Novel Artemisinin and Curcumin Micellar Formulations: Drug Solubility Studies by NMR Spectroscopy

SILVIA LAPENNA,[1] ANNA RITA BILIA,[1] GARETH A. MORRIS,[2] MATHIAS NILSSON[2]

[1]Department of Pharmaceutical Sciences, University of Florence, Via Ugo Schiff 6, 50019 Sesto Fiorentino (FI), Italy

[2]School of Chemistry, University of Manchester, Oxford Road, Manchester M13 9PL, United Kingdom

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ABSTRACT: Artemisinin, a potent antimalarial drug derived from *Artemisia annua* L., and curcumin, a polyphenol extracted from the roots of *Curcuma longa* L., are reported to exert a synergistic antimalarial action, albeit manifesting a low bioavailability. In fact, both these molecules are poorly soluble in aqueous environments. In this study, we report a DOSY investigation of the solubilisation capacity of micelles of sodium dodecyl sulphate (SDS) for artemisinin and curcumin, individually and in combination. The aqueous solubility of artemisinin was enhanced approximately 25-fold by 40 mM SDS, and 50-fold by 81 mM SDS, while that of curcumin was increased to 2 mM by 81 mM SDS. In addition, we performed model studies on the use of the surface-active radical scavenger octanoyl-6-*O*-ascorbic acid (ASC8) to combine solubilisation with protection against oxidation for the chemically labile artemisinin. A 16-fold enhancement of artemisinin solubility was measured in a solution containing 40 mM SDS and 60 mM ASC8. Even after treatment with 60 mM hydrogen peroxide, more than a 30-fold excess, almost half the artemisinin remained, suggesting a potentially useful combination of the surface activity and antioxidant properties of the novel binary SDS:ASC8 system. © 2009 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 98:3666–3675, 2009

Keywords: NMR; spectroscopy; DOSY; micelle; surfactants; SDS; ASC8; artemisinin; curcumin; solubility

INTRODUCTION

Artemisinin (**1**; Fig. 1), also known as qinghaosu, is the active constituent of *Artemisia annua* L. (sweet wormwood, Asteraceae), a Chinese herb that has been used for over two millennia in traditional Chinese medicine to treat fevers including malaria.[1] The compound demonstrated clinical efficacy against chloroquine- and quinine-resistant strains of the malarial parasite, *Plasmodium falciparum*, with no signs of serious toxicity for patients.[2,3] Artemisinin-based combination therapy (ACT), employing **1** or its semi-synthetic derivatives, such as arteether or artesunate, in combination with a conventional antimalarial drug,[4] is recommended by the World Health Organisation in those areas affected by multi-drug resistance mutants of the plasmodium.[5,6] Moreover, cytotoxicity towards several tumour cell lines have been recently reported.[7] However, clinical use of artemisinin is hampered by its low solubility in both water and pharmaceutical oils. The lactone endoperoxide group is essential for both the antimalarial and anticancer activities.[8,9] Yet, the highly energetic functional

Silvia Lapenna's present address is C.R.O.M. Oncology Research Centre "Fiorentino Lo Vuolo" Via Ammiraglio Bianco, 83013 Mercogliano (AV), Italy.

Correspondence to: Gareth A. Morris (Telephone: +44-161-275-4665; Fax: +44-161-275-4598; E-mail: g.a.morris@manchester.ac.uk)

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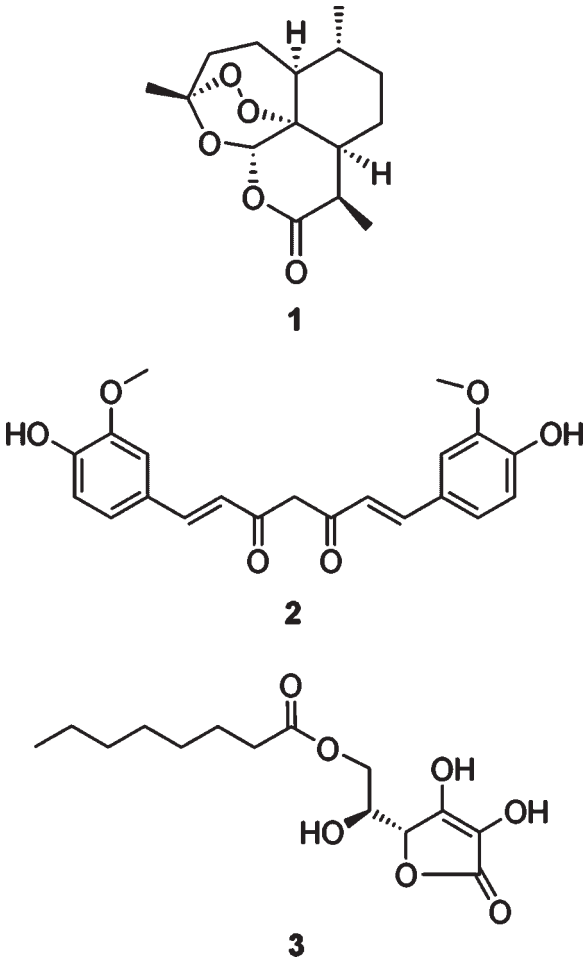

