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Solubilisation in aqueous micellar solutions of block copoly(oxyalkylene)s

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Abstract

The solubilisation capacities of micellar solutions of diblock and triblock copolymers composed of hydrophilic poly(ethylene oxide) and hydrophobic poly(styrene oxide) have been compared using the poorly water-soluble drug griseofulvin as a model solubilisate. Our results showed an increase of solubilisation capacity (expressed as mg griseofulvin per gram of hydrophobic block) with temperature and, for spherical micelles, with core volume before reaching limiting values. A change of micelle shape from spherical to cylindrical (or worm-like) resulting from an increase in micelle aggregation number was accompanied by a further enhancement of solubilisation capacity. Comparison with the solubilisation of the same drug in micellar solutions of block copolymers of poly(ethylene oxide) and poly(1,2-butylene oxide) showed that the solubilisation capacity of a poly(styrene oxide) block was approximately four times that of a poly(1,2-butylene oxide) block for spherical micelles. Solubilisation capacity at 25 °C was approximately doubled when griseofulvin was incorporated into a copolymer melt and micelles initially formed from the drug-loaded melt at 65 °C rather than by loading the drug into pre-micellised solution at 25 °C in the usual manner.

Keywords: Solubilisation; Block copolymers; Micellar solutions; Griseofulvin

1. Introduction

A widely recognised challenge in the formulation of oral delivery systems is the design of liquid dosage forms for poorly water-soluble drugs. Of the various formulations that have been explored, those utilizing

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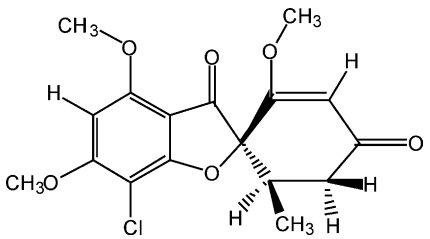
copolymer micelles have received recent attention because of the many advantageous features of micellar carrier systems of this type (Kataoka et al., 1992; Jones and Leroux, 1999; Rosler et al., 2001; Adams et al., 2003). In most block copolymer systems the hydrophilic block has been poly(oxyethylene) which serves as a stabilizing interface between the hydrophobic core and the external aqueous medium and also, in some cases, as a locus for the solubilisation of more hydrophilic solutes. In addition, the poly(oxyethylene) shell allows the micelles to evade scavenging by the mononuclear phagocyte system (MPS) resulting in increased blood circulation times (Yokoyama, 1992). The hydrophobic blocks of the copolymer, which form the core of the micelle, serve as a microenvironment for the incorporation of lipophilic drugs and allow their transportation at concentrations exceeding their intrinsic water solubility. An advantage of block copolymers is the ease with which the size and chemical composition of their micelles can be readily varied through the choice of hydrophobic block and the relative block lengths of hydrophobic and hydrophilic components. Micelles formed from copolymers with a wide range of hydrophobic blocks have been explored for their solubilising potential for poorly soluble drugs (see reviews by Hurter et al., 1995; Allen et al., 1999). Following early work in our laboratory (Collett and Tobin, 1979), micellar solutions of the commercially available triblock copolymers, e.g., Pluronics (BASF) and Synperonics (Uniqema), in which the hydrophobe is poly(oxypropylene) have been examined as solubilisers (Lin and Kawashima, 1985; Saettone et al., 1988; Kabanov et al., 1989, 2002; Hurter and Hatton, 1992; Gadelle et al., 1995; Oh et al., 2004). Other workers have explored the potential of copolymers with biodegradable hydrophobic blocks such as poly(γ-benzyl-L-aspartate) (La et al., 1996), poly(D,L-lactic acid) (Yasugi et al., 1999; Zhang et al., 1996) and poly(ε-caprolactone) (Kim et al., 1998; Shin et al., 1998).

