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## Determination of physicochemical properties of halofantrine

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### Summary

The physicochemical properties of halofantrine hydrochloride (HF HCl) - a phenanthrene methanol antimalarial, were determined practically in this study since such experimental values are still unknown. The solubility in different solvents were determined and found to be 0.67%w/v in methanol (slightly soluble); 0.4%w/v in both n-octanol and acidified acetonitrile (slightly soluble); 0.09%w/v (very slightly soluble) and less than 0.002%w/v in warm water (50°C) (practically insoluble). Halofantrine hydrochloride was found to be practically insoluble in water (at room temperature), n-hexane and phosphate buffer solution of pH 7.4. The partition coefficient between n-octanol and water gave a log p in the range of 3.20 - 3.26 (mean  $3.20 \pm 0.04$ ) and this was at variance with the log P of 8.5 estimated theoretically in literature. The value also confirms the lipophilicity of HF HCl. The ionisation constant (pKa) determined in partly aqueous solvent (40% methanol) ranged between 8.10 and 8.20 (mean  $8.18 \pm 0.05$ ) and confirms the monobasicity of halofantrine. This value also differed from the theoretical estimation of 9.6. The values obtained confirm the often unpredictable and erratic absorption of HF HCl, which bears direct relationship to the physicochemical properties and support the need for better formulations with improved drug delivery potentials.

**Keywords:** *Halofantrine, solubility, partition coefficient, and ionisation constant*

### Résumé

Les propriétés physicochimiques de l'halofantrine hydrochloride (HFHCL) un antipaludien /antimalaria de phenanthrene méthanol étaient déterminés pratiquement dans cette étude du fait que telles valeurs expérimentales ne sont pas encore connu. La solubilité dans différent solvants était déterminé et variait de 0.67% w/v dans du méthanol, 0.4%w/v dans du méthanol et l'acide acétonitrile 0.09%w/v (faible solubilité) et moins de 0.002% w/v dans de l'eau chaude (insoluble). L'halofantrine hydrochloride était pratiquement insoluble dans l'eau (à la température normale), n-hexane et solution neutre de phosphate et de sulfate d'un PH de 7.4. Le coefficient de partition entre n-octanol et l'eau donnaient logP variant entre 3.20-3.26 (moyenne  $3.2 \pm 0.04$ ). Il avait une variance avec un logP de 8.5 estimé théoriquement en littérature. Cette valeur confirme la lipophilicité du HFHCL. La constante d'ionisation (Pka) déterminant partiellement dans du solvant aqueux (40% méthanol) variait de 8.0-8.2 (moyenne  $8.18 \pm 0.05$ ) et confirme la monobasicité de l'HF. Cette valeur était différent de la valeur théorique de 9.6. Les valeurs obtenues confirment le plus souvent l'absorption imprédictable et erratique du HFHCL qui montre une relation directe entre les propriétés physiochimiques et le support nécessaire pour une bonne formulations avec des potentielles améliorations en livraisons.

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### Introduction

Halofantrine (HF) (Fig. 1) belongs to a new class of antimalarials - phenanthrene methanols and is available as the hydrochloride salt. HF is effective in the treatment of acute uncomplicated malaria and multi-drug resistant malaria [1,2], where it is administered orally as the tablet or suspension. HF is reported to be highly lipophilic and poorly soluble in aqueous media [1]. Its poor and erratic absorption after oral administration has been implicated in treatment failure [3]. Oral absorption also shows wide intra- and inter- subject variability and fatty meal is reported to increase the bioavailability of HF by 6-9 fold due to its high lipophilicity [1,4,5]. Dose dependent cardiotoxicity has been reported with its use [7] leading to sudden death in some case [6].

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 [8]. Physicochemical properties help to control the absorption, distribution, metabolism, excretion and interaction of a drug at the active sites [8]. Although HF hydrochloride (HF HCl) has been in use since 1988, its physicochemical properties such as solubility in various solvents, partition coefficient (log P) and ionisation constant (pKa) are yet to be fully elucidated and documented in literature. However, theoretical estimates have been reported in the product manual of SmithKline Beecham [9] namely: solubility in water at room temperature as 0.01 % w/v, ionisation constant (basic pKa) as 9.7 and partition coefficient (log P) as 8.5. There is therefore the need for practical determination of these physicochemical properties. This study is therefore aimed at elucidating some of the properties of HF HCl such as solubility in different solvents, partition coefficient, UV-VIS spectral characteristics in some solvents and ionisation constant experimentally.

### Methodology

#### *Apparatus and reagents*

UV-Visible scanning spectrophotometer (CAMP SEC M 350, double beam and pH meter (Metler Delta 340) were used for analysis. Halofantrine hydrochloride reference standard was obtained as a gift from SmithKline Beecham PLC, Nigeria and Halfan ® tablets (SB France, Batch No. 550) were purchased commercially. All reagents used were of analytical grade.