Figure 1. Structures of artemisinin (**1**), curcumin (**2**) and octanoyl-6-*O*-ascorbic acid (**3**).

groups of artemisinin determine its chemical lability and tendency to decomposition.[10]

Curcumin (**2**; Fig. 1), a hydrophobic polyphenol extracted from the roots of *Curcuma longa* L., is a staple of traditional Indian medicine including Ayurveda.[11] Recent studies have shown a synergistic effect of curcumin and artemisinin against the malarial plasmodium both *in vitro* and *in vivo*.[12,13] Curcumin is well tolerated even at very high doses and is a cheap substance, thus its combination with artemisinin may offer several advantages over conventional ACT, including a decreased artemisinin dosage and reduced cost of therapy.[13] However, the poor water solubility, incomplete adsorption and rapid clearance of both these compounds challenge their pharmaceutical formulations.[14,15]

To circumvent the pitfall of poor bioavailability, we have investigated the solubilisation properties of micelles of sodium dodecyl sulphate (SDS) towards artemisinin and curcumin, singly and in combination. By hosting lipophilic molecules in their inner hydrophobic core, micelles may be used both to increase their availability in aqueous environments and to minimise their degradation and loss.[16] Precedents in which the solubility properties of **1** and **2** have been studied in surfactant aggregates include the micellar system formed by cetyltrimethylammonium bromide for curcumin,[17] and micelles of octanoyl-6-*O*-ascorbic acid (ASC8; **3**) for artemisinin.[18] Aqueous solutions of the latter surfactant above the critical micelle concentration (cmc) showed up to a fivefold increase in artemisinin solubility. Other types of delivery systems have emerged in an attempt to increase the bioavailability of these two natural products, including artemisinin[19] and curcumin[20] liposomes, artemisinin complexes with cyclodextrins,[21] cetyltrimethylammonium bromide micelles of curcumin,[17] and polymeric nanoparticles of curcumin ("nanocurcumin").[22]

The extent of micellar-assisted aqueous solubilisation of **1** and **2** was investigated by diffusion-ordered spectroscopy (DOSY), a high-resolution NMR technique which has been used extensively to study supramolecular aggregates and solubilisation processes.[18,23–26] DOSY uses the results of pulsed field gradient spin echo experiments to determine diffusion coefficients for the individual signals in a spectrum, and hence to distinguish between the signals of different components in a mixture.[27] In our applications, two-dimensional (2D) DOSY spectra were used, plotting chemical shifts against diffusion coefficients.

One goal of this study was to assess whether SDS micelles are as effective in solubilising mixtures of artemisinin and curcumin as they are at increasing the solubilities of the individual drugs. By studying the solubilisation behaviour of **1** and **2** in micellar dispersions of SDS, we hope to contribute to the development of novel formulations of artemisinin and curcumin with enhanced bioavailability. In the second part of this article, we describe the behaviour of binary mixtures of the surfactants SDS and ASC8 in water with respect to their ability to form micelles and to solubilise artemisinin. ASC8 is an amphiphilic molecule, which retains the antioxidant activity of vitamin C. We reasoned that the use of micelles containing this special surfactant could improve artemisinin stability and reduce its decomposition in formulation and in the physiological environment. Thus, another goal of this study was to evaluate whether the mixed SDS:ASC8 solutions may improve, retain, or decrease the solubilising

power for artemisinin of micelles formed from each of the two surfactants individually. In contrast to encapsulating carriers such as liposomes, micelles are dynamic entities, in which both surfactant and solubilised solute molecules are exchanged continuously and rapidly with free solution. Thus, to protect solubilised artemisinin from oxidation it is necessary to scavenge oxidants very efficiently, for example by giving the surfactant antioxidant properties. Finally, therefore, to test the potential of ASC8 to provide sacrificial protection against artemisinin oxidation, severe oxidative stress was simulated by treating micellar solutions of artemisinin with a large excess of hydrogen peroxide, and the effects on artemisinin concentration and surfactant diffusion measured.

EXPERIMENTS

Materials

Artemisinin, SDS, 3-(trimethylsilyl)-propionic-2,2,3,3-d_4 acid sodium salt (TSP), and D_2O (99.8%) were purchased from Sigma–Aldrich (Milan, Italy and Gillingham, UK), and curcumin from Extrasynthese (Milan, Italy), and used as received. Octanoyl-6-*O*-ascorbic acid (ASC8) was synthesised according to a previously reported method[28] involving the reaction between L-ascorbic acid (98+%; Aldrich, Steinheim, Germany) and octanoic acid (99+%; Aldrich) in concentrated sulphuric acid (Aldrich). SDS solutions in D_2O were prepared with concentrations from 2 to 81 mM. ASC8 solutions in D_2O/SDS (40 mM) were prepared with concentrations from 2 to 60 mM. Samples of artemisinin and/or curcumin solution in D_2O or surfactant:D_2O were prepared by saturation with the relevant drug, sonication for 20 min, cooling to room temperature (22°C) and filtering through Iso-Disc™ filters (4 mm, 0.2 μm; Sigma–Aldrich, Gillingham).

