Extraction of erythromycin-A using colloidal liquid aphrons: I. Equilibrium partitioning

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Abstract: Colloidal liquid aphrons (CLAs) are surfactant-stabilised solvent droplets which have recently been explored for use in pre-dispersed solvent extraction (PDSE). In this work, the equilibrium partitioning of a microbial secondary metabolite, erythromycin, has been studied using both CLAs (formulated from 1% (w/v) Softanol 120 in decanol and 0.5% (w/v) SDS in water) and surfactant-containing, two-phase systems. The equilibrium partitioning of erythromycin was found to be strongly influenced by the extraction pH, and exhibited a marked change either side of the pK_a of the molecule. A modified form of the Henderson–Hasselbach equation could be used as a simple design equation to predict the equilibrium partition coefficient ($m_{eryt} = C_{org}/C_{aq}$) as a function of pH. For extraction experiments with dispersed CLAs where pH > pK_a, m_{eryt} values as high as 150 could be obtained and the erythromycin could be concentrated by factors of up to 100. Experiments were also carried out in surfactant-containing, two-phase systems to determine the effect of individual surfactants used for aphron formulation on erythromycin partitioning. For extraction at pH 10 neither the Softanol (a non-ionic surfactant) nor SDS (an anionic surfactant) had any influence on the equilibrium erythromycin partition coefficients. For stripping at pH 7, however, it was found that recovery of erythromycin from the organic phase decreased with increasing concentration of SDS, although again the Softanol had no influence on the equilibrium. The effect of SDS was attributed to a specific electrostatic interaction between individual erythromycin and SDS molecules under stripping conditions. The m_{eryt} values and concentration factors achievable in the two-phase systems were considerably less than those for the PDSE experiments. The physical properties of the two-phase systems, ie density, viscosity, interfacial tension, etc, and the equilibrium distribution of the surfactants were also determined in relation to subsequent studies on the kinetics of erythromycin extraction.

Keywords: erythromycin; aphrons; extraction equilibria; surfactants; SDS; Softanol

NOTATION

cmc	Critical micelle concentration (mol dm^{-3})
C	Concentration (g dm^{-3})
CLAs	Colloidal liquid aphrons
CTMAB	Cetyltrimethylammonium bromide
CV	Coefficient of variance (dimensionless)
d_{ov}	Sauter mean diameter (μm)
Er	Erythromycin
ErH^+	Protonated form of erythromycin
HLB	Hydrophile–lipophile balance (dimensionless)
m	Equilibrium partition coefficient (dimensionless)
PDSE	Predispersed solvent extraction
PVR	Phase volume ratio ($= V_{org}/V_{aq}$) in polyaphron (dimensionless)
SDS	Sodium dodecyl sulfate
V	Volume (dm^3)
V_r	Volume ratio (dimensionless)

Subscripts

aq	Aqueous
eryt	Erythromycin
max	Maximum
org	Organic
SDS	Sodium dodecyl sulfate
S30	Softanol 30

1 INTRODUCTION

Colloidal liquid aphrons (CLAs) are micron-sized solvent droplets surrounded by a thin aqueous film which is stabilised by a mixture of non-ionic and ionic

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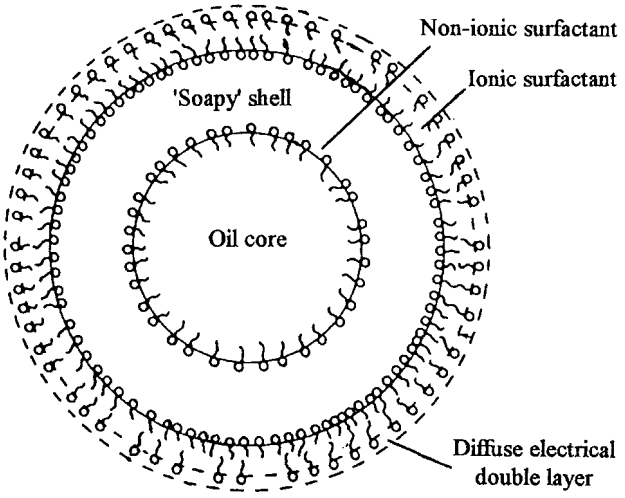

Figure 1. Schematic diagram of a single colloidal liquid aphron (CLA) dispersed in a continuous aqueous phase (redrawn from Sebba[1]).

surfactants; a schematic diagram of a single CLA is shown in Fig 1. This structure was first proposed by Sebba[1] in order to account for the enhanced stability of aphrons relative to oil droplets in conventional oil–water emulsions. Since their discovery in 1972, aphrons have been investigated in relation to the predispersed solvent extraction (PDSE) of a range of organic molecules,[2,3] and as a novel support for enzyme immobilisation in multi-phase biocatalytic processes.[4–6]