#### *Experimental*

Extractions of HF HCl from the tablets were performed in different solvents such as methanol, acetonitrile and 0.7% acidified acetonitrile. The 0.7% acidified acetonitrile (0.7 ml conc. HCl, 11.4M in 100 ml acetonitrile) produced the largest and most crystalline yield with comparable properties (such as Rf and melting point) with the reference sample. TLC determinations were carried out in different mobile phases but the best resolution was obtained with methanol : water (100:1.5) and this gave the same Rf values for reference and extracted sample. The melting point of the extracted sample (200-202°C) was also in agreement with that of the reference sample.

Solubility in different solvents (methanol, *n*-octanol, acidified acetonitrile, acetonitrile and water) was carried out by placing 10 mg of HF HCl 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 in water, buffer solution and *n*-hexane was done by placing 10 mg of the drug into 500, 250 and 500 ml flasks respectively. Solubility in water at elevated temperature (50°C) was carried out by sonicating for 2 hr.

Log P was determined according to Leo Hansch method for lipophilic substances using a ratio of organic phase to aqueous phase of 1:100 [10]. 2 ml of 1 mg/ml of HF HCl in *n*-octanol was placed in a separatory funnel and partitioned with water (200 ml). 100 inversions were made and the solution was allowed to settle for 45 minutes before separation of the two layers. The *n*-octanol phase was analyzed spectrophotometrically while the drug in water layer was obtained by difference as the levels were below detection limit in UV. The partition coefficient was also carried out using warm water at 40°C. Prior to this, HF HCl was scanned in UV spectrophotometer to obtain maximum absorption ( $\lambda_{max}$ ) of 257.9 nm in *n*-octanol and 205.3 nm in water. Calibration curves of HF HCl (0–20 µg/ml) were obtained at these  $\lambda_{max}$  for *n*-octanol and water respectively. Partition coefficient, P was calculated as conc. of HF HCl in *n*-octanol divided by conc. of HF HCl in water  $\{P = [HF]_{oct} / [HF]_{water}\}$ , from which log P was determined.

Ionization constant (pKa) was determined potentiometrically according to the method of Benet and Goyan 1967 [11] for poorly soluble compounds by using partly aqueous solvent of methanol: water (4:6) or 40% methanol. Titration was by 0.01M NaOH after standardisation. pKa of the halofantrine hydrochloride salt was determined graphically as pH at half-neutralisation (pH = pKa) and confirmed with first order derivative plot [12]. From that value the basic pKa of halofantrine base was calculated as pKw – pKa(salt).

All determinations carried out in this study were done in quadruplicates and at room temperature (27°C) with both reference and extracted HF HCl except where indicated otherwise.

## Results

The solubility in different solvents (methanol, *n*-octanol, acetonitrile, acidified acetonitrile, water, *n*-hexane and phosphate buffer), UV-VIS spectral characteristics (in methanol, *n*-octanol and water) log P and pKa of HF HCl are shown in Table 1.

water, *n*-hexane and phosphate buffer pH 7.4. The solubility in water of < 0.002% w/v was effected at 50°C, and the drug crystallised out on cooling. The log P ranged between 3.20 and 3.29 (3.25 ± 0.04) when water at room temperature was used and ranged between 3.36 and 3.40 (3.38 ± 0.03) when water at elevated temperature (40°C) was used. The ionisation constant (pKa) was between 8.10 and 8.20 (mean, 8.18 ± 0.05). Similar values were obtained for reference and extracted halofantrine samples.

**Table 2:** Interpretation of solubility of halofantrine hydrochloride in various solvents [13]

Solvent	Mean Solubility (%w/v)	Parts of solvent per 1 part of solute	Inference
Methanol	0.685 ± 0.02	1 part in 145 parts	Slightly soluble
<i>n</i> -octanol	0.400 ± 0.000	1 part in 250 parts	Slightly soluble
Acidified acetonitrile	0.400 ± 0.000	1 part in 250 parts	Slightly soluble
Acetonitrile	0.091 ± 0.001	1 part in 1100 parts	Very slightly soluble
Water	0.002 ± 0.000	1 part in 50,000 parts	Practically insoluble
<i>n</i> -hexane	0.000 ± 0.000	1 part in >100,000 parts	Practically insoluble
Phosphate buffer (7.4)	0.000 ± 0.000	1 part in >100,000 parts	Practically insoluble

## Discussion

The UV-VIS spectral characteristics obtained for halofantrine HCl in these solvents used will serve to identify and authenticate batch samples, tablets and active pharmaceutical ingredient. When combined with melting point and TLC studies, fake, adulterated or substandard HF HCl will be detected.