NMR Experiments

NMR experiments were performed at a proton resonance frequency of 500 MHz, on a Varian VNMRS 500 spectrometer equipped with a 5 mm triple indirect detection probe capable of providing pulsed field gradients of up to 60 $G\,cm^{-1}$. Artemisinin and curcumin solubilities were determined by proton signal integration, employing the trimethylsilyl signal of TSP (0.232 mM) as reference. Diffusion measurements were carried out at a temperature of 25°C, using the Oneshot pulse sequence[29] with a diffusion delay of 0.25 s and bipolar pulse pairs in which each pulse of each pair was of 1.25 ms duration. Between 8 and 25 measurements, each of 4–64 transients, of diffusion-attenuated stimulated echo spectra were made for each sample, using z field gradient strengths between 3 and 60 $G\,cm^{-1}$ spaced at equal intervals of gradient squared. The data were then processed as described previously,[27] using least-squares fitting of peak heights to derive estimated diffusion coefficients and standard errors from the attenuation of signal as a function of the square of field gradient pulse area. Nonexponential fitting based on experimental measurements of the spatial distribution of pulsed field gradient strength was used, in order to improve both the relative and the absolute accuracy of diffusion measurement. DOSY spectra were constructed with the NMR dimension showing the normal NMR line shape for each peak, and the diffusion dimension showing a Gaussian shape centred on the experimental diffusion coefficient with a width governed by the standard error of the fit. Tabulated diffusion coefficients were those found for the chain-head methylene group of SDS at 4.03 ppm, the chain-head methylene group of ASC8 at 2.37 ppm and the acetal proton signal of artemisinin at 6.05 ppm, these being the signals least affected by overlap. The mean value of the diffusion coefficients found for the olefinic and aromatic signals (6.65–7.65 ppm) of curcumin was used as the best approximation to the curcumin diffusion coefficient.

Oxidation Experiments

To investigate any protective effect of ASC8 against artemisinin oxidation, a 60 mM aqueous solution of ASC8 saturated with artemisinin was treated with 60 mM hydrogen peroxide and artemisinin concentration measured for 24 h using HPLC/DAD/ESI MS. The effect of oxidation on the micellisation of ASC8 was investigated by performing DOSY measurements as above on a 60 mM solution of ASC8 in D_2O before and after treatment with 60 mM hydrogen peroxide.

RESULTS AND DISCUSSION

Artemisinin and Curcumin in SDS Micelles

Proton NMR and 2D DOSY spectra were recorded for a range of SDS concentrations from 2 to 81 mM

in D_2O, before and after saturation with artemisinin and curcumin used singly and in combination. Drug concentration and drug and surfactant diffusion coefficient varied as a function of SDS concentration in the sample, as shown in Table 1.

Typical 2D DOSY spectra, of D_2O solutions saturated with artemisinin and curcumin and containing SDS below (2 mM) and above (81 mM) the cmc, are shown in Figure 2. All the species in solution show single sets of signals, indicating that molecular exchange between the free form in solution and the bound form in micelles is rapid on the chemical shift timescale, for both surfactant and solutes. The 2D DOSY display permits the diffusion coefficients of all species to be easily read on the diffusion dimension. Diffusion coefficients reported include error estimates that combine estimated uncertainties from temperature and gradient calibration with the random error estimates obtained in the least-squares fitting. The relatively high estimated errors of 20–30% for the curcumin data reflect the poor signal-to-noise ratio caused by the low concentrations.

SDS micellisation may be followed through the concentration dependence (Fig. 3) of the SDS diffusion coefficient, which is averaged between free and micellar SDS. If the system is approximated by an equilibrium between free molecules of surfactant and micelles of a fixed size, then in the absence of obstruction effects the measured

Table 1. Diffusion Coefficients of SDS (D_{SDS}), Artemisinin (D_{artem}), Curcumin (D_{curc}) and Measured Concentrations of the Solutes as a Function of Total [SDS]