The generally low solubilisation capacity of these and other block copolymer micellar systems has been the motivation for studies of the factors influencing solubilisation efficiency (Hurter et al., 1995; Allen et al., 1999; Nagarajan, 1999, 2001). For a copolymer with a given poly(ethylene oxide) block length, an increase in the length of the core-forming block has been found to increase the partition coefficient of a lipophilic solubilisate between the micelles and the external medium (Tian et al., 1995; Xing and Mattice, 1997). Nagarajan et al. (1986) have emphasised the relationship between the solubilisation capacity of block copolymer micelles and the compatibility of the solubilisate with the core-forming block. On this basis it may be reasoned that block copolymers with hydrophobic blocks formed from monomers bearing aromatic groups should be efficient solubilisers of aromatic drugs. However, the studies of Elworthy and coworkers on poly(oxyethylene) alkyl ethers with long alkyl chains showed that enhancement of solubilisation capacity by increase of the chain length of the hydrophobe is limited by the necessity of maintaining a 'fluid-like' micellar core (Anarson and Elworthy, 1981; Elworthy and Patel, 1982). It is known that copolymers of poly(ethylene oxide) and poly(styrene) do not have fluid cores, a drawback which has been remarked upon by Reiss and coworkers (Jada et al., 1996; Hurtrez et al., 1998), the glass-transition temperature (T_g) of lengthy poly(styrene) chains being ca. 100 °C. Recently we have investigated the possibility of using copolymers of poly(ethylene oxide) and poly(styrene oxide) for drug solubilisation (Rekatas et al., 2001), the T_g of lengthy poly(styrene oxide) chains being much lower than that of poly(styrene), i.e., ca. 40 °C (Allen et al., 1967). We have shown that micelle cores formed from poly(styrene oxide) blocks in solution at 40 °C are highly mobile, i.e., sufficiently so to respond to NMR frequencies of the order of 10^6 Hz, which implies satisfactory mobility for uptake and release of drug molecules under quiescent conditions at temperatures some 30 °C lower (Rekatas et al., 2001).

In this paper we focus on the solubilisation of the aromatic drug griseofulvin (see Scheme 1) in micellar solutions of block copolymers of poly(ethylene



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Scheme 1.

oxide) and poly(styrene oxide). Comparison is made with the solubilisation of the same drug in micellar solutions of block copolymers of poly(ethylene oxide) and poly(1,2-butylene oxide). Griseofulvin has very poor water solubility, less than 1 mg dL^{-1} (Yalkowsky and He, 2003), and has been used by many laboratories, including our own, as a standard for testing a range of micellar hosts. In our preliminary study (Rekatas et al., 2001) we demonstrated an advantage for solubilisation of this drug in micellar solutions of copolymers with poly(styrene oxide) hydrophobic blocks compared with those of copolymers with poly(propylene oxide) and poly(butylene oxide) hydrophobic blocks. In the present study we were able to investigate both a wider range of block copolymer compositions, including two designed to produce cylindrical micelles, and a wider range of methodology for solubilisation of the drug.

We use the following notation to describe the copolymers: E for an oxyethylene unit [OCH_2CH_2], S (from styrene oxide) for an oxyphenylethylene unit [$OCH_2CH(C_6H_5)$], B for an oxybutylene unit [$OCH_2CH(C_2H_5)$], and m and n for number-average block lengths in chain units. Thus E_mS_n denotes a diblock copolymer prepared by polymerisation of ethylene oxide (EO) followed by styrene oxide (SO) starting from a monofunctional initiator, $E_mS_nE_m$ a triblock starting from a difunctional initiator, and so on. The copolymers used are listed in Table 1, together with relevant molecular characteristics: the number-average molar mass (M_n), the ratio of the mass-average to the number-average molar mass (M_w/M_n, an indicator of the width of the chain length distribution), and w_h, the mass-fraction of the hydrophobic (S or B) block of the copolymer. The methods used can be found in the references quoted in Table 1, those without references being prepared and characterised using similar methods. Copolymer $E_{43}B_{14}E_{43}$ is a product of The Dow Chemical Company (see Yu et al., 1996; Booth et al., 2000).

Values of the critical micelle concentration measured in previous work (Yu et al., 1996; Mingvanish et al., 1999; Chaibundit et al., 2002; Crothers et al., 2002; Yang et al., 2003a,b) for a number of the copolymers in aqueous solution at 25 °C range from 0.07 wt.% ($E_{11}B_8$) to less than 0.0001 wt.% ($S_{20}E_{67}$). The solubilisation experiments were carried out on solutions with copolymer concentrations at least thirty-times the cmc, conditions under which the copolymers were effectively fully micellised. Values of the micelle association number are described in Section 3.2.

