly soluble
Distilled H_2O	1.46 ± 0.30	1Part in 68 parts	Sparingly soluble

MATERIALS AND METHODS

Apparatus and reagents

The apparatus used in the study are; UV-Visible spectrophotometer (Helios-Pye Unicam, Cambridge U.K.), analytical balance (Mettler AE160) and pH meter (Cole Parmer, Illinois). Pyronaridine base and pyronaridine tetraphosphate (melting points of 128-132°C and 190-194°C respectively), synthesised in the Institute of Parasitic Disease, Chinese Academy of Preventive Medicine were gifts to CPB; methanol (GPR, BDH Poole, England), Ethanol, Chloroform, liquid paraffin, *n*-hexane, hydrochloric acid, *n*-octanol, sodium hydroxide pellets, all of analytical reagent from BDH, were used as materials and reagents.

Analytical thin layer chromatography (TLC)

Silica gel GF_{254} (precoated) was used for all TLC analyses. Solutions of pyronaridine tetraphosphate in ethanol (1mg/ml) were used in all cases. Normal phase TLC was eventually investigated using mobile phase combinations of methanol: strong ammonia (20:1), methanol: acetic acid (7:3), chloroform: methanol (90:10) and methanol: strong ammonia (100:1.5). The developed plates were examined under daylight and under the UV lamp at 254nm.

UV-VIS spectral characteristics

Absorption spectra of pyronaridine tetraphosphate were recorded in water and in 0.1 mol. dm^{-3} HCl (10 μg/ml concentration), ethanol and *n*-octanol (saturated solution). The UV spectral analysis of the pyronaridine base was carried out in *n*-octanol, methanol, chloroform (10 μg/ml) and in water (saturated solution). All measurements were taken over the range of 190 to 780 nm wavelength. The wave lengths of maximum absorbance (λ_{max}) as well as the absorptivity at each principal λ_{max} were determined.

Solubility profile in various solvents

Solubility of pyronaridine tetraphosphate in several solvents (methanol, distilled water, *n*-octanol, ethanol and chloroform) was determined by placing 5 mg of the drug in a test tube and adding 0.5 ml aliquots of solvent with continuous agitation until all particles were completely dissolved and at a point when addition of few crystals will remain undissolved i.e. saturation point. Solubility of pyronaridine was similarly carried out in the same set of solvents.

Determination of partition coefficient (log P)

Log P for both the base and the tetraphosphate was determined using the Leo-Hansch method (Leo *et al.,* 1971). However, determinations were carried out in screwed-capped extraction tubes instead of a separatory funnel. Both *n*-octanol and water used were saturated with each other prior to analysis.

For the tetraphosphate, 10 mg of the drug was dissolved in 10ml of distilled water to give a 1mg/ml solution. 3ml of this solution was saturated with drops of *n*-octanol and then added into 10ml of *n*-octanol previously saturated with distilled water. This larger volume of *n*-octanol was used since pyronaridine tetraphosphate is more soluble in water than in *n*-octanol. The extraction tube was inverted 100 times in approximately 5 minutes and was then allowed to stand for 45 minutes before separation of the two layers. The concentration of pyronaridine tetraphosphate in the aqueous layer was analyzed spectrophotometrically after appropriate dilutions. Prior to the partition coefficient experiment, a calibration curve (0-20µg/ml) for pyronaridine tetraphosphate in water was prepared at the λ_{max} of 260 nm. The concentration of the drug in *n*-octanol was obtained by difference. Log P was calculated as the concentration of pyronaridine tetraphosphate in *n*-octanol divided by the concentration of the drug in water i.e. log {P = $[\text{pyronaridine}]_{oct}$ /$[\text{pyronaridine}]H_2O$.

Log P for pyronaridine base was similarly determined using pyronaridine base dissolved in *n*-octanol and partitioning into 10ml of distilled water already saturated with *n*-octanol. The concentration of the base dissolved in *n*-octanol was obtained by extrapolation from a calibration curve (0-20µg/ml) of the base in *n*-octanol at 280 nm (λ_{max}).