[SDS]/mM	[art]/mM	[curc]/mM	$D_{SDS}/10^{-10}$ ($m^2 s^{-1}$)	$D_{artem}/10^{-10}$ ($m^2 s^{-1}$)	$D_{curc}/10^{-10}$ ($m^2 s^{-1}$)
0	0.26 ± 0.05	<0.005		5.64 ± 0.4	—
2.02	0	0	4.60 ± 0.06		
2.02	0.22 ± 0.05	<0.005	4.61 ± 0.06	5.30 ± 0.4	—
3.97	0	0	4.77 ± 0.06		
3.97	0.19 ± 0.05	0	4.66 ± 0.06	5.32 ± 0.4	
3.97	0	<0.005	4.58 ± 0.06		—
3.97	0.30 ± 0.05	<0.005	4.96 ± 0.06	5.33 ± 0.4	—
6.97	0	0	4.56 ± 0.03		
6.97	0.34 ± 0.06	0	4.25 ± 0.03	4.00 ± 0.2	
6.97	0	<0.005	4.51 ± 0.03		—
6.97	0.22	<0.005	4.39 ± 0.03	4.42 ± 0.2	—
8.10	0	0	4.63 ± 0.08		
8.10	0.42 ± 0.06	<0.005	4.04 ± 0.08	3.38 ± 0.2	—
8.99	0	0	3.53 ± 0.08		
8.99	0.49 ± 0.06	0	3.89 ± 0.08	3.01 ± 0.2	
8.99	0	0.01 ± 0.005	4.20 ± 0.08		—
8.99	0.64 ± 0.07	0.01 ± 0.005	3.75 ± 0.08	3.02 ± 0.2	—
14.01	0	0	2.66 ± 0.05		
14.01	1.30 ± 0.1	0	2.54 ± 0.05	1.51 ± 0.1	
14.01	0	0.32 ± 0.07	2.85 ± 0.05		1.25 ± 0.2
14.01	1.43 ± 0.11	0.32 ± 0.07	2.45 ± 0.05	1.78 ± 0.1	0.84 ± 0.2
20.00	0	0	2.05 ± 0.04		
20.00	2.8 ± 0.2	0	1.61 ± 0.04	1.02 ± 0.05	
20.00	0	0.39 ± 0.08	1.86 ± 0.04		0.82 ± 0.2
20.25	2.3 ± 0.2	0.48 ± 0.09	1.60 ± 0.04	1.16 ± 0.05	0.68 ± 0.13
40.01	0	0	1.18 ± 0.04		
40.01	5.9 ± 0.3	0	1.07 ± 0.04	0.88 ± 0.05	
40.01	0	0.82 ± 0.12	1.06 ± 0.04		0.63 ± 0.2
40.50	5.5 ± 0.3	1.08 ± 0.15	1.01 ± 0.04	0.78 ± 0.05	0.66 ± 0.13
65.04	9.4 ± 0.5	0	0.85 ± 0.03	0.78 ± 0.03	
65.04	0	1.10 ± 0.15	0.85 ± 0.03		0.58 ± 0.2
81.00	0	0	0.77 ± 0.03		
81.00	10.7 ± 0.6	0	0.64 ± 0.03	0.64 ± 0.03	
81.00	0	2.04 ± 0.24	0.64 ± 0.03		0.49 ± 0.2
81.00	10.8 ± 0.6	2.3 ± 0.3	0.64 ± 0.03	0.71 ± 0.03	0.64 ± 0.13

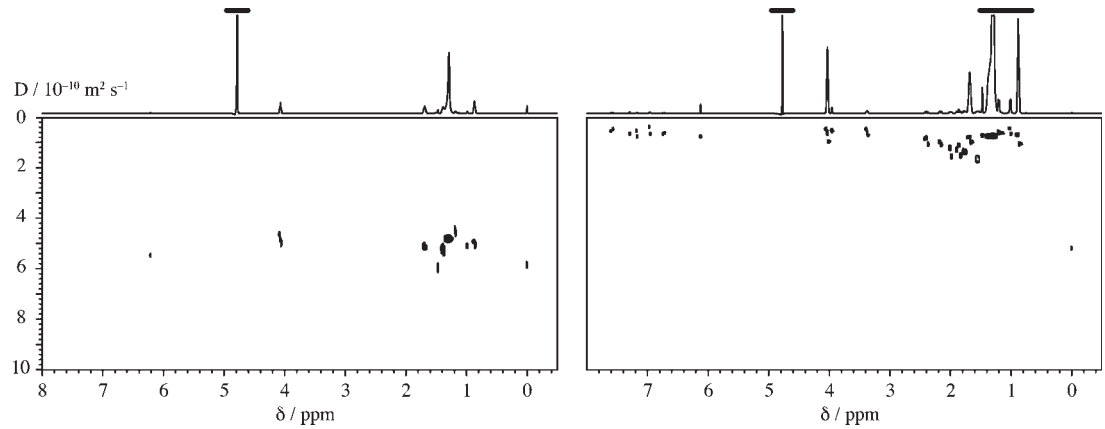

Figure 2. 500 MHz ^{1}H DOSY spectra of D_2O solutions saturated with artemisinin and curcumin and containing SDS below (2 mM) and above (81 mM) the cmc of SDS.