2. Experimental

The extent of drug solubilisation was determined either by UV spectroscopy after calibration or absolutely by ^{1}H-NMR spectroscopy. Copolymer concentrations were in the range 1–2.5 wt.%. In method 1 a portion of stock copolymer solution (9.9 g) was added to finely ground (1 mm^2 mesh) griseofulvin powder (Sigma–Aldrich, Poole, Dorset, UK, 0.1 g). The mix-

Table 1
Molecular characteristics of the block copolymers

Copolymer	M_n (g mol^{-1})	M_w/M_n	w_h	References
$E_{17}S_8$	1700	1.05	0.562	Yang et al. (2003a)
$E_{45}S_8$	2940	1.06	0.327	–
$E_{45}S_{10}$	3180	1.04	0.377	Crothers et al. (2002)
$S_{10}E_{135}$	7140	1.04	0.168	–
$S_{15}E_{63}$	4570	1.04	0.394	Crothers et al. (2002)
$S_{17}E_{65}$	4940	1.04	0.416	Crothers et al. (2002)
$S_{20}E_{67}$	5300	1.05	0.449	Crothers et al. (2002)
$E_{82}S_8E_{82}$	8150	1.07	0.117	Yang et al. (2003b)
$E_{20}S_{10}E_{20}$	2960	1.03	0.405	–
$E_{66}S_{13}E_{66}$	7370	1.04	0.212	Yang et al. (2003b)
$E_{67}S_{15}E_{67}$	7700	1.04	0.234	Yang et al. (2003b)
$E_{11}B_8$	1060	1.03	0.543	Chaibundit et al. (2002)
$E_{96}B_{18}$	5520	1.03	0.235	Mingvanish et al. (1999)
$E_{43}B_{14}E_{43}$	4790	1.08	0.307	Yu et al. (1996)

ture was stirred at constant temperature (25, 37 or 40 °C) for 3–5 days before being filtered (0.45 μm Millipore) to remove unsolubilised drug. This method is equivalent to the so-called Shake-Flask method.

Because griseofulvin has a low solubility, we were concerned that the solubilisation should not be limited by slow diffusion of the drug into the micelle core. Accordingly, in certain experiments (method 2) finely ground griseofulvin (0.1 g) was added to the copolymer melt at 65 °C, allowing 3 h for dissolution, followed by addition of the required amount of water at 65 °C. This temperature was maintained for 1 h before cooling to 25 °C, stirring for 5 days and then filtering to remove excess drug.

In order to assess the extent of solubilisation in the E-block corona of the micelles the solubilisation of griseofulvin in solutions of polyethylene glycol $M_n = 6000\ g\ mol^{-1}$ (PEG6000, E_{136}, 5–30 wt.% in water) was also investigated.

2.1. UV spectroscopy

A UV–vis spectrometer (Cecil CE1020) was used, calibrated by recording the absorbance (wavelength range 200–350 nm) of methanol solutions of griseofulvin (2–20 mg dm^{-3}) against a solvent blank. The strong absorbance at 292 nm gave a satisfactory Beer's law plot. The sample was then diluted with methanol to enable analysis by UV spectroscopy. The water content after dilution was low enough to allow the calibration for methanol solutions to be used without correction. The absorbance of the copolymer solution at the same dilution was measured as a blank: for E/B copolymers the correction was negligible, while for E/S copolymers the correction was 10–20% of the total absorbance. All measurements were carried out in triplicate and the results averaged.

2.2. ^{1}H-NMR spectroscopy

NMR spectroscopy provides absolute measurements of the ratio of drug to polymer, avoiding the need for calibration. A solution with solubilised drug was prepared and filtered as described for the UV method, but was then freeze-dried (24 h, $<10^{-3}$ mmHg) to remove water. The entire sample was dissolved in $CDCl_3$ and its ^{1}H-NMR spectrum recorded at ambient temperature (ca. 20 °C) using a Varian Associates Unity 500 spectrometer operating at 500 MHz. The pulse sequence consisted of 10 s delay time followed by a 90° pulse and then 2 s for acquisition of the free induction decay. This sequence was time-averaged for 256 acquisitions. The recycle time of 12 s was sufficient to allow complete relaxation of all relevant nuclei. The amount of griseofulvin solubilised per gram of copolymer was determined using appropriate peak integrals as described previously (Rekatas et al., 2001).

3. Results and discussion

Solubilisation capacity (s) was recorded as the amount of drug dissolved in 100 cm^3 of solution in excess of that dissolved in an equivalent volume of water. Each value was the average of results from at least three experiments. These values were divided by the copolymer concentration in wt.% to obtain s_{cp}, the solubilisation capacity per gram of copolymer.