Liquid–liquid extraction is a widely used industrial process for the recovery of microbial secondary metabolites produced by fermentation.[7,8] Successive stages of extraction and stripping facilitate the initial purification and concentration of the target antibiotic from the fermentation broth in a process that can be operated continuously on a large scale. Antibiotic purification is potentially one area where PDSE, using CLAs, may be applied in order to reduce purification costs for both new and existing products. Antibiotic extraction is driven by a favourable partitioning of the target molecule between the aqueous feed solution and the solvent core of the dispersed CLAs, while the extremely large interfacial area available for mass transfer (due to the small diameter of the individual CLAs of 5–20 μm)[9,10] allows equilibrium partitioning to be achieved in seconds.[11] The claimed advantages of PDSE over conventional solvent extraction include reductions in the capital costs of mixer-settler units, power requirements for solvent dispersion and solvent inventories, though these remain to be verified experimentally.[1] For extraction from such complex media, however, it will be necessary to determine the effects of the broth components on antibiotic partitioning as these have previously been shown to alter the extraction equilibria of low molecular weight products.[12]

In this work, erythromycin was chosen as a model compound as it is produced commercially by large-scale fermentation of *Streptomyces* sp and is currently purified by solvent extraction.[8] Erythromycin-A is a macrolide antibiotic of molecular weight 734 Da consisting of a 14-membered oxygenated ring to which two sugar moieties are attached.[13] The molecule has a pK_a of 8.6 which allows the aqueous–organic partition of the molecule to be controlled by manipulation of the extraction/stripping solution pH. The objective of this work was to investigate the influence of the surfactants used for aphron formulation on erythromycin partitioning for both PDSE and extraction in surfactant-containing two-phase systems. This, together with data on the physical properties of the phases, will provide the basis for subsequent investigations into the kinetics of erythromycin extraction in the same systems.[11]

2 MATERIALS AND METHODS

2.1 Materials

The solvents used in this work, decan-1-ol, decane, chloroform and propan-2-ol were purchased from Aldrich and were >99% pure. The anionic surfactant used was sodium dodecyl sulfate, SDS (99%, Sigma), the cationic surfactant was cetyltrimethylammonium bromide, CTMAB (Fluka), while the non-ionic surfactants used, Softanol 30 (HLB 7.9) and Softanol 120 (HLB 14.5), were of the alcohol ethoxylate type (Honeywill and Stein Ltd); all were used as supplied. The non-ionics had E_o mole numbers of 3 and 12 respectively. Erythromycin-A (98% w/w) was purchased from Sigma. All the buffer salts used were AnalaR grade from Merck, while the indicator solutions used in the determination of SDS concentration (phenolphthalein, dimidium bromide, disulfhide blue VN and 0.004 mol dm^{-3} Hyamine 1622) were also purchased from Merck, and were of the highest purity available. Hydranal anode and cathode reagents for Karl Fischer titrations were purchased from Fisons. Fresh deionised water obtained from a Purite RO50HP unit had a conductivity of $<0.1\,\mu S\,cm^{-3}$, and was filtered through a 0.2 μm filter before use.

2.2 Polyaphron formulation and manufacture

Polyaphron phases were prepared by dropping the organic phase, containing 1% (w/v) Softanol 120 in decanol, from a burette into a well-stirred (≈800 rpm), foaming aqueous solution containing 0.5% (w/v) SDS. The initial volume of the aqueous phase was typically 1.5 cm^3, and the organic phase was added at an average flow rate of approximately 0.5 cm^3 min^{-1} until the desired phase volume ratio (PVR = V_{org}/V_{aq}) was reached. The organic phase initially disperses easily and can be added at a higher rate but, as the maximum PVR (PVR$_{max}$) is approached, the mixture becomes extremely viscous and the rate of addition must be reduced. The PVR$_{max}$ for the aphron formulation used here has previously been determined to be around nine.[9] The polyaphron phases formed in this way can be considered as 'biliquid foams', ie with a structure like a normal gas–liquid foam except in which the air

pockets are replaced by solvent.[1] These have a creamy-white appearance and showed no signs of phase separation over a period of months. When these polyaphron phases are subsequently dispersed in a continuous aqueous solution, as in a solvent extraction process, the 'cells' of the polyaphron separate to form individual, spherical colloidal liquid aphrons, as shown in Fig 1.[1,9] The influence of various solvents, surfactants and formulation procedures on the stability of the polyaphrons[10] and the half-lives of dispersed CLAs[9] have been published previously.

2.3 Equilibrium partition experiments

Equilibrium distribution experiments between 20 mmol dm^{-3} buffered aqueous phases and dispersed CLAs were performed in test-tubes using a typical volume of the continuous aqueous phase of 5 cm^3. The ratio of CLA:continuous aqueous phase was in the range 1:50 to 1:300 v/v for extraction experiments, while for stripping experiments it was fixed at 1:11 v/v. The polyaphrons were initially dispersed by repeated vortex mixing over 30 min which previous experiments had shown was sufficient time for extraction and stripping equilibria to be reached.[3] Phase separation subsequently occurred for 24 h at 25 °C after which time a sample of the lower aqueous phase was removed for erythromycin analysis. The concentration of erythromycin in the aphron phases at equilibrium was calculated by mass balance given the small volumes of the aphron phases used. In all experiments the test tubes were tightly covered to prevent losses by splashing or evaporation and no change in the pH of the aqueous phase was measured during the extraction/stripping steps. Equilibrium distribution experiments in aqueous buffer–decanol two-phase systems were carried out in a similar manner but with equal volumes of the two phases. In this case both phases could be sampled at equilibrium and analysed for erythromycin, water and surfactant concentrations as required.