diffusion coefficient D below the cmc will be simply the free monomeric diffusion coefficient (D_{free}), and above the cmc will be given by

$$D = D_{mic} + \frac{(D_{free} - D_{mic})cmc}{[SDS]} \quad (1)$$

sometimes referred to as Lindman's law, where D_{mic} is the diffusion coefficient of the SDS micelles and [SDS] is the total concentration of the surfactant. Figure 3 summarises the SDS average diffusion coefficient in solutions containing SDS only, containing SDS and saturated with artemisinin, containing SDS and saturated with curcumin, and containing SDS and saturated with both artemisinin and curcumin. The SDS data show good general agreement with Lindman's law, and confirm that the presence of solutes does not cause any gross disturbance of micellisation. Fitting the data above and below the cmc separately gives a free SDS diffusion coefficient SDS (D_{free}) of $4.6 \pm 0.1 \times 10^{-10}$ $m^2 s^{-1}$ and the expected cmc of 7.0 ± 0.5 mM, but a surprisingly low extrapolated micellar SDS diffusion coefficient (D_{mic}) of $3.9 \pm 2 \times 10^{-11}$ $m^2 s^{-1}$. Eq. (1) assumes that the micellar diffusion coefficient D_{mic} is independent of concentration. However, as noted by Trembleau and Rebek,[30] the viscosity of SDS solutions rises significantly above 10–20 mM. This causes the plot of D versus inverse concentration to deviate from the bilinear form of Eq. (1) and leads to an anomalously low extrapolated value for D_{mic}. The relatively high cmc of SDS means that its diffusion coefficient, averaged between free and micellar, is a less sensitive reporter of micellar diffusion than that of a solubilised nonpolar solute such as artemisinin, since the latter has a much lower concentration as free molecule in solution. As a result, the concentration and diffusion data for artemisinin are rather more informative.

At SDS concentrations below the cmc, artemisinin concentration is *ca*. 0.22 mM (average of values obtained with the three samples with [SDS] < cmc). However, in solutions with [SDS] above the cmc (from 7.0 to 81 mM), artemisinin concentration rises approximately linearly with SDS concentration (Fig. 4). Thus, artemisinin solubility in D_2O was enhanced approximately 25-fold (to 5.9 mM) with 40 mM SDS, and 50-fold

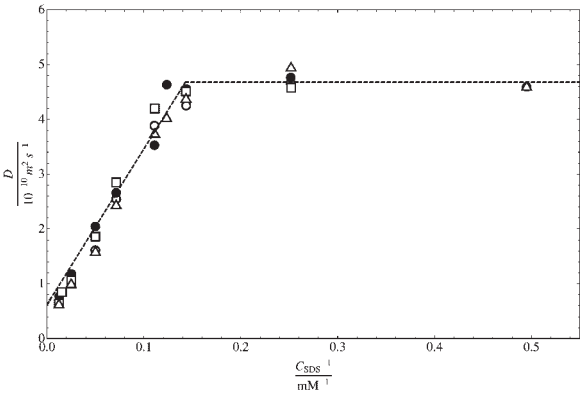

Figure 3. Variation of the SDS diffusion coefficient D_{SDS} with the inverse of SDS concentration, with and without saturation with artemisinin and curcumin used singly and in combination. Filled circle, SDS alone; open circle, solution saturated with artemisinin; square, saturated with curcumin; triangle, saturated with both artemisinin and curcumin. Dotted lines: below cmc, average D_{SDS}; above cmc, Eq. (1) with a cmc of 7.0 mM and a D_{mic} of 6.2×10^{-11} $m^2 s^{-1}$ estimated by extrapolation of the data of Figure 5 (see text).

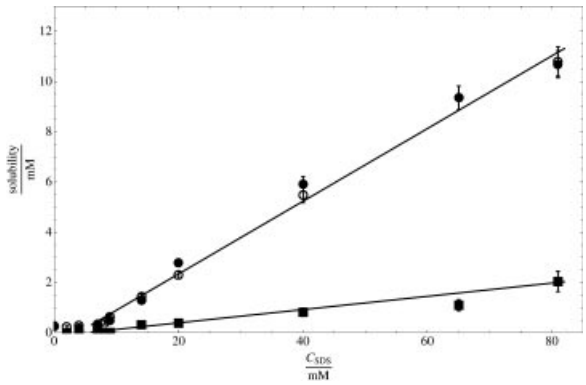

Figure 4. Solubilities of artemisinin and curcumin, singly and in combination, as a function of SDS concentration. Filled circles and filled squares, saturated artemisinin and curcumin concentrations, respectively, in single solution with SDS; open symbols, in mixed solution.

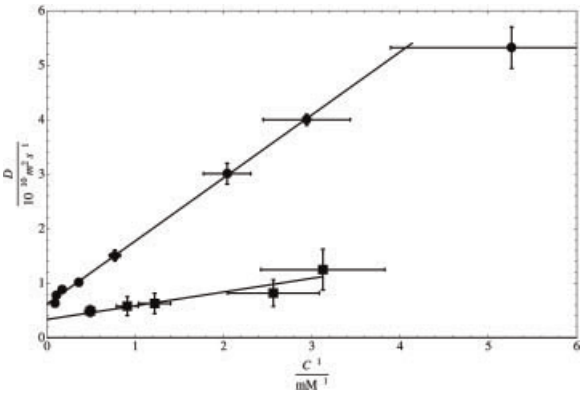

Figure 5. Dependence of the artemisinin (circles) and curcumin (squares) diffusion coefficients on the inverse of the total respective solute concentration, for single solutes in aqueous SDS.