Determination of hydrophobicity index (R_m)

The R_m values were determined for pyronaridine tetraphosphate and pyronaridine base using 30%, 50%, 60%, and 70% dimethylformamide (DMF)/water mixtures as mobile phase on silica gel coated with 5% liquid paraffin in *n*-hexane. The two drugs were spotted beside each other on the chromatoplate for each organic modifier's concentration. The mobile phase composition over which the R_m theory is applicable was determined by the linear range in the plot of R_m versus fraction of organic modifier in the mobile phase. The R_m values determined were subjected to linear regression analysis. The R_m for both the base and tetraphosphate at 100% water concentration were obtained by extrapolation using the equation $R_m = \log\{1/R_f - 1\}$.

Determination of ionization constant (pK_a)

The ionization constant (pK_a) for pyronaridine tetraphosphate was determined potentiometrically. Titration was by 0.05M NaOH after standardization. The pK_a's of the pyronaridine tetraphosphate were determined graphically as pH values at half-neutralization ($pH=pK_a$) and these were confirmed with first order derivative plot (Olaniyi 2000a).

All determinations carried out in this study, except R_m, were done in quadruplicates and results are reported as mean ± S.D.

RESULTS

On TLC analysis, significant solute migration was observed with the following mobile phases: methanol: strong ammonia (20:1) ($R_f = 0.22$); methanol: acetic acid 7:3 ($R_f = 0.2$); methanol: strong ammonia 100:1.5 ($R_f = 0.92$); chloroform: methanol 90:10 ($R_f = 0.17$). The last two mobile phases were employed on silica gel plates previously treated with methanolic KOH and dried. No significant movement was obtained for the pyronaridine tetraphosphate in all reverse-phase TLC adopted.

In all the solvents used for recording the absorption spectra of both the pyronaridine base and the salt, three major peaks were observed in each solvent. Table 1 shows the respective $A_{(1\%.1cm)}$ for both drugs in the various solvents.

The pKa, log P and the hydrophobicity index for pyronaridine base and tetraphosphate are presented in table 2 while table 3 presents the solubility in different solvents, and their respective interpretation according to (Alfred *et al.,* 1983). The log P value for the base ranged between 0.233 and 0.265 (mean 0.26 ± 0.02) while that of the salt ranged between –1.394 to –0.933 {mean – (1.24 ± 0.21)}.

Four prominent points of inflexion were observed on plotting the ratio of changes in pH and volume against volume of titrant. This confirms that pyronaridine has four pKa values. The values obtained were 7.08 ± 0.05, 7.39± 0.07, 9.88 ± 0.05 and 10.25 ± 0.10.

DISCUSSION

The UV-VIS spectra of pyronaridine base (as shown in table 1) in *n*-octanol, methanol and chloroform showed three main absorption peaks. In general, both pyronaridine base and its tetraphosphate salt exhibits λ_{max} values basically in the UV region (190-380nm). The λ_{max} values in the visible region (428nm in *n*-octanol and methanol) had low absorptivity since there is lack of extensive conjugation in the molecule, this is in spite of the yellow and orange colours of pyronaridine salt and base respectively. However, this particular peak completely disappeared in chloroform. This may prove some lack of interaction between chloroform and the drug. For the tetraphosphate, three main absorption peaks were also observed in water, *n*-octanol, 0.1 mol. dm^{-3} HCl and ethanol. On changing the solvent from water to HCl, slight bathochromic shift was observed (420 to 424 nm) with an improved absorptivity, though significantly less than the properties of the peaks in the UV region. The spectrum of pyronaridine base in water was recorded using a saturated solution while similar saturated solution was utilized for the tetraphosphate in *n*-octanol, and ethanol due to solubility limitations. This precluded the calculation of $A^{(1\%,1cm)}$ for the drugs in these solvents as exact concentration is not known. A proper specification of the λ_{max} values (especially the number of peaks) and the respective absorptivity index when combined with melting point characteristics and TLC studies should serve as a means of authenticating batch samples of pyronaridine and possibly its formulations.