3.1. Poly(oxyethylene) solutions

Fig. 1 illustrates the results obtained for solubilisation of griseofulvin in solutions of PEG6000. The UV method of assay was used. The results are plotted as mg griseofulvin solubilised in excess of that in water per gram of polymer in solution (s_E). Assuming no penetration of water and E blocks into the micelle core, the concentration of E blocks in the water-swollen corona of a micelle can be estimated with sufficient accuracy for our purposes from the micelle molar mass and the micelle volume, properties available from light scattering measurements, and the mass fraction of E in the copolymer ($w_E = 1 - w_h$, available from Table 1). For

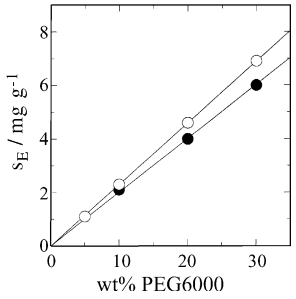

Fig. 1. Excess solubility of griseofulvin in aqueous solutions of polyethylene glycol 6000 over that in water: (●) 25 °C, (○) 37 °C.

example, the molar mass of the micelles of copolymer $S_{17}E_{65}$ at 25 °C is 7.4×10^5 g mol^{-1} (Crothers et al., 2002) and $w_E = 0.584$, whence the mass of the E blocks is 0.72×10^{-18} g. The hydrodynamic volume (effective hard-sphere volume) from dynamic light scattering is 8.5×10^{-18} cm^3 (Crothers et al., 2002), so the average concentration of E units in the micelle fringe is no greater than 10 wt.%. Similar calculations for other copolymers give similar results. Considering Fig. 1, and noting that in a 1 wt.% solution of copolymer the micelle coronas will occupy less than 10 vol% of the solution, we conclude that the amount of griseofulvin solubilised in the E-block coronas of our micelles, in excess of that solubilised in the same volume of water, is rather small at 25 and 37 °C, i.e., $s_E \approx 0.2$ mg/g of copolymer.

3.2. Copolymer solutions: method 1

Values of the solubilisation capacity of the copolymer solutions for griseofulvin (s_{cp}) obtained by method 1 are listed in Table 2. The values for copolymers $E_{11}B_8$ and $E_{96}B_{18}$ have been reported previously (Chaibundit et al., 2002). Changes in copolymer concentration (range 1–2.5 wt.%) produced no systematic differences in s_{cp}, and the results shown are averages for all concentrations. Values obtained using UV and NMR assays were in good agreement (see Table 2). Also listed are values of s_h, the solubilisation capacity per gram of hydrophobic block, calculated using the values of w_h listed in Table 1, averaging values of s_{cp} when both UV and NMR assays are reported and, as an approximate correction for drug solubilised in the micelle corona, deducting $s_E = 0.2$ mg g^{-1} from each value of s_{cp}. This quantity gives a direct measure of the efficiency of solubilisation of the drug in the micelle core. Considering all sources of error, we estimate a maximum uncertainty in s_h of ±4 mg g^{-1}.

3.2.1. Effect of core size and shape

Values of the mass-average association numbers of the micelles (N_w) are listed in Table 2. These values are either taken directly from references (Yu et al., 1996; Mingvanish et al., 1999; Chaibundit et al., 2002; Crothers et al., 2002; Yang et al., 2003a,b), interpolating where necessary, or are estimated using correlations established for these and related copolymers (Crothers et al., 2002; Yang et al., 2003b). The estimated values are marked with an asterisk. The values of N_w serve as indicators of the shape of the micelle core as well as its size. As reported previously, the high association numbers ($N_w > 240$) of the micelles of copolymer $E_{17}S_8$ in solution at 25 and 30 °C (Yang et al., 2003a), and of $E_{11}B_8$ ($N_w = 340$) in solution at 40 °C (Chaibundit et al., 2002), indicate the formation of highly elongated (probably worm-like) micelles. The determining factor is the value of the association number in relation to the average length of the hydrophobic block: a copolymer with a short hydrophobic block cannot form a large spherical micelle core.