(to 10.7 mM) with 81 mM SDS. Linear regression of the data above the cmc for pure SDS (7 mM) shows a high molar solubilisation ratio of 0.14, corresponding to a mole ratio artemisinin:SDS of 1:7.

The solubility of curcumin in water is much less than that of artemisinin, and its increase in the presence of the surfactant is also much more modest than that of artemisinin. Curcumin concentrations in D_2O and in SDS solutions below the cmc were too low to be measured by proton NMR, but less than 5 μM; this detection limit was imposed by the presence of more soluble impurities with overlapping signals. However, above the cmc of SDS, curcumin concentration rises approximately linearly with SDS concentration, although with a lower slope than that for artemisinin (Fig. 4).

Artemisinin solubilisation by SDS is, as expected, accompanied by a substantial reduction in the (averaged) diffusion coefficient D_{artem} of the drug (Tab. 1). At low SDS concentrations (2–4 mM) D_{artem} is similar to that with no surfactant (5.6×10^{-10} m^2 s^{-1}). Above the cmc of SDS, D_{artem} falls in approximately inverse proportion to the concentration of artemisinin, as expected for simple micellar solubilisation. Assuming that incorporating artemisinin does not have a large effect on the SDS micelles, and that the partition coefficient between aqueous and micellar artemisinin is constant, as supported by Figures 2 and 3, respectively, Lindman's law for the artemisinin diffusion coefficient becomes

$$D_{\mathrm{artem}} = D_{\mathrm{mic}} + \frac{(D_{\mathrm{free}} - D_{\mathrm{mic}})\mathrm{sol}}{[\mathrm{art}]} \qquad (2)$$

where sol is the concentration of artemisinin in saturated aqueous solution, [art] is the total artemisinin concentration and D_{free} is now the diffusion coefficient of free aqueous artemisinin. Figure 5 shows the dependence of D_{artem} on the inverse of the total artemisinin concentration, in good agreement with Eq. (2). Because the aqueous solubility of artemisinin is much less than the cmc of SDS, the averaged artemisinin diffusion coefficient reports on the micellar diffusion coefficient D_{mic} even at relatively low micelle concentrations, where the changes in solution viscosity are very small, so the extrapolation for D_{mic} is much more reliable than is the case for SDS. The value for D_{mic} obtained by linear regression of the artemisinin data of Figure 5 for SDS concentrations between 7 and 20 mM is $6.2 \pm 0.4 \times 10^{-11}$ m^2 s^{-1}. This corresponds to a Stokes–Einstein hydrodynamic radius of *ca*. 3 nm, consistent with values obtained by neutron scattering.[31,32] The linear regression also yields an independent estimate of the solubility, of 0.24 ± 0.01 mol dm^{-3}, in excellent agreement with the direct measurement by integration of the artemisinin signal at 6.05 ppm. The slight downturn in artemisinin diffusion coefficient at high SDS (at the very left of Fig. 5) confirms the effects of viscosity and/or obstruction on the micellar diffusion coefficient at high SDS concentration noted above.

The averaged diffusion coefficient of artemisinin is exquisitely sensitive to SDS concentration near the cmc, so a good estimate of the SDS cmc in the presence of artemisinin can be found by nonlinear least-squares fitting, as illustrated in

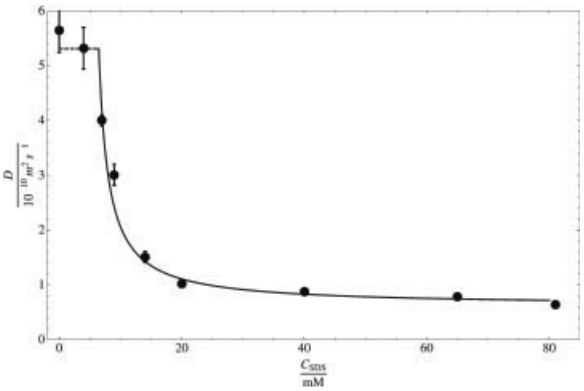

Figure 6. Artemisinin diffusion coefficient (circles) as a function of SDS concentration, with (solid line, above the cmc) the results of nonlinear least-squares fitting to Eq. (3) using the solubilisation ratio K_{artem} from the data of Figure 4 and the micellar diffusion coefficient D_{mic} from the data of Figure 5, and (dotted line, below the cmc) the averaged estimate of the free artemisinin diffusion coefficient.

Figure 6, of the artemisinin diffusion coefficient as a function of SDS concentration to the expression

$$D_{artem} = \frac{D_{mic} + (D_{free} - D_{mic})sol}{sol + K_{artem}([SDS] - cms)} \qquad (3)$$

where D_{free} is fixed at the average (5.4×10^{-10} $m^2 s^{-1}$) of all the available measurements below the cmc, D_{mic} is fixed at the value ($6.2 \pm 0.4 \times 10^{-11}$ $m^2 s^{-1}$) found by linear regression of the data of Figure 5, and K_{artem} (0.14) is the artemisinin:SDS solubilisation ratio found from linear regression of the data of Figure 4. Given the very high mole ratio of artemisinin to SDS at saturation (*ca.* 1:7), it is interesting that the cmc found, 6.5 ± 0.5 mM, indicates that the micellisation of SDS is so little affected.