2.4 Two-phase system selection

Experiments to determine the kinetics of erythromycin extraction were also carried out in a modified Lewis cell which is a non-dispersive, liquid–liquid contactor with defined interfacial area.[11] In order to identify suitable two-phase systems for use in the Lewis cell, a series of 200 aqueous and organic phases were first prepared. The initial composition of the organic phase was varied between either 0–5% (v/v) Softanol 120 or 0–2% (v/v) Softanol 30 in decanol. The initial composition of the aqueous phase was varied between 0–0.5% (w/v) SDS in either 0.02–0.1 mol dm^{-3} sodium phosphate buffer (pH 7) or sodium carbonate–bicarbonate buffer (pH 10). A 5 cm^3 aliquot of each phase was then mixed in sealed test-tubes and allowed to separate for 24 h at 25 °C. The appearance of all the two-or three-phase systems formed in this way was then recorded. The criteria for selection of the phase systems is discussed later.

2.5 Analytical techniques

2.5.1 Determination of erythromycin concentration

The concentration of erythromycin was determined using a colorimetric assay based upon the reaction of the two sugar moieties on each erythromycin molecule with 9 mol dm^{-3} H_2SO_4 to yield a yellow product absorbing at 479 nm. The method has been described previously by Danielson and co-workers,[13] and allows detection down to levels of 1–10 μg cm^{-3}. The maximum coefficient of variance (CV) over the range of erythromycin concentrations analysed in this work was 4%.

2.5.2 Determination of SDS concentration and SDS–erythromycin interactions

The concentration of SDS was determined by two-phase titration using a mixed indicator solution.[14] A 20 cm^3 aliquot of an appropriately diluted aqueous sample was placed in a 100 cm^3 conical flask and 1–2 drops of a phenolphthalein solution were added (1% w/v in propan-2-ol). This was then neutralised to a faint pink colour with a few drops of 1 mol dm^{-3} NaOH or H_2SO_4. Ten cm^3 distilled water, 25 cm^3 chloroform and 10 cm^3 of an acid indicator solution were then added to the flask (the acid indicator solution was previously made by adding 100 cm^3 distilled water, 10 cm^3 of a dimidium bromide/disulfide blue VN mixed indicator solution and 10 cm^3 2.5 mol dm^{-3} H_2SO_4 to a graduated flask and diluting to a total volume of 250 cm^3). At this stage the anionic surfactant, SDS, in the sample forms a salt with the cationic dimidium bromide dye which is extracted into the chloroform layer and generates a red-pink colour. The two-phase mixture was then titrated with 0.004 mol dm^{-3} Hyamine 1622 solution. The Hyamine cation displaces the dimidium from the salt and the end-point of the titration is reached when the chloroform layer becomes clear (excess Hyamine will form a salt with the anionic disulfide blue VN dye to produce a blue colour). To ensure accuracy the two-phase system was mixed intensively with a magnetic stirrer and the Hyamine 1622 solution was added in 20 mm^3 aliquots. After addition of each aliquot the mixture was left stirring for 5 min before being allowed to settle in order to observe the colour of the chloroform layer. It was also found that the concentration of SDS in decanol phases could be determined using a slight modification of the above assay in which 1 cm^3 of the initial aqueous solution was replaced with an equal volume of a decanol phase sample containing SDS. The maximum CV for this assay was 8%.

The two-phase titration method could also be used to measure the interaction between SDS and erythromycin molecules. Control titrations in which erythromycin was added to SDS-containing solutions were observed to produce a blue colour, indicating an excess of titrant, after the first 20 mm^3 Hyamine aliquot was added to the mixture. This indicated that an interaction occurred between the erythromycin and SDS molecules which prevented the SDS from

forming the initial red-pink salt with the dimidium bromide dye. To quantify the interaction between the erythromycin and SDS molecules, known concentrations of erythromycin were added to a fixed concentration of SDS in decanol and the solution titrated as described above to determine the concentration of 'free' SDS, ie that not interacting with the erythromycin present.

2.5.3 Measurement of dispersed CLA size

The size distributions of dispersed CLAs were determined by a light scattering technique using a Malvern 2600 (Model 3.0) particle size analyser.[9] One drop of the CLA suspension was added to 15 cm^3 of deionised water present in a magnetically-stirred sample cell situated in the light path of the Malvern instrument. Data collection was begun after the CLAs were evenly dispersed throughout the sample cells and was always complete within 30 s of adding the sample. Results for the CLA diameter, d_{ov}, are given here as the Sauter mean diameter which is a measure of the ratio of the total volume of particles to the total surface area. The maximum CV for the determination of d_{ov} was 5%. Various routines were available in the software for analysing the scattering pattern obtained from the CLAs. The one which gave the best correlation between experimental and theoretical scattering patterns was that which assumed the sample consisted of spherical particles having a mono-modal size distribution.