Table 2
Solubilisation of griseofulvin in block copolymer solutions (method 1) expressed as solubilisation capacity per gram of copolymer (s_{cp}) and per gram of hydrophobic block (s_h)

Copolymer	s_{cp} (mg g^{-1})[a]		s_h (mg g^{-1})	N_w[b]
	UV	NMR		
25 °C				
$E_{17}S_8$	29.5	–	52	245
$E_{45}S_8$	7.5	–	22	65*
$E_{45}S_{10}$	11.2	–	29	103
$S_{10}E_{135}$	6.0	–	35	70*
$S_{15}E_{63}$	11.2	11.6	28	140
$S_{17}E_{65}$	–	11.7	28	150
$S_{20}E_{67}$	11.8	13.0	27	189
$E_{82}S_8E_{82}$	2.7	–	21	11
$E_{20}S_{10}E_{20}$	11.8	–	28	>40*
$E_{66}S_{13}E_{66}$	–	4.0	18	21
$E_{67}S_{15}E_{67}$	–	5.6	22	25
$E_{11}B_8$	3.9	–	7	63
$E_{96}B_{18}$	3.3	–	13	163
$E_{43}B_{14}E_{43}$	3.3	–	10	9
37 °C				
$E_{17}S_8$	35.0	–	62	≫500
$E_{45}S_{10}$	19.1	–	50	108
$S_{15}E_{63}$	–	15.0	38	145
$S_{17}E_{65}$	–	17.2	41	163
$S_{20}E_{67}$	–	17.5	39	191
$E_{66}S_{13}E_{66}$	–	4.3	19	24
$E_{67}S_{15}E_{67}$	–	7.1	29	27
40 °C				
$E_{11}B_8$	21	–	38	340
$E_{96}B_{18}$	3.9	–	16	174

Values shown with asterisk (*) have been estimated using known correlation, as noted in the text.

[a] Estimated uncertainty ±1 mg g^{-1}.

[b] Mass-average association numbers of the micelles, N_w, are taken from the references given in Table 1.

Inspection of Table 2 shows that the values of s_h are indeed high for solutions of $E_{17}S_8$ (at 25 and 37 °C) and $E_{11}B_8$ (at 40 °C) in comparison with those of the other diblock copolymers. There is evidence from experimental work on other systems (Allen et al., 1999) and from theory (Nagarajan, 1999) that solubilisation in the micelle core is enhanced if the micelles are cylindrical rather than spherical.

The other copolymers, having longer hydrophobic blocks and/or lower association numbers, all form spherical (or near spherical) micelles, as does copolymer $E_{11}B_8$ in solution at 25 °C. Raising the temperature of micellar solutions of block copoly(oxyalkylene)s results in an increase in association number (Booth and Attwood, 2000; Chu and Zhou, 1996). However, for spherical micelles a limit is reached when the hydrophobic blocks are highly stretched (Ryan et al., 2001; Frielinghaus et al., 2001), and any further increase in N_w initiates a change from spherical to cylindrical geometry. The small increases in the values of N_w when the temperature is raised from 25 to 37–40 °C seen for the micelles which remain spherical on heating, e.g., particularly for the large micelles of diblock copolymers $E_{45}S_{10}$, $S_{15}E_{63}$, $S_{17}E_{65}$, $S_{20}E_{67}$ and $E_{96}B_{18}$, show that they are near to their limiting size for sphericity. Copolymer $E_{20}S_{10}E_{20}$ is a similar case even though N_w, estimated as if the micelles are spherical, is only 40: the S block of $E_{20}S_{10}E_{20}$ loops in the core, so its effective length is only S_5 while the core has the volume of forty S_{10} blocks. Calculating as described previously (Yang et al., 2003a) the core radius of micelles with $N_w = 40$ is 2.7 nm but the S_5 block is only 1.8 nm long on average, which is shorter than that required for a spherical micelle. However, the distribution of S-block lengths may well suffice to maintain a spherical (or near spherical) core.

Fig. 2 shows that micelle association number can be used in a semiquantitative way as a crude indicator of core solubilisation capacity across the range of copolymers investigated. All data for E/S copolymers are plotted. As judged by the values of N_w they clearly show: (i) low solubilisation capacity for micelles with small cores; (ii) limiting values of $s_h \approx 28\,mg\,g^{-1}$ at 25 °C and $s_h \approx 42\,mg\,g^{-1}$ at 37 °C for spherical micelles; and (iii) higher values of $s_h = 52\,mg\,g^{-1}$ (25 °C) and $s_h = 62\,mg\,g^{-1}$ (37 °C) for the cylindrical micelles of copolymer $E_{17}S_8$. The values of s_h found for copolymer $E_{20}S_{10}E_{20}$ at 25 °C is $28\,mg\,g^{-1}$, i.e., at the limit

