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Photoreactivity of biologically active compounds. XVII. Influence of solvent interactions on spectroscopic properties and photostability of primaquine

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The influence of solvent interactions on absorption properties, fluorescence properties (emission spectra and quantum yields) and relative photochemical degradation rates of primaquine has been investigated, in order to evaluate photochemical reaction mechanisms and chemical properties of the compound. The first absorption band ($n - \pi^*$) of primaquine is only slightly dependent on properties of the solvent, which can be ascribed to a strong, intramolecular hydrogen bond between the quinoline N and amine group in the ground state (S_0). Amphiprotic solvents with predominant acidic properties (water and methanol) will to some extent stabilize the molecule and initiate hypsochromic shifts of the absorption band by protic interactions, while the other solvents (amphiprotic, basic and neutral) influence the absorption spectrum by general solvent effects only. The excited singlet (S_1^*) state of primaquine interacts more efficiently with the surrounding solvents than the S_0 state, as evaluated by the Stokes shifts. The pKa value of the quinoline N is likely to increase in the S_1^* state, which is important for the observed protic interactions with amphiprotic solvents of predominant acidity. Specific solvent effects are highly important for the efficiency of the fluorescence (fluorescence quantum yields; Φ_f). The fluorescence is quenched by amphiprotic solvents, likely due to a rupture of the intramolecular bond and protonation of the quinolone N, and enhanced by polar, non-protic (basic) solvents, probably by stabilization of the δ intramolecular hydrogen bond. The observed photochemical degradation rates of primaquine in amphiprotic media are positively correlated with Φ_f, indicating that the photochemical degradation of primaquine is dependent on intramolecular hydrogen bonding and non protonated lone-pair electrons at the quinoline N. The intramolecular ring-formation with a subsequent increased lipophilic character and (lack of) interactions with the surroundings, are important factors for biological behavior as well as pharmaceutical properties of primaquine. Knowledge about solvent interactions with primaquine in the S_0 and S_1^* states is essential for the proceeding evaluation of photostability and phototoxicity of the drug.

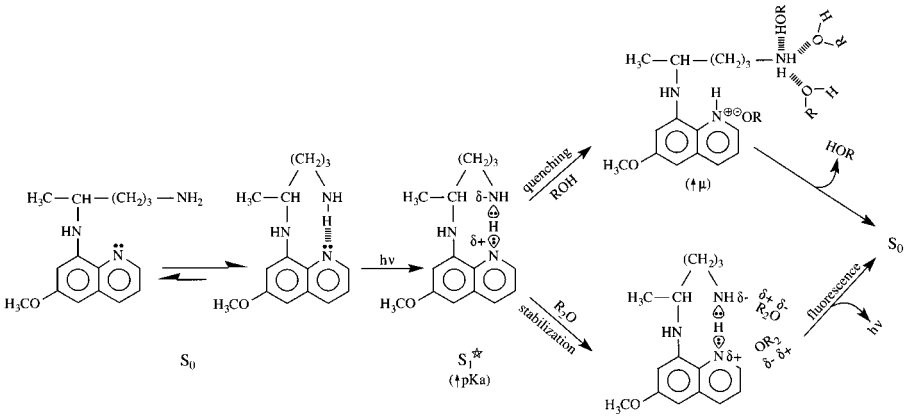
1. Introduction

Primaquine is an 8-aminoquinoline with antimalarial activity. The drug acts as a tissue schizontocide, and is mainly used curatively to eliminate latent liver forms of *Plasmodium ovale* and *P. vivax*. Due to severe haematological adverse effects (methaemoglobinaemia and haemolysis), primaquine is not suitable for general prophylaxis (WHO Drug Information 1988).

Primaquine is a photolabile drug, absorbing optical irradiation in the UV-visible range of the spectrum (up to 430 nm under physiological conditions). The drug is decomposed photochemically into several degradation products at physiological pH, reactions which are highly dependent on the oxygen level of the medium (Kristensen et al. 1993; 1998). Primaquine acts as an *in vitro* photosensitizer, inducing photohaemolysis, photopolymerization of lens proteins and photoreduction of cytochrome C (Kristensen et al. 1994, 1995, 1997). We have confirmed the formation of free radicals ($OH^\bullet$ and $O_2^{\bullet -}$) in the photochemical reactions, by addition of radical scavengers during irradiation. These intermediates are important mediators in the *in vitro* oxidiation of haemoglobin induced by primaquine (Summerfield and Tudhope 1978; Thornalley et al. 1983). Light above 320 nm is penetrating Caucasian skin (Megaw and Drake 1986), thus the haematological adverse effects observed after medication with primaquine can (partly) be ascribed to photochemical reactions of the drug *in vivo* (Kristensen 1997).

The present work was undertaken to obtain further knowledge on the mechanisms of primaquine photoreactivity *in vitro*, with a focus on the influence of solvent-solute interactions. This knowledge is important in the evaluation of *in vitro* photostability and *in vivo* phototoxicity of primaquine. The study also gives valuable information concerning chemical properties of pharmaceutical interest, which is essential in the development of new drug formulations, e.g. primaquine loaded liposomes (Stensrud et al. 2000).