2.5.4 Determination of phase physical properties

The physical properties of the two-phase systems used in the equilibrium partition and Lewis cell[11] experiments were determined at 25 °C after pre-equilibration for 24 h at 25 °C. All results are the average of at least three determinations. The densities of the individual phases were measured using a Paar Scientific densitometer (DMA 35) with a maximum CV of 1%. The kinematic viscosity of each phase was measured using a Technico BS/U U-tube viscometer with a capillary diameter appropriate to the viscosity of the phase being analysed. The viscometers were mounted vertically in a thermostatted water-bath and all flow times recorded were greater than 200 s. The dynamic viscosity was calculated by multiplying the kinematic viscosity by the density of the sample. The maximum CV for the kinematic viscosity determinations was 1%. The water content of the organic phases was determined by Karl Fischer titration using a Metler DL-37 coulometer coupled to an analytical balance to determine the precise mass of sample injected into the coulometer. The maximum CV of the Karl Fischer assay was 9%.

The interfacial tensions between the various equilibrated phases were measured using a Krüss K10 digital tensiometer fitted with a platinum ring (RI 10). The sample chamber was carefully filled with 20 cm^3 of each phase and measurements were made by slowly pulling the ring up through the interface. The weight of the liquid lamella around the circumference of the ring was accounted for by the Zuidema–Waters equation, using the appropriate phase density value, and the maximum CV of the interfacial density determination was 2%. Measurements over a period of 30 min showed no significant variation in the measured value of the interfacial tension. Between each sample the ring was thoroughly cleaned by flaming and the sample bowl was cleaned, rinsed in deionised water and dried with acetone.

3 RESULTS AND DISCUSSION

3.1 Pre-dispersed solvent extraction equilibria

Knowledge of the extent to which erythromycin will partition into the solvent core of the CLAs, and the factors which control this, are vital in relation to the design and operation of a pre-dispersed solvent extraction process. Figure 2 shows the influence of feed solution pH on the equilibrium partition coefficients of erythromycin for both extraction and stripping. The equilibrium partition coefficient, m, is defined as the concentration of antibiotic in the organic (aphron) phase, C_{org}, divided by the concentration in the continuous aqueous phase, C_{aq}; all results are the average of three separate determinations. The large values of m are a consequence of being able to operate at extreme volume ratios of CLA:feed solution, 1:100 in these extraction experiments, which allows the concentration of erythromycin in the CLAs up to the level of 30 g dm^{-3} in this case (which represents a 60-fold increase in erythromycin concentration), and the recovery of >90% (w/w) of the antibiotic in a single equilibrium stage.

Given that erythromycin is a weak base (pK_a of the amino group is approximately 8.6), and that the initial

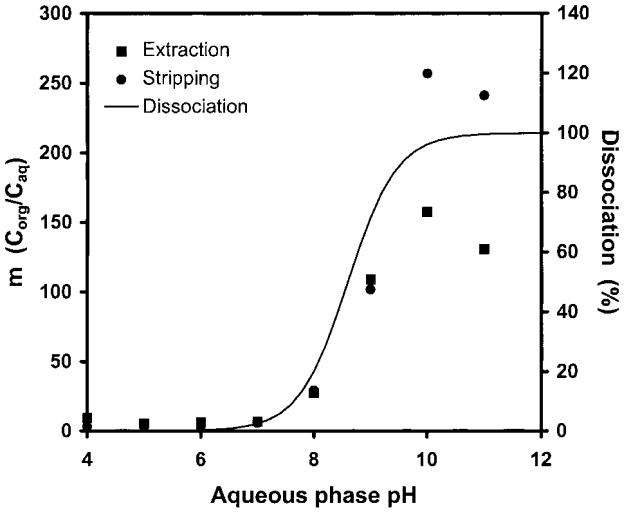

Figure 2. Effect of pH on erythromycin partitioning between 20 mol dm^{-3} buffered aqueous phases and dispersed CLAs; solid line calculated according to eqn (1). Extraction of 0.5 g dm^{-3} erythromycin at a CLA:feed ratio of 1:100 v/v and stripping of 10 g dm^{-3} erythromycin at a CLA:stripping solution ratio of 1:11 v/v. Aphrons formulated from 1% (w/v) Softanol 120 in decanol and 0.5% (w/v) SDS in water to PVR 9.

concentration of the antibiotic is known, the ratio of undissociated to dissociated species at each solution pH can be calculated using a modified form of the Henderson–Hasselbach equation, as shown below:

$$\mathrm{pH} = \mathrm{p}K_a - \log_{10}\left(\frac{[\mathrm{ErH}^+]}{[\mathrm{Er}]}\right) \qquad (1)$$

where $[\mathrm{ErH}^+]$ is the concentration of the protonated form of erythromycin, and [Er] is the concentration of the non-protonated form. As shown by the solid line in Fig 2, calculation of the degree of dissociation/undissociation from eqn (1) enables satisfactory prediction of the partitioning behaviour of erythromycin as a function of pH and particularly the change in m_{eryt} values around the $\mathrm{p}K_a$ of the molecule. Thus eqn (1) is a useful design equation for the initial stages of process synthesis. From Fig 2 it is clear that extraction is best carried out at a feed $\mathrm{pH} > \mathrm{p}K_a$ where the more non-polar (Er) species predominates, while stripping of erythromycin from the CLAs is favoured at a $\mathrm{pH} < \mathrm{p}K_a$ where the charged (ErH^+) species predominates.