The solubility profiles of pyronaridine base as summarized in table 3 reveal that the drug is slightly soluble in both methanol and ethanol and sparingly soluble in chloroform. This is expected for chloroform as the presence of naphthyridine and phenyl ring residues will confer some solubility in non-aqueous media. The slight solubility in methanol and ethanol may be attributed to either hydrogen bond interaction or similarity of structure (due to 4`-hydroxyl and 2-methoxyl groups). The interaction producing appreciable solubility in methanol and ethanol for the base may likely not be hydrogen bond formation as the pyronaridine base was only very slightly soluble in water. The solubility in octanol, though classified as very slightly soluble, gave more appreciable value than water. This, in addition to that of chloroform, proves the lipophilic nature of the base; which is anyway anticipated from the structure.

Pyronaridine tetraphosphate was found to be sparingly soluble in water and very slightly soluble in other solvents. Usually majority of important drugs belongs to the class of weak acids and weak bases and, within definite ranges of pH, exists as ions that are ordinarily soluble in water (Martin *et al.,* 1983). Thus, the higher solubility of the tetraphosphate in water should aid absorption from oral route since dissolution will be faster. The use of pyronaridine base in injectables (especially in buffered solutions) will however yield better bioavailability since absorption will be better therefrom. Likewise, these solubilities will help in selection of solvents for analysis.

The log P in *n*-octanol-water obtained for the base (0.26±0.02) is significantly more positive than that of the salt {-(1.24±0.21)}. This is anticipated and confirms the liposolubility of the base. However, the value obtained for the base is not in itself extensively positive compared to some weak bases such as halofantrine, 3.25 (Babalola *et al.,* 2003) and quinine, 3.44 (Zimmerman *et al.,* 1989) and this can be attributable to the polar functional groups in the molecule. The low partition coefficient can affect oral absorption with concomitant low oral bioavailability.

The hydrophobicity index, R_m, which also indicates extent of liposolubility (table 2), reveals not too different partitioning behaviour between the base and the salt. However, the salt showing higher value (0.872), when extrapolated to 100% water, than the base (0.773) once again reveals the higher polar characteristics of the salt.

The ionization constants (pK_a) of 7.08, 7.39, 9.88 and 10.25 showed that there are four residues with basic and nearly neutral nature in the pyronaridine molecule. The pK_a value of 9.88 can be assigned to the nitrogen atoms in the pyrolidine molecules as is common with these moieties. The value of 10.25 however could be due to the secondary amino bridge, which has additional hydroxyl group *para* to it and hence appearing most basic of all the centers in the molecule. The two nitrogen centers on the 1,5-naphthyridine ring therefore are assigned the 7.39 and 7.07 values. The N-1 position will have the 7.39 value due to activating influence of the methoxyl group. The presence of these four centres on the pyronaridine molecule should profoundly affect the oral bioavailability of the drug. Using the Hendersson-Hasselbalch equation for weak bases % ionized = $100/\{1+10^{(pH-pka)}\}$, and for all pK_a values over 99.99% of the drug will be ionized in the stomach (pH 1.4). This should favour the transfer of the drug to the small intestine. The fraction of the drug available for absorption in the intestine is the fraction unionized. Depending on the pH of the intestinal content (usual range 6-8), the fraction unionized range from 92.32% at pH 6 to as low as 10.73% at pH 8 unionized in the intestine. The poor oral bioavailability reported for pyronaridine may therefore be due this low amount that is unionized at intestine where majority of absorption takes place, and may also be governed by the type of food taken as food is known to determine the pH of intestinal content. Even if the contribution of some of the pK_a values is significant, the sparingly soluble nature of the drug in aqueous medium will preclude sufficient solubility that can aid absorption. Interplay of these factors will at the end determine the % of the drug that is absorbed.

In conclusion, this study points to the need to improve on the properties of pyronaridine in order to enhance its usefulness. The choice of other salt forms with better solubility, partition and distribution properties may help in improving the already observed poor bioavailability of this drug. Improvement of the physicochemical characteristics of the drug may aid good formulation and enhance its disposition.

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Received: 29-11-05 – Accepted: 30-1-2006