Scheme



2. Investigations, results and discussion

2.1. Absorption spectra

Properties of the solvents used in this investigation are presented in Table 1, and the absorption and emission data of primaquine-base in these solvents are given in Table 2. Primaquine-base is a sticky, viscous liquid, which is hard to weigh out at a high accuracy prior to dissolution. The extinction coefficients of primaquine were therefore not determined in this study. The first absorption band of primaquine represents the transition of the lone-pair electrons ($n - \pi^*$ transition) at the quinoline N (Kristensen et al. 1998). This conclusion is based on the value of the extinction coefficient ($\varepsilon = 3300\,l\ cm^{-1}\ mol^{-1}$ of primaquine diphosphate in aqueous phosphate buffer at pH 7.4), the large shift of the first absorption maximum by acidification of the medium ($A_{max} = 414$ nm in aqueous H_2SO_4 pH 2.0; pKa of the quinoline N = 3.2; Hufford and McChesney 1983), the unchanged absorption band in basic aqueous media (pKa of the amine group = 10.4; Hufford and McChesney 1983) and the blue shift when changing the solvent to a polar medium (Table 2). Due to the very low pKa value of the aniline N (<−1; Hufford and McChesney 1983), the lone-pair electrons of this group are likely delocalized in the aromatic ring.

The first absorption band of primaquine is only slightly dependent on solvent properties (Table 2), despite of the fact that the quinoline N is polar and has an excellent potential for hydrogen bonding to the lone-pair electrons. Substituted quinolines are known to form intramolecular hydrogen bonds (Wilk and Rochlitz 1967), and a bonding between the quinoline N and the amine group of primaquine will lead to reduced intermolecular interactions with the solvent. Water is the only solvent giving a distinct shift in the absorption band compared to all the other solvents investigated, but the maximum energy difference detected (between water and cyclohexane) is limited ($1100\ cm^{-1}$). The first absorption band has a smooth shape with one distinct maximum in all the solvents evaluated.

General solvent effects are present for all dissolved molecules, and the influence on primaquine can be observed by evaluation of the absorption maxima. Considering all the solvents, there is a slightly increased bathochromic (red) shift as a function of increased solvent electronic polarizability (refractive index; n), as measured by the refractive index term $(n^2 - 1)/(2n^2 + 1)$ (Lakowicz 1983) (Kundt's rule; Lerosen and Reid 1952). Increased solvent polarity, measured as the π^* parameter (Kamlet et al. 1983), leads to slightly increased hypsochromic (blue) shift. The increased shift to shorter wavelengths is linearly dependent on the increased polarity in the amphiprotic solvents (water and the alcohols); $R^2 = 0.978$. Specific interactions also seem to have some influence on the absorption properties. A plot of absorption maximum (nm) ver-

Table 1: Solvent properties

Category	Solvent	ε	n	$(\varepsilon - 1)/(2\varepsilon + 1)$	$(n^2 - 1)/(2n^2 + 1)$	Δf	π^*	α	β
Polar	Water	78.39	1.3325	0.490	0.170	0.320	1.09	1.17	0.18
Amphiprotic	Methanol	32.70	1.3265	0.477	0.168	0.309	0.60	0.93	0.62
	Ethanol	24.55	1.3594	0.470	0.181	0.289	0.54	0.83	0.77
	Isopropanol	19.92	1.3752	0.463	0.186	0.277	0.48	0.76	0.95
	n-Butanol	17.51	1.3973	0.458	0.194	0.264	0.47	0.79	0.88
Polar	Acetone	20.70	1.3560	0.465	0.179	0.285	0.71	0.08	0.48
Non-protic	THF	7.58	1.4050	0.407	0.197	0.210	0.58	0.00	0.55
(Basic)	Ethyl acetate	6.02	1.3698	0.385	0.184	0.201	0.55	0.00	0.45
	Diethyl ether	4.34*	1.3495	0.345	0.177	0.168	0.27	0.00	0.47
Non-polar	Cyclohexane	2.02*	1.4235	0.202	0.203	−0.001	0.00	0.00	0.00
(Neutral)	Isooctane	1.94*	1.3890	0.193	0.191	0.001	–	–	–
	n-Heptane	1.92*	1.3851	0.190	0.190	0.000	−0.08	0.00	0.00
	n-Hexane	1.88	1.3723	0.185	0.185	0.000	−0.08	0.00	0.00

THF = tetrahydrofuran. The refractive index (n) and dielectric constant (ε) taken from Suppan and Nagwa (1997) are measured at 25 °C (or at 20 °C when denoted *). Solvent polarity (π^* parameter), proton donor property (α parameter) and proton acceptor property (β parameter) are taken from Kamlet et al. (1983). Solvent orientation polarizability, Δf, is calculated according to Lakowicz (1983); $\Delta f = (\varepsilon - 1)/(2\varepsilon + 1) - (n^2 - 1)/(2n^2 + 1)$