While curcumin also shows the expected decrease in diffusion coefficient, this is less marked because almost all of the curcumin detectable by NMR is in micelles. Unfortunately, the extremely low solubility of curcumin in neutral water prevented the measurement of its diffusion coefficient in nonmicellar solutions. Figure 5 shows that there is a slight dependence of the curcumin diffusion coefficient on concentration, but in the absence of an independent measure either of the aqueous solubility or of the free diffusion coefficient, it is not possible to interpret the slope of the regression line. The intercept of the line suggests that the micelles containing curcumin may be significantly larger than those containing artemisinin, the limiting micellar diffusion coefficient being $3.5 \pm 2 \times 10^{-11}$ $m^2 s^{-1}$, but the very limited data set does not allow firm conclusions to be drawn. While it is tempting to speculate on the role of the extended linear curcumin structure in influencing micellar size and shape, experiments on mixed solutions (see below) do not provide any supporting evidence.

A similar series of proton NMR and DOSY experiments was carried out for solutions simultaneously saturated with both artemisinin and curcumin, at similar SDS concentrations and under the same experimental conditions as for the single-drug series. The solubility data of Figure 5 show that the solubilisation of the two drugs is substantially independent, with a small possible synergistic effect on the solubilisation of curcumin (i.e. the partition coefficient between aqueous and micellar curcumin is slightly higher for SDS micelles saturated with artemisinin than for pure SDS micelles). The only significant difference seen in the pattern of diffusion coefficients is that the trend towards a lower limiting D_{mic} seen in the data for curcumin alone is absent in the mixed solutions.

Artemisinin in Mixed SDS:ASC8 Micelles

A series of D_2O solutions of SDS (40 mM) containing increasing concentrations of ASC8 (from 2 to 60 mM), before and after saturation with artemisinin, was investigated by quantitative proton NMR and 2D DOSY experiments. As shown in Table 2, the observed diffusion coefficients of ASC8 and SDS were similar throughout the solution series, ranging from 0.7 to 1.1×10^{-10} $m^2 s^{-1}$, indicating the formation of slowly diffusing, mixed SDS:ASC8 micelles. As shown in Figure 7, both diffusion coefficients diminish progressively with increasing [ASC8]. The main reason for this reduction is that as the total surfactant concentration increases, the proportion of free surfactant, and hence the weighted average diffusion coefficient, decreases (as described by Eq. (1)). The decrease observed is slightly bigger than that expected from this source, almost certainly because of increased obstruction/viscosity effects for surfactant concentrations above 20 mM, as seen in the data for SDS alone. As noted earlier, the low aqueous solubility of artemisinin means that the diffusion

Table 2. Diffusion Coefficients of ASC8 (D_{asc8}), SDS (D_{SDS}) and Artemisinin (D_{artem}) as a Function of Total [ASC8] in D_2O Solutions Containing 40 mM SDS

[ASC8]/mM	[art]/mM	$D_{ASC8}/10^{-10}$ ($m^2 s^{-1}$)	$D_{SDS}/10^{-10}$ ($m^2 s^{-1}$)	$D_{artem}/10^{-10}$ ($m^2 s^{-1}$)
1.98		1.12 ± 0.05	1.12 ± 0.03	
1.99	6.1 ± 0.3	1.05 ± 0.05	1.08 ± 0.03	0.83 ± 0.04
3.96		1.16 ± 0.07	1.04 ± 0.03	
3.97	6.2 ± 0.4	1.01 ± 0.08	0.95 ± 0.03	0.86 ± 0.04
6.66		1.04 ± 0.07	1.04 ± 0.02	
6.68	5.5 ± 0.3	0.96 ± 0.07	1.02 ± 0.02	0.95 ± 0.04
7.56		1.00 ± 0.08	1.03 ± 0.03	
7.58	5.2 ± 0.3	0.96 ± 0.08	0.96 ± 0.03	0.83 ± 0.06
9.72		0.96 ± 0.09	0.99 ± 0.04	
9.75	5.1 ± 0.3	0.82 ± 0.09	0.82 ± 0.04	0.82 ± 0.08
13.56		0.90 ± 0.07	0.90 ± 0.02	
13.60	5.1 ± 0.3	0.85 ± 0.08	0.79 ± 0.02	0.86 ± 0.06
19.98		0.83 ± 0.03	0.83 ± 0.03	
20.05	4.5 ± 0.3	0.76 ± 0.03	0.82 ± 0.03	0.87 ± 0.04
39.96		0.83 ± 0.02	0.83 ± 0.03	
40.09	4.0 ± 0.2	0.69 ± 0.02	0.69 ± 0.03	0.73 ± 0.04
60.00		0.71 ± 0.03	0.71 ± 0.03	
60.20	3.5 ± 0.2	0.70 ± 0.04	0.62 ± 0.03	0.76 ± 0.04

coefficients measured for artemisinin are much closer to those for the micelles, and show only a slight decrease with increasing ASC8 concentration. It would thus appear that micellar size is relatively little affected either by the comicellisation of SDS and ASC8 or by the incorporation of artemisinin.