The influence of phase volume ratio on erythromycin extraction at pH 9.5 is shown in Fig 3 where the data are plotted in the form of an equilibrium adsorption isotherm. It can be seen that saturation of the CLAs with erythromycin is not reached up to absorbed concentrations of 60 g dm^{-3} though it was not possible experimentally to reach any saturation limit due to the low solubility of erythromycin in the initial aqueous feed solutions at this pH. The results in Fig 3 indicate that volumetric erythromycin concentration factors up to 100-fold can be reached. However, in order to minimise erythromycin levels in the aqueous raffinate after extraction either low CLA:feed ratios must be used (<1:50 v/v) or else multi-stage processes must be employed.

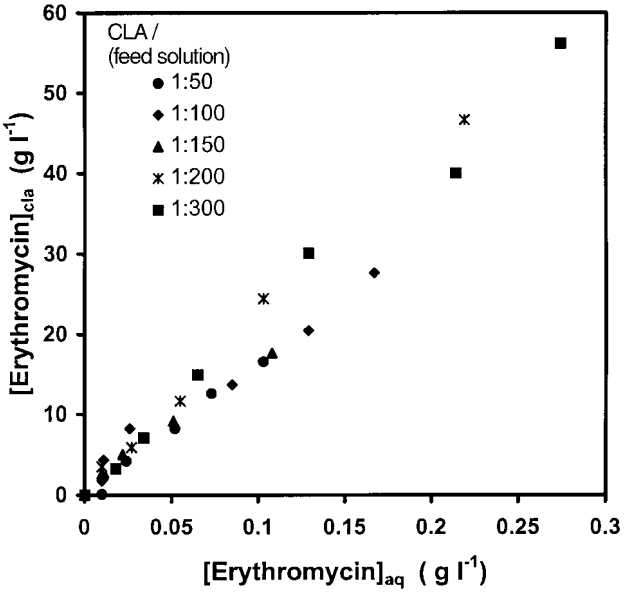

Figure 3. Equilibrium adsorption isotherms for erythromycin extraction (pH 9.5) at various CLA:feed solution ratios (v/v). Aphron formulation as in Fig 2.

3.2 Extraction equilibria in two-phase systems

3.2.1 Two-phase system selection

In related work to the current investigation, the kinetics of erythromycin extraction and stripping were determined for both pre-dispersed solvent extraction processes and in a Lewis cell contactor.[11] A requirement of the Lewis cell experiments is that the two liquid phases could be pre-equilibrated in the absence of a single transferring solute, erythromycin in this case, so that all the other species present (solvent, surfactants, water, ions etc) could attain partition equilibrium before addition of erythromycin to one of the two phases. This phase equilibration was achieved by first vigorously mixing 250 cm^3 of each of the two phases in a separating funnel and then allowing the phases to separate for 24 h at 25 °C. Unfortunately, when the organic (1% w/v Softanol 120 in decanol) and aqueous (0.5% w/v SDS in water) phases typically used for aphron formulation were equilibrated in this way a cloudy, three-phase system formed which could not be separated by centrifugation.

In order to identify a suitable surfactant-containing two-phase system for the Lewis cell studies a range of 200 organic and aqueous phases were prepared, of varying surfactant type and concentration, pH, ionic strength, etc, and then equilibrated. The criteria for selection of the two-phase systems for use in the Lewis cell experiments was that they should separate to produce two clear phases with no interfacial precipitate. The final systems selected consisted of an organic phase containing 0–2% (v/v) Softanol 30 in decanol and an aqueous phase consisting of 0–0.02% (w/v) SDS in 0.05 mol dm^{-3} aqueous buffer at either pH 7 or pH 10. Softanol 30 proved to be a better choice of surfactant than Softanol 120 because of its lower HLB number and hence reduced ability to stabilise the aqueous–organic interface. It can, however, still be used to form CLAs.[10] Ionic strengths greater than 0.1 mol.dm^{-3} also improved phase separation but previous studies have shown that the half-lives of CLAs dispersed in aqueous solutions of this ionic strength or greater are considerably reduced.[9] Table 1 shows the physical properties of the selected aqueous and organic phases before and after phase equilibration. The main points to note are the high viscosity of the decanol phases compared with the aqueous ones, and the larger reduction in interfacial tension for the systems containing SDS compared with Softanol 30.