Table 2: Absorption and fluorescence properties of primaquine in pure solvents

Solvent	λ_{abs} (nm)	$\nu_{abs} \cdot 10^4$ (cm^{-1})	λ_{em} (nm)	$\nu_{em} \cdot 10^4$ (cm^{-1})	Stokes shift (cm^{-1})	$\Phi_f \cdot 10^{-4}$
			500	2.00		
Water	353	2.83	534	1.87	9600	2.9 (± 0.1)
Methanol	361	2.77	474	2.11	6600	6.6 (± 0.1)
Ethanol	364	2.75	474	2.11	6400	9.0 (± 0.3)
Isopropanol	364	2.75	472	2.12	6300	17.6 (± 2.2)
n-Butanol	365	2.74	471	2.12	6200	17.8 (± 3.0)
Acetone	364	2.75	480	2.08	6600	190.2 (± 3.3)
THF	366	2.73	472	2.12	6100	95.4 (± 4.1)
Ethyl acetate	363	2.75	473	2.11	6400	135.1 (± 4.0)
Diethyl ether	364	2.75	463	2.16	5900	63.0 (± 0.5)
Cyclohexane	367	2.72	444	2.25	4700	33.9 (± 1.3)
Isooctane	365	2.74	443	2.26	4800	27.4 (± 1.6)
n-Heptane	366	2.73	443	2.26	4700	28.0 (± 1.2)
n-Hexane	365	2.74	444	2.25	4900	24.6 (± 1.7)

First absorption maximum (λ_{abs}), corrected fluorescence emission maximum (λ_{em}), fluorescence quantum yield (Φ_f) and calculated Stokes shift of primaquine-base in pure solvents

sus proton donor properties (measured as the α parameter; Kamlet et al. 1983) for all the solvents, show small hypsochromic shifts at $\alpha > 0.8$. This increased shift to shorter wavelengths is linear in the amphiprotic solvents ($R^2 = 0.968$). The effect is predictable, as molecules with N atoms lone-pair electrons form strong hydrogen bonds with acidic solvents, leading to blue shifts of the $n - \pi^*$ absorption band (Suppan and Nagwa 1997). An absorption plot of the proton acceptor properties (as measured by the β parameter; Kamlet et al. 1983) for all the solvents, indicate that basic solvent properties are not important for the first absorption band. Hydrogen bonding with proton acceptors is not expected to affect the $n - \pi^*$ electronic transition (Suppan and Nagwa 1997), and the diverging effect of water in this plot is likely due to the comparatively strong acidic properties of this solvent.

Amphiprotic solvents can interact with functional groups of the solute by both proton donor- and acceptor mechanisms during the continually forming and reforming interactions. The importance of the relative acidic/basic properties of the solvent on the $n - \pi^*$ excitation was evaluated by a plot of absorption maximum (nm) versus the relative value of α/β (polar solvents). The plot indicates that acidic properties have to be predominant ($\alpha/\beta > 1$) to influence the absorption spectrum of primaquine, which is the case for water and methanol only. The effect can be exemplified by the absorption properties of primaquine in ethanol and ethyl acetate. These solvents possess similar polarity (π^* parameter), but ethanol is a better proton acceptor (β parameter) than ethyl acetate and has additional proton donor properties (α parameter). The similar absorption maxima in these solvents (energy difference $< 100\ cm^{-1}$) can be explained by the amphiprotic properties of ethanol ($\alpha/\beta = 1.1$), and lack of acidic interactions with the solute. Only general solvent effects will influence the absorption spectra in solvents without predominant acidic properties.

Marked blue absorption shifts in amphiprotic solvents are generally observed for $n - \pi^*$ transitions (Suppan and Nagwa 1997). In the present study, the blue shifts are detectable only in solvents with predominant acidic properties (water and methanol), and the shifts are less than expected. This can be explained by the intramolecular hydrogen bond between the quinoline N and amine group of primaquine (Scheme). The lone-pair electrons can be delocalized between the molecular groups, resulting in a strong intramolecular bond which is hard to break. Only excellent proton donors are capable of opening the intramolecular ring, by formation of hydrogen bonds with the quinoline N lone-pair electrons. Since intermolecular hydrogen bonds usually involve electrostatic interactions, which are weaker forces than electronic delocalization, the interactions between the solute and the solvent will be suppressed in competition with the intramolecular interaction. Even water, as a highly polar molecule with excellent proton donor properties, is not able to extensively stabilize the molecule. Since general solvent effects are also small, the energy level of the ground state of primaquine (S_0) and thus the $n - \pi^*$ absorption band, is quite similar in all the solvents.

2.2. Fluorescence emission spectra

The excited singlet (S_1^* state) of primaquine interacts with the surrounding solvent to a larger extent that the ground state (S_0), as evaluated by the Stokes shifts (Table 2). The Stokes shifts in the non-polar solvents (4700–4900 cm^{-1}) represent energy loss due to dissipation of vibrational energy and redistribution of electrons in the surrounding solvent, while the Stokes shifts in the polar solvents ($\geq 5900\ cm^{-1}$) are also results of reorientation of solvent molecules around the excited state dipole (μ^*), and specific interactions with the solvent (Lakowicz 1983). The increased Stokes shifts when changing from non-polar to polar media (energy difference $\delta \geq 1000\ cm^{-1}$) indicate that an increase in dipole moment (μ) takes place during excitation ($\mu^* > \mu$), which is expected for aromatic compounds (Lakowicz 1983).