Incorporating ASC8 in the SDS micelles decreased slightly the solubilisation of artemisinin compared to that obtained with micelles of pure SDS (Fig. 8). The saturated artemisinin concentration in an aqueous solution of 40 mM SDS and 60 mM ASC8 was 3.5 mM, an approximately 15-fold enhancement of aqueous solubility, compared to 6.0 mM for 40 mM SDS alone. Although the extent of artemisinin solubilisation in mixed SDS:ASC8 micelles is lower than that for SDS alone, it is much higher than is the case for pure ASC8 (1 mM artemisinin solubility in 60 mM ASC8, only a fourfold enhancement).[18] Therefore, from a pharmaceutical point of view, combined SDS and ASC8 surfactant formulations may be advantageous as they combine a

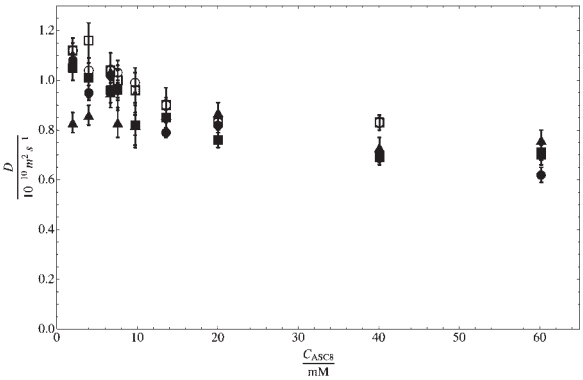

Figure 7. Variation of the diffusion coefficients of SDS, ASC8 and artemisinin with ASC8 concentration, with and without saturation with artemisinin. Open circles and squares, SDS and ASC8, respectively, in the absence of artemisinin; filled symbols, saturated with artemisinin; and triangles, artemisinin.

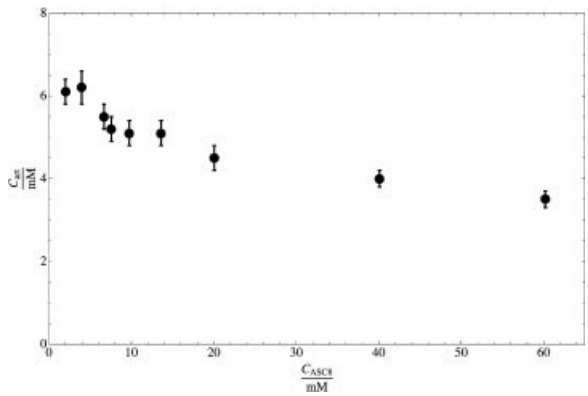

Figure 8. Variation of artemisinin solubility with [ASC8] in mixed aqueous SDS/ASC8 solutions.

substantial enhancement of artemisinin solubilisation with the antioxidant protection of vitamin C, which may be useful to increase the stability of the critical endoperoxide moiety of artemisinin.

Artemisinin Oxidation in ASC8 Micelles

As an extreme test of the ability of ASC8 to protect solubilised artemisinin from oxidation, a saturated solution of artemisinin in 60 mM aqueous ASC8 was treated with equimolar hydrogen peroxide and the artemisinin concentration was measured. Reaction was essentially complete after 6 h; approximately 40% of the artemisinin (0.7 mM, compared to the initial 1.9 mM) remained after this time, despite the treatment with a 30-fold excess of peroxide. DOSY experiments showed that the diffusion coefficients of the unreacted and reacted ASC8 were the same within experimental error, at 8.0×10^{-9} $m^2 s^{-1}$, suggesting strongly that the surface activity, and hence the solubilising capacity, of ASC8 is retained on oxidation.

CONCLUSIONS

Artemisinin (**1**) and curcumin (**2**) are plant-derived bioactive compounds that show a synergistic action in contrasting *P. falciparum* and *P. berghei* infections. However, the poor water solubilities of both substances restrict their absorption after oral administration and compromise drug efficacy and safety. This article reports an investigation of the micellar solubilisation of **1** and **2** by aqueous solutions of SDS, and of **1** by aqueous mixtures of SDS and ASC8. The composite information gained by quantitative ^{1}H-NMR and DOSY measurements indicates that the novel SDS-based supramolecular systems can increase the solubilities of artemisinin and curcumin, both singly and in combination. Moreover, the novel mixed SDS:ASC8 micellar formulations of **1**, combining solubility enhancement with the radical scavenging properties of ASC8, may prove suitable for increasing molecular stability, particularly of the labile endoperoxide group of **1** which is essential for antimalarial activity. In view of these characteristics, these new micellar systems of artemisinin and curcumin should be considered for bioavailability tests *in vivo*.

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