3.2.2 Effect of surfactants on erythromycin partitioning

To compare the partitioning of erythromycin using either CLAs or surfactant-containing two-phase systems, a series of extraction and stripping experiments as performed with two-phase systems containing different concentrations of SDS and/or Softanol 30. The results in terms of the measured equilibrium partition coefficients are shown in Fig 4. For extraction at pH 10, as shown in Fig 4(a), neither surfactant had any significant effect on the partitioning of erythromycin over the range of concentrations inves-

Table 1. Physical properties of individual phases and two-phase systems after phase equilibration (All results are the average of at least three determinations)

Phases and equilibrated systems[a] *used in extraction and stripping experiments*	*Density* ($kg\,m^{-3}$)	*Viscosity* $\times 10^{-3}$ ($kg\,m^{-1}\,s^{-1}$)	*Interfacial tension* ($mN\,m^{-1}$)
Decanol	829	13.08	NA
50 mmol dm^{-3} carbonate buffer (pH 10)	1003	1.007	NA
50 mmol dm^{-3} phosphate buffer (pH 7)	1005	1.014	NA
Decanol	832	12.57	8.73
50 mmol dm^{-3} carbonate buffer (pH 10)	1003	1.003	
Decanol	832	12.98	7.11
50 mmol dm^{-3} carbonate buffer (pH 10) + 0.02% (w/v) SDS	1003	1.025	
Decanol + 2% (w/v) Softanol 30	835	12.89	7.83
50 mmol dm^{-3} carbonate buffer (pH 10)	1003	1.024	
Decanol + 2% (w/v) Softanol 30	834	12.81	6.5
50 mmol dm^{-3} carbonate buffer (pH 10) + 0.02% (w/v) SDS	1003	1.025	
Decanol	832	13.02	ND
50 mmol dm^{-3} phosphate buffer (pH 7)	1005	1.017	

[a] Table shows initial composition of phases before equilibration.

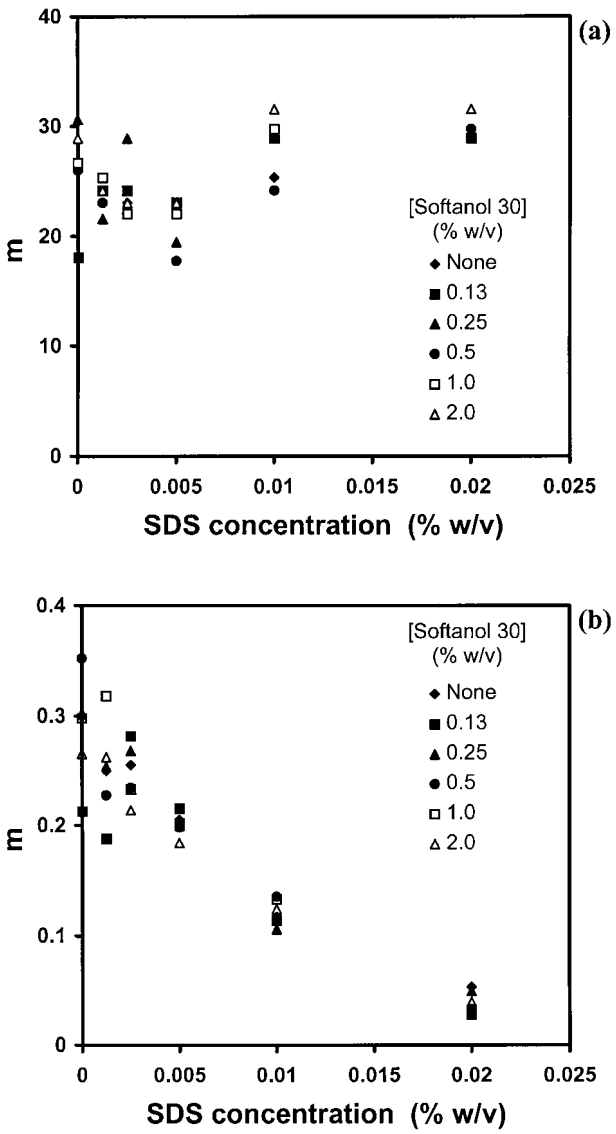

Figure 4. Effect of surfactant type and concentration on (a) erythromycin extraction at pH 10 and (b) stripping of erythromycin at pH 7 in decanol–50 mmol dm^{-3} buffer two-phase systems (V_r=1). Initial erythromycin concentration in feed phase was 0.5 g dm^{-3} in both cases.

tigated. The measured m_{eryt} values, however, were nearly an order of magnitude less than those found for extraction with CLAs (see Fig 2). This is a consequence of the large phase volume ratios which can be used with pre-dispersed solvent extraction. For stripping of erythromycin at pH 7, Fig 4(b) shows that, while Softanol 30 has no significant effect on erythromycin partitioning, the equilibrium partition coefficient of erythromycin decreases with increasing concentrations of SDS. Thus, at higher SDS concentrations, less erythromycin can be recovered from the organic phase back into the conjugate aqueous phase.