General solvent effects, which are present for all dissolved molecules, have some influence on the S_1^* state of prima-

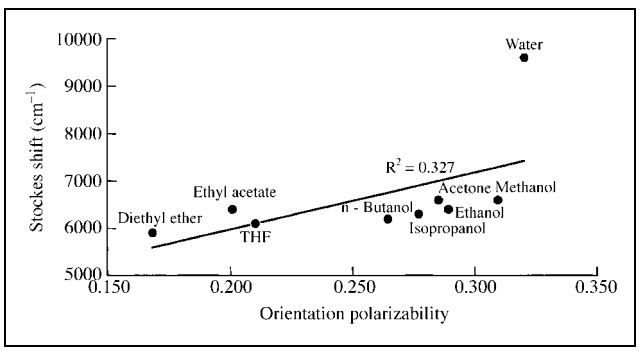


Fig. 1: Lippert plot (Stokes shift as a function of solvent orientation polarizability; Δf) of primaquine-base in polar solvents

quine. There is a decrease in the Stokes shift as a function of increased solvent electronic polarizability, measured as the refractive index term $(n^2 - 1)/(2n^2 + 1)$. The effect is linear in the alcoholic solvents ($R^2 = 0.997$). Increased solvent orientation polarizability, expressed as $\Delta f = (\varepsilon - 1)/(2\varepsilon + 1) - (n^2 - 1)/(2n^2 + 1)$ (Lakowicz 1983), result in a linear increase in the Stokes shift in the alcohols; $R^2 = 0.995$. The effect is emphasized by the increased spectral shift in acetone compared to diethyl ether (energy difference = 700 cm^{-1}), which are polar, basic solvents of similar β parameters but highly different Δf values (Table 1). Primaquine has identical absorption maxima in these solvents, thus the effect is likely caused by general solvent interactions with the S_1^* state. These general solvent effects are predicted, but the Lippert plot of primaquine (Fig. 1) shows that specific solvent effects are also involved, due to deviation from linearity; $R^2 = 0.327$ (Lakowicz 1983).

A plot of all solvents shows an increase in the Stokes shift with an increase in the α parameter > 0.8, which is close to linearity in the amphiprotic solvents ($R^2 = 0.909$). The spectral shifts do not show any clear dependency on basic solvent properties (β parameter), and the divergent effect of water in the plot (Stokes shift versus β parameter of all solvents) is likely due to the comparatively strong acidity of water. The relative influence of acidic and basic solvent properties was evaluated by plotting the Stokes shift of all the polar solvents as a function of α/β. As seen by the plot, the solvent has to possess predominant acidic properties ($\alpha/\beta > 1$) to influence the Stokes shift.

When the acidic properties of the solvent are low ($\alpha/\beta < 1$), only general solvent effects will influence the Stokes shift. The spectral shifts in ethanol and acetone are close (energy difference = 200 cm^{-1}), despite of very different protic properties (α and β parameters). The solvents have comparable solvent orientation polarizabilities (Δf values) and refractive index terms, and acidity is not predominant in any of the solvents (Table 1). The influence of general solvent interactions with primaquine in the S_1^* state is thus emphasized, since the absorption maxima are identical in the two solvents. However, as seen by the Lippert plot (Fig. 1), primaquine is not highly sensitive to Δf of the organic solvents. Thus the change in dipole moment upon excitation ($\mu \rightarrow \mu^*$) is narrow in the organic media (Lakowicz 1983).

Water leads to a large Stokes shift compared to the other solvents. The hypsochromically shifted absorption maximum in water accounts for only some of the effect, hence μ^* is clearly larger in water than in the polar, organic solvents. Due to predominant acidic properties (Table 1), water has the potential to break the intramolecular hydrogen bond by competing intermolecular hydrogen bonding with the quinoline N lone-pair electrons. An opening of the intramolecular ring system in the S_1^* state will lead to exposure of polar molecular groups and an increase in μ^*. Interactions with the solvent stabilizes the S_1^* state of primaquine, and leads to the increased Stokes shift. The basicity of quinoline N is reported to increase in the excited state, leading to acid-base reactions with protic solvents (Wilk and Rochlitz 1966). This seems to be the case also for primaquine, since water interacts stronger with the S_1^* state of primaquine than with the ground state molecule (S_0), as evaluated by the Stokes shifts and first absorption maxima of primaquine in water and organic solvents.

The fluorescence spectrum in water contains a new spectral component compared to the organic solvents, as illustrated by the fluorescence spectra in water and methanol (Fig. 2). The fluorescence emission spectrum generally appears to be a mirror image of the absorption spectrum. Deviation usually indicates a different geometric arrangement of nuclei in the excited state as compared to the ground state, and the appearance of a new spectral component with changes in solvent is a characteristic feature of specific solvent effects (Lakowicz 1983). The spectral deviation is not due to ionic equilibriums, as primaquine dication (pH 2.0) is non-fluorescent, and the fluorescence emission spectrum of the neutral form (pH 11.4) has the same structured shape as the monocation (pH 6.0). The interaction between water and the S_1^* state of primaquine and opening of the intramolecular ring system thus seems to be substantiated.