This study also confirms the poor solubility of halofantrine in many solvents especially in aqueous media [15,16]. Solubility in *n*-octanol was 200 times more than that of water thus confirming the lipophilic nature of the drug. The solubility in water (< 0.002% w/v) at 50°C was 5 times less than the estimated theoretical value of 0.01%w/v at room temperature [9]. Interpreting the solubility according to standard terms specified by Alfred et al (1983) [13] shows that HF HCl is practically insoluble in water, physiological buffer and *n*-hexane; very slightly soluble in acetonitrile; slightly soluble in acidified acetonitrile, *n*-octanol and methanol (Table 2). The moieties contributing to this characteristic are the CF<sub>3</sub>, the bulky dibutyl alkyl side chain and the phenanthrene nucleus (Fig. 1). The only polar group -OH seems to be sterically overshadowed by the hydrophobic moieties. The

**Table 1:** Physicochemical properties of halofantrine hydrochloride

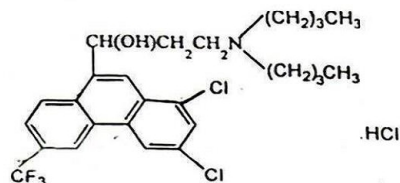
UV-VIS spectral characteristics <sup>a</sup> Solvent $\lambda_{max}$ A (1%,1cm)	log P <sup>b</sup>	pKa <sup>c</sup>	Solubility (%w/v) <sup>d</sup>						
			methanol	<i>n</i> -octanol	acidified acetonitrile	acetonitrile	Water <sup>e</sup>		
Methanol	255	317	3.25 ± 0.04	8.18 ± 0.05	0.685 ± 0.02	0.40 ± 0.00	0.40 ± 0.00	0.091 ± 0.005	0.002 ± 0.000
<i>n</i> -octanol	257.9	1.09							
Water	205.3	0.26							

<sup>a</sup> a = same result obtained for both reference and extracted samples, b = log P for reference sample 3.25 ± 0.02, c = basic pKa for reference 8.18 ± 0.03, d = HF HCl was insoluble in *n*-hexane and phosphate buffer pH 7.4, solubility assessment done with reference samples, e = solubility effected in 500 mls of water at 50°C

Table 2. Shows the interpretation of the solubility result. Solubility was best in methanol followed by acidified acetonitrile, *n*-octanol and water. HF HCl was practically insoluble in ordinary

salt form of the drug (hydrochloride) did not improve solubility in aqueous solvents. The insolubility in readily available solvents such as *n*-hexane, water and buffer solution with physiological

pH will pose a problem in analysis and affect absorption and distribution across body tissues.



1. 3-Dichloro- $\alpha$ -(2-(dibutylamino)-ethyl)-6-(trifluoromethyl)-9-phenanthrene) methanol hydrochloride.

Fig. Structure of halofantrine hydrochloride

The log P in n-octanol - water obtained in this study (range, 3.20 - 3.29) is about 2.5 times lower than the log P of 8.5 estimated theoretically [9,15]. However, the present value of log P is high enough to guarantee easy passage through cell membrane in the body once the drug is in solution. According to Fick's law, the higher the log P, the higher the rate of diffusion [12]. This value of log P explains why highly lipid meals increase absorption of HF by up to 9-fold [5]. This is because solubility in lipid content makes the drug unionised aiding easy passage across cell membrane. Partitioning with water at higher temperature (40°C) gave a slightly higher log P value, an indication that temperature can affect partition coefficient determination, and possibly help in absorption and distribution of HF HCl in acute malaria attacks since there is always a raised body temperature.

The ionisation constant (pKa) obtained in the present (mean, 8.18) study also differs from the theoretical estimation of 9.6 [9]. However, the present practical value is a proof that HF is a weak base just as most antimalarials. The position of one molecule of nitrogen in its structure (Fig. 1) makes it readily available to form a monobasic salt such as the hydrochloride. Using Henderson-Hasselbalch equation for a weak base [% ionised =  $100 / (1 + 10^{(pH - pK_a)})$ ], about 99.9% of HF will be ionised leaving 0.01 % unionised in the gastric juice of the stomach (pH 1.4), while in the intestine (pH 6-8) about 93% of HF will be unionised [14]. Absorption of most drugs take place in the intestine due to its large surface area and only the unionised moieties are capable of transversing the cell membrane [10]. Therefore the high amount of unionised HF and its log P will favour absorption in the intestine but the poor solubility in water and physiological pH [7.4] may limit its absorption orally because absorption is subject to solubility of the drug in body fluids. An interplay of all these factors may either contribute to effective or erratic absorption with consequences on the oral bioavailability. The values obtained however confirm the often poor, unpredictable and sometimes erratic absorption of HF HCl. Recent studies have attempted to formulate HF as nanocapsules [17], colloidal mixtures [18] and formation of 1:1 molecular complexes with caffeine and nicotinamide [16], all aimed at improving its oral bioavailability.

In conclusion, the differences between the experimental values obtained in our study and the theoretical estimates suggest that extrapolation of theoretical data to experimental should be done with caution.

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