One explanation for the observed stripping behaviour is that there is an interaction between the SDS and erythromycin molecules. Since the majority of erythromycin molecules will possess a single positive charge at pH 7 there is the potential for an electrostatic interaction with the negatively charged head-groups of the SDS molecules. Such an SDS–erythromycin complex would be preferentially soluble in the organic phase, and consequently reduced values of m_{eryt} would be expected, ie it would be more difficult to extract the complex from the organic phase. No such interaction would occur in the case of extraction at pH 10 where the erythromycin molecules are uncharged. To confirm the presence of this electrostatic interaction at pH 7 the experiments shown in Fig 4(b) were repeated, but with SDS replaced by the cationic surfactant CTMAB. The head-groups of the CTMAB molecules each possess a positive charge and would not, therefore, be expected to interact electrostatically with the positively charged erythromycin molecules. The results of these experiments (data not shown) showed that m_{eryt} did not vary significantly for CTMAB concentrations in the range 0–0.02% (w/v) which tends to confirm the existence of an electrostatic interaction between the SDS and erythromycin molecules at pH 7. An alternative explanation could be that there was the formation of reverse micelles in the organic phase in which erythromycin was preferentially solubilised.

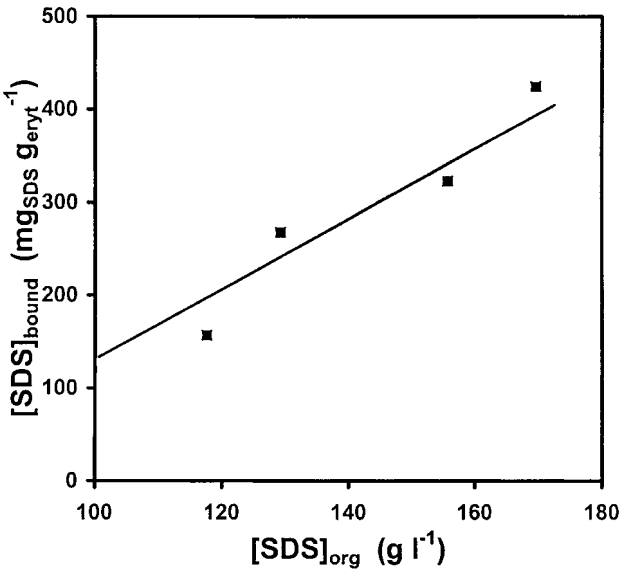

Figure 5. Equilibrium adsorption isotherm for the binding of SDS to erythromycin in decanol. Solid line fitted by linear regression.

Measurement of the water content of the organic phase (for each point in Fig 4), however, showed no significant variation with increasing concentrations of SDS, Softanol 30 or CTMAB. For extraction at pH 10 the mean water content of the decanol phase at equilibrium was 20.3 ± 1.8 g dm^{-3} while for stripping at pH 7 it was 22.2 ± 0.91 g dm^{-3} based on the errors involved this difference in water content was not statistically significant.

Previous studies on the type and concentration of the surfactants used for aphron formulation have focused primarily on the formation and stability of either polyaphron phases[1,10] or dispersed CLAs.[9] In work related to the current investigation[11] we also studied the effect of surfactants on erythromycin extraction kinetics. It is well known that surfactants can adversely influence solute mass transfer rates due to factors such as alteration of the hydrodynamics near the interface[15,16] or the development of an interfacial resistance.[17] Thus there is an opposing situation in which higher surfactant concentrations will increase the stability of the CLAs, but will simultaneously reduce solute mass transfer rates. The current work also shows that certain surfactants can alter the extraction equilibrium via a specific interaction with the erythromycin molecules. Although little is known concerning surfactant–antibiotic systems in general, there are reports in the literature that β-lactam antibiotics can form micelles with cationic and non-ionic surfactants.[18] Since the interaction discovered here between erythromycin and SDS will potentially have a detrimental effect on the quantity of erythromycin that could be recovered from the CLAs during the stripping operation it was worthwhile investigating the SDS–erythromycin interaction further.

The interaction between SDS and erythromycin molecules could be quantified by the two-phase titration technique described earlier. Figure 5 shows a partial adsorption isotherm describing the binding of SDS to erythromycin over the range of concentrations permitted by the sensitivity of the assay. On a molar basis there would appear to be one or two molecules of SDS interacting with each erythromycin molecule. Given the limitations of the two-phase assay this result again supports the hypothesis of a single electrostatic interaction between SDS and erythromycin molecules rather than, say, the formation of colloidal aggregates such as reverse micelles.

3.2.3 Partitioning of other system components

In addition to the partitioning of erythromycin between the two liquid phases, both the Softanol 30 and SDS could potentially be solubilised in each phase. Table 2 shows the equilibrium partition coefficients for erythromycin, SDS and Softanol 30 in the systems selected for use in the subsequent Lewis cell experiments.[11] It is clear that in addition to the erythromycin the SDS also partitions strongly towards the organic phase, while the Softanol 30 remains entirely solubilised in the organic phase (no Softanol 30 could be detected in the aqueous phases at equilibrium). The water content of the decanol phases is also seen to increase greatly after equilibration with the various aqueous phases (the decanol was initially

Table 2. Equilibrium partitioning of system components (all results are the average of at least three determinations)