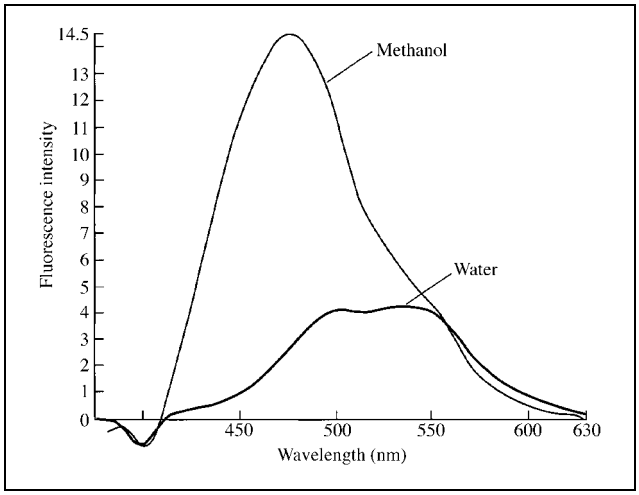


Fig. 2: Corrected emission fluorescence spectra of primaquine-base in methanol and water. (Absorbance at the excitation wavelength; methanol 0.067, water 0.057)

The time-dependent relaxation of fluorophores can be a limitation in the evaluation of steady-state emission spectra. However, in low viscous solvents at room temperature, most molecules reach the relaxed state prior to luminescence (Suppan and Nagwa 1997). In this study, it is assumed that solvent relaxation is complete prior to fluorescence emission, but only time-resolved spectra can verify this assumption.

2.3. Fluorescence quantum yields

The fluorescence quantum yields (Φ_f) of primaquine are highly influenced by properties of the solvents, as presented in Table 2. Primaquine is not a very efficient fluorophore, and the lowest Φ_f is measured when the drug is dissolved in water. General solvent effects have some influence, as demonstrated by the linear increase of Φ_f as a function of increased refractive index term $(n^2 - 1)/(2n^2 + 1)$ in the alkanes ($R^2 = 0.979$). The influence of solvent polarity, evaluated as the π^* parameter and the solvent orientation polarizability, $\Delta f = (\varepsilon - 1)/(2\varepsilon + 1) - (n^2 - 1)/(2n^2 + 1)$, can be studied in non-protic solvents, which do not possess quenching by protonation (Suppan and Nagwa 1997). The basic solvents enhance the fluorescence of primaquine as a function of increased polarity, with some deviation from linearity; $R^2 = 0.874$ (Φ_f as a function of π^*) and 0.855 (Φ_f as a function of Δf), including the alkanes in the plots. This effect is not typical when $\mu^* > \mu$ (as seems to be the case for primaquine), but can result from the crossing of electronically

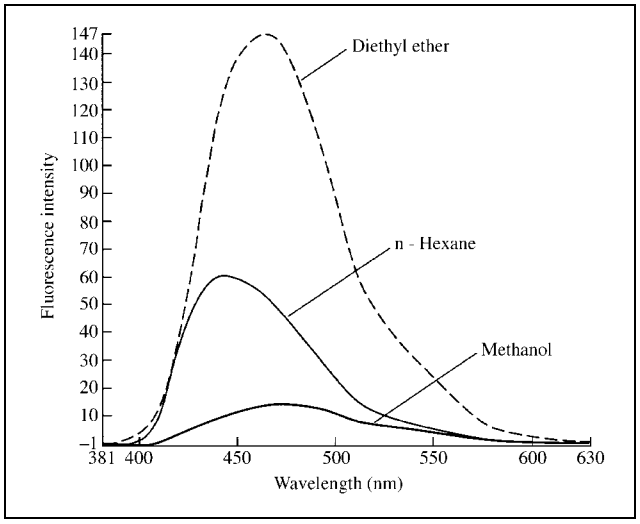


Fig. 3: Corrected emission fluorescence spectra of primaquine-base in n-hexane (neutral solvent), diethyl ether (basic solvent) and methanol (protic solvent). (Absorbance at the excitation wavelength; n-hexane 0.056, diethyl ether 0.057, methanol 0.067)

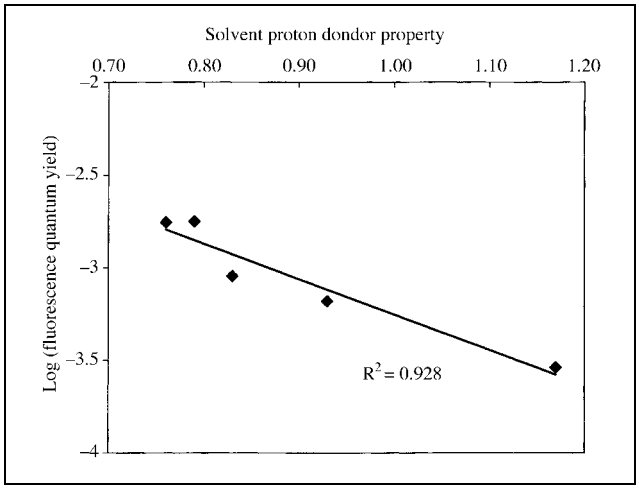


Fig. 4: Fluorescence quantum yields (Φ_f) of primaquine in amphiprotic solvents (water, methanol, ethanol, isopropanol, n-butanol) as a function of solvent proton donor properties (α parameter)

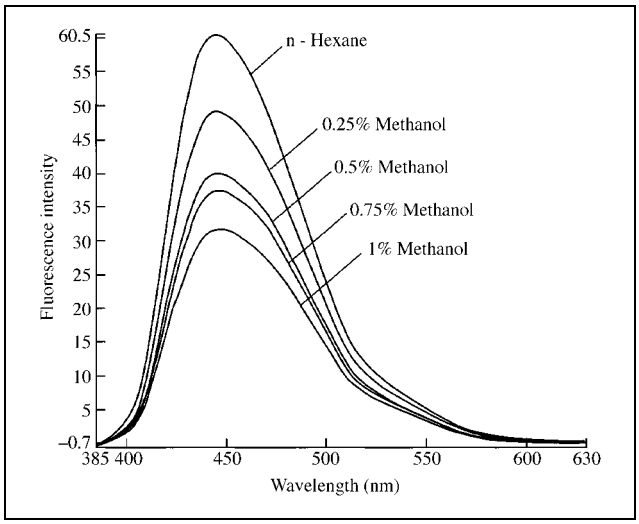


Fig. 5: Corrected emission fluorescence spectra of primaquine-base in solvent gradients of methanol (0–1%) in n-hexane

excited states ($n - \pi^* > \pi - \pi^*$) of different luminescent properties in polar solvents (Suppan and Nagwa 1997). However, specific solvent interactions are also involved between primaquine and the basic solvents.

Specific solvent effects are important for the fluorescence of primaquine. When evaluating the Φ_f of primaquine in polar solvents with the non-polar solvents as references, the amphiprotic solvents are quenchers of fluorescence, while the basic solvents enhance the emission (Table 2). The effect is illustrated by the fluorescence emission spectra of primaquine-base in n-hexane, methanol and diethyl ether (Fig. 3). The increased basicity of the quinoline N in the excited state and subsequent acid-base reactions with protic solvents (Wilk and Rochlitz 1966) will lead to quenching of the S_1^* state (Suppan and Nagwa 1997). Moreover, the fluorescence intensity of substituted quinolines is reported to increase with an increase in intramolecular hydrogen bonding (Wilk and Rochlitz 1967), and a rupture of the intramolecular bond by formation of hydrogen bonds with the solvent will lead to a decrease in Φ_f of primaquine. Quenching by protic reactions is detected as a logarithmic decrease of Φ_f by an increase in acidity (α-parameter) of the amphiprotic solvents (Fig. 4). The specific interactions with the protic solvents are further demonstrated by a solvent gradient of methanol in n-hexane (Table 3). The addition of methanol is too small ($\leq 1\%$) to alter the bulk properties of the solvent (Lakowicz 1983), but results in increased quenching of the fluorescence with an increase in methanol concentration (0–1%) which is close to linearity ($R^2 = 0.905$). Addition of only 1% methanol decreases the Φ_f as much as 43%,

Table 3: Absorption and fluorescence properties of primaquine in solvent gradients

Bulk solvent	Second solvent (%)	λ_{abs} (nm)	λ_{em} (nm)	Stokes shift (cm^{-1})	$\Phi_f \cdot 10^{-4}$
n-Hexane		365	444	4900	24.6 (± 1.7)
n-Hexane	Methanol (0.25%)	365	444	4900	20.6 (± 0.2)
n-Hexane	Methanol (0.50%)	365	445	4900	16.4 (± 0.5)
n-Hexane	Methanol (0.75%)	365	445	4900	13.7 (± 0.8)
n-Hexane	Methanol (1.0%)	365	445	4900	14.0 (± 0.3)
Methanol		361	474	6600	6.6 (± 0.1)
n-Hexane		365	444	4900	24.6 (± 1.7)
n-Hexane	Diethyl ether (0.50%)	365	443	4800	25.2 (± 0.9)
n-Hexane	Diethyl ether (0.75%)	365	443	4800	30.2 (± 0.3)
n-Hexane	Diethyl ether (1.0%)	365	443	4800	28.9 (± 0.2)
n-Hexane	Diethyl ether (2.0%)	365	443	4800	28.8 (± 1.5)
Diethyl ether		364	463	5900	63.0 (± 0.5)

First absorption maximum (λ_{abs}), corrected fluorescence emission maximum (λ_{em}), fluorescence quantum yield (Φ_f) and calculated Stokes shift of primaquine-base in solvent gradients

while the spectral properties are not affected, as illustrated by Fig. 5.