Phases and equilibrated systems[a] *used in extraction experiments*	$[H_2O]_{org} \times 10^3$ (ppm)	m_{eryt} ($=C_{org}/C_{ao}$)	m_{SDS} ($=C_{org}/C_{ao}$)	m_{S30} ($=C_{org}/C_{ao}$)
Decanol	0.43 ± 0.005	NA	NA	NA
Decanol	33.7 ± 0.9	13	NA	NA
50 mmol dm^{-3} carbonate buffer (pH 10)				
Decanol	32.9 ± 0.8	18	90	NA
50 mmol dm^{-3} carbonate buffer (pH 10) + 0.02% (w/v) SDS				
Decanol + 2% (w/v) Softanol 30	38.0 ± 0.5	14	NA	>100
50 mmol dm^{-3} carbonate buffer (pH 10)				
Decanol + 2% (w/v) Softanol 30	36.9 ± 0.8	15	90	>100
50 mmol dm^{-3} carbonate buffer (pH 10) + 0.02% (w/v) SDS				
Decanol	32.1 ± 0.6	0.233	NA	NA
50 mmol dm^{-3} phosphate buffer (pH 7)				

[a] Table shows initial composition of phases before equilibration.

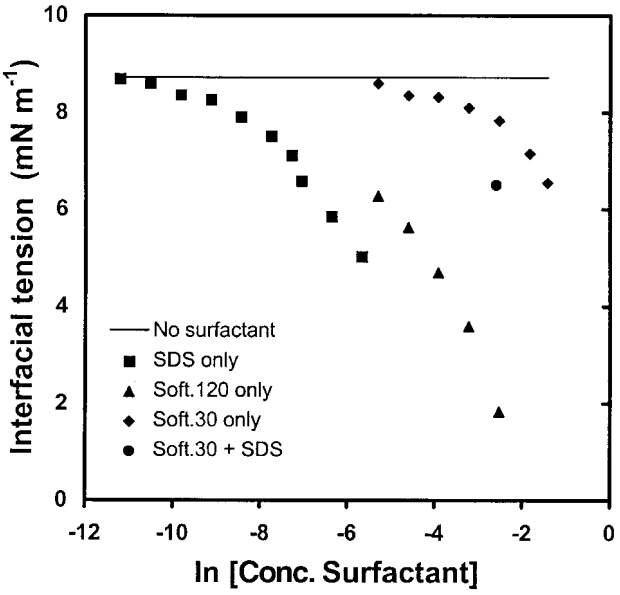

Figure 6. Effect of surfactant type and concentration on equilibrium interfacial tension in decanol–buffer two-phase systems at 25 °C. Organic phase initially consisted of decanol plus varying concentrations of Softanol 30 or Softanol 120 while aqueous phase initially consisted of 50 mmol dm^{-3} carbonate–bicarbonate buffer, pH 10, and varying concentrations of SDS.

'dry' though not anhydrous before phase equilibration) although, as found earlier, there is no evidence of reverse micelle formation with either individual surfactants present or in combination.

3.2.4 Surface activity of system components

The surfactants used in aphron formulations play two important roles. Firstly they alter the surface energies of the two liquid phases to allow the oil phase droplets to spread on an aqueous liquid film during polyaphron formation.[1] Secondly, their adsorption at the oil-core–'soapy-shell' interface stabilises the CLAs by a reduction of the interfacial tension. The presence of the surfactant molecules at the interface of both dispersed CLAs and the surfactant-containing two-phase systems studied here would also be expected to influence the rate of erythromycin partitioning as mentioned previously. Because of this, the interfacial tensions of the two-phase systems were determined at pH 10 using the surfactants used for aphron formulation over a wide range of concentrations. As can be seen from Fig 6, the relative surface activities of the surfactants follows the order SDS > Softanol 120 > Softanol 30. The interfacial tension of a decanol–buffer system in the absence of any surfactants was 8.73 mN m^{-1}. Also, there was no evidence of a critical micelle concentration (cmc) having been reached for any of the surfactants, in which case the interfacial tension would have become constant with increasing surfactant concentration, which again precludes the formation of micelles or reverse micelles in the aqueous and organic phases respectively. It is noticeable that the interfacial tension of the mixed surfactant system (2% w/v Softanol 30 and 0.02% w/v SDS) is less than that with Softanol 30 alone which indicates that the SDS molecules adsorb more strongly at the interface and displace those of the non-ionic surfactant. Control experiments showed that the measured values of interfacial tension were not influenced by the presence of erythromycin in the system.

4 CONCLUSIONS

In this work we have shown that a predispersed solvent extraction process for the extraction of erythromycin using CLAs is an attractive alternative to conventional liquid–liquid extraction. The benefits of PDSE include high equilibrium partition coefficients and volume concentration factors, which are a consequence of the small CLA:feed solution ratios that can be employed. The equilibrium partitioning of erythromycin was found to be strongly influenced by the extraction pH and this could be adequately predicted using a modified form of the Henderson–Hasselbach equation. A specific, one-to-one interaction between erythromycin and SDS molecules, which are required for aphron formulation, was also shown to occur. This may reduce the quantity of erythromycin which can be recovered from the CLAs during the stripping step. The equilibrium partitioning data for erythromycin, together with data on the physical properties of the phases, also provided the basis for studies on the kinetics of erythromycin extraction in both PDSE and conventional liquid–liquid extraction processes.[11]

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