The influence of basic properties (β parameter) is harder to evaluate, as there is no correlation between Φ_f and β-parameter among the basic solvents. There is a logarithmic increase in Φ_f as a function of β-parameter in the amphiprotic solvents (log Φ_f as a function of β-parameter leads to a straight line; $R^2 = 0.948$), but due to quenching by protic reactions, the relative value α/β is of greater interest. Considering all the solvents possessing some acidity (i.e. the amphiprotic solvents including acetone), there is a steep decline in Φ_f as a function of increased α/β at relative acidity $\ll 1$. It is interesting to note that the Φ_f of primaquine has the largest value in acetone. The acidic properties of acetone ($\alpha/\beta = 0.17$) are not sufficient to quench the luminescence. As described, polarity is important for the Φ_f of primaquine in basic solvents, but other properties also influence Φ_f. The polarity of THF and ethyl acetate are in the same range, expressed as π^* and Δf values (Table 1), but the Φ_f are highly different (Table 2). Functional groups and steric configurations can be of importance, since solvents with acyl groups (acetone and ethyl acetate) lead to higher Φ_f than the ethers (THF and diethyl ether). Stabilization of the intramolecular bonding by solute solvent interactions can lead to an increase in the Φ_f (Scheme). The efficiency and stability of these interactions will highly depend on sterical properties of the solvent and the dissolved drug. In general, an increase in the Stokes shift is corresponding with a reduction in the Φ_f (Lakowicz 1983), as observed in the amphiprotic and non-polar solvents. However, the opposite correlation is established in basic solvents; there is a linear increase in Φ_f as a function of increased Stokes shift ($R^2 = 0.975$). This observation supports the previous conclusions: Interactions with protic solvents (leading to increased Stokes shift) is followed by a rupture of the intramolecular bond and protonation of the quinoline N (leading to reduced Φ_f), while interactions with basic solvents (leading to increased Stokes shifts) will stabilize the intramolecular bridge (leading to increased Φ_f) (Scheme). The specific interactions between primaquine and basic solvents are further demonstrated by the solvent gradient of diethyl ether ($\leq 2\%$) in n-hexane (Table 3). Addition of only 1% diethyl ether increases the Φ_f of 17%, while the spectral properties are not affected.

2.4. Photochemical degradation

Photochemical degradation was evaluated by irradiation (300–600 nm) of primaquine diphosphate in four of the amphiprotic solvents (water, methanol, ethanol and isopropanol). At the concentration studied ($4 \cdot 10^{-5}$ M), primaquine has a substantial absorption above 300 nm, and an extensive overlap with the incident irradiation lines (Kristensen 1997). As the sample absorption is rather high (>0.1), the rate limiting factor is then the intensity of the incident irradiation, and the photochemical degradation rate is predicted to follow observed zero-order degradation kinetics (Moore 1996a). The decomposition of primaquine was followed to >50% conversion in isopropanol, necessary to verify the applicability of zero order reaction kinetics (Moore 1996a; Sande 1996). The calculated rate constants and half lives are applicable only under the experimental conditions obtained by the specific lamp used (see 3.2.), but are valuable when evaluating the influence of media properties on photochemical reactivity. The influence of the solvents on the photochemical stability of primaquine diphosphate is presented in Table 4.

Table 4: Observed photochemical degradation rates of primaquine diphosphate

Solvent	$k_0 \cdot 10^{-8}$ (M min^{-1})	R^2	$t_{1/2}$ (min)
Water	6.2	0.867	321
Methanol	6.8	0.857	294
Ethanol	9.3	0.979	214
Isopropanol	20.8	0.947	96

Observed photochemical zero order degradation rates (k_0) and calculated half lives ($t_{1/2}$) of primaquine diphosphate in amphiprotic solvents. R^2 is the squared correlation coefficient of the calculated line

There is a linear relationship between an increase of the observed photochemical degradation rate (k_0) and an increase in Φ_f ($R^2 = 0.938$), as well as decreased photochemical half life ($t_{1/2}$) and increased Φ_f ($R^2 = 0.969$) in the amphiprotic solvents (Fig. 6). Thus photochemical stability is well correlated with fluorescence properties, at least in protic media. Rupture of the intramolecular hydrogen bond and protonation of the quinoline N in the S_1^* state by protic solvents are thus processes that reduce photochemical degradation as well as the Φ_f.

Primaquine has the highest photochemical stability in water, due to the decreased photochemical decomposition at increased acidity (α parameter) of the medium. Primaquine is mainly decomposed photochemically by electron transfer processes (Kristensen 1997), reactions that are favoured in strongly polar solvents such as water (Moore 1996b). Despite of this fact, the photochemical degradation reactions are favored in alcohols of reduced polarity (π^* parameter), but which have correspondingly lower acidity (α-parameter). Photochemical degradation products formed are likely to be addition products of the solvent used (Oppenländer 1996). Secondary alcohols, as isopropanol, are good scavengers of free radicals, and will often protect the therapeutic substance from degradation (Moore 1996b). Isopropanol forms several photochemical degradation products that can condensate with fragments of the decomposed drug (Pacakova et al. 1985; Nord et al. 1991). The large viscosity of isopropanol (1.90 cP) compared to water (0.89 cP), methanol (0.54 cP) and ethanol (1.08 cP) may lead to increased lifetimes, reduced diffusion distances and changed reactivity of short lived intermediates formed in the photochemical processes. However, the most important factor influencing photochemical stability of primaquine in pure, protic media seems to be the

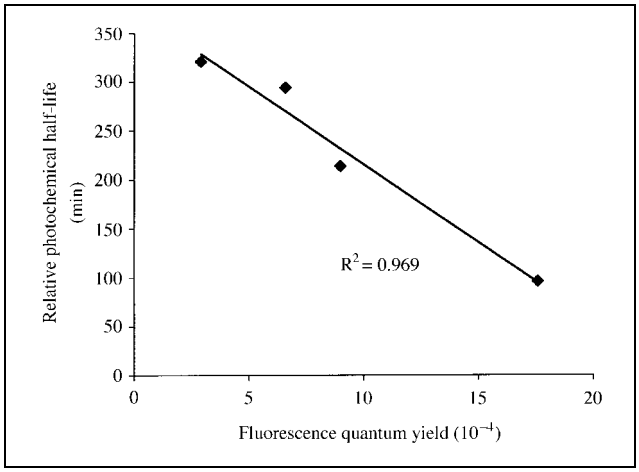


Fig. 6: Relationship between observed photochemical half-lives ($t_{1/2}$; relative values) and fluorescence quantum yields (Φ_f) of primaquine in amphiprotic solvents (water, methanol, ethanol, isopropanol)

quenching by break of the intramolecular bond and protonation of the quinoline N, as demonstrated by the observed correlation between photodecomposition rate (half-life) and the Φ_f.

2.5. Conclusions

At physiological pH, primaquine mainly exists as a monocation, but formation of the intramolecular bond can lead to a shielding of the positive charge. Thus primaquine possesses higher lipophilic character than expected, and diffusion across cell membranes is easily performed. Primaquine concentrates in erythrocytes (Kennedy and Frischer 1990), which is important for the severe haematological adverse effects induced by the drug (Warhurst 1987; WHO Drug information 1988). These side effects may be ascribed to photochemical reactions of primaquine *in vivo*. Due to the influence of the surrounding environment on photochemical reactivity, phototoxic reactions *in vivo* will depend on localizations of the drug in tissues (e.g. blood stream, skin) and cell compartments (cytoplasm, membrane, organelles) during light exposure.
The intramolecular bond of primaquine seems to be essential also for the pharmaceutical properties of the drug. We have obtained an efficient loading of preformed liposomes with primaquine monocation by use of a pH gradient (Stensrud et al. 2000). The lipophilic character of primaquine by shielding of the positive charge was the reason for the diffusion of the drug across the liposome membrane, and necessary for the high degree of encapsulation obtained (98%). The photochemical stability of liquid primaquine formulations (e.g. liposome preparations) will highly depend on the physicochemical properties of the surroundings, as evaluated. An investigation of the influence of excipients on the photochemical stability of primaquine in aqueous media will be published in an upcoming paper.

3. Experimental

3.1. Materials

Primaquine diphosphate (>99% pure) was obtained from Aldrich, Germany. PQ-base was produced by extraction with $CHCl_3$ from a basic aqueous solution of the phosphate salt. Quinine sulphate was purchased from Fluka, Switzerland. The water used was deionised and purified. All other chemicals were of p.a. grade.

3.2. Equipment

UV-visible absorption spectra (190–700 nm) were recorded by a Schimadzu UV-2101PC spectrophotometer. Corrected fluorescence emission spectra were recorded at 25 °C from a Perkin-Elmer LS 50 luminescence spectrometer. Irradiation source: Heraeus immersion lamp system (120 W high pressure Hg-arc), emission wavelengths 240–600 nm, equipped with a glass filter to obtain irradiation above 300 nm. The system was cooled by rinsing, cold water.

3.3. Methods

3.3.1. UV-visible absorption properties

The UV-visible absorption spectra of primaquine-base were recorded, to evaluate the influence of solvent properties on the first absorption band of primaquine.

3.3.2. Fluorescence properties

Corrected fluorescence emission spectra ($n = 3$) were recorded from primaquine-base in various solvents at 25 ± 0.01 °C. The absorbance at the excitation wavelength (maximum of the first absorption band) was adjusted to ≤ 0.07. Fluorescence quantum yields (Φ_f) were obtained by using quinine sulphate dissolved in 0.05 M H_2SO_4 as reference ($\Phi_f = 0.55$; Calvert and Pitts 1966). The Stokes shifts were measured as the energy difference between the band maximum of the emission spectrum and the corresponding absorption band (Suppan and Nagwa 1997).

3.3.3. Photochemical degradation of primaquine

Primaquine diphosphate ($4 \cdot 10^{-5}$ M) was dissolved in water, methanol, ethanol and isopropanol, and irradiated for 120 min in the Heraeus immersion lamp system. The samples were circulated when analyzed. The degradation of primaquine was followed by HPLC (3.3.4.) after 10, 20, 30, 40, 60, 80, 100, and 120 min exposure. Each experiment was performed in duplicate.
The observed zero order rate constants were calculated by correlation of the zero order plots ($n = 2$) to straight lines. The deviation from linearity was calculated as the squared correlation coefficients of the calculated lines (R^2). The half lives were calculated by use of the observed rate constants.

3.3.4. HPLC

The concentration of primaquine was measured by reverse phase HPLC, as previously described (System 2; Kristensen et al. 1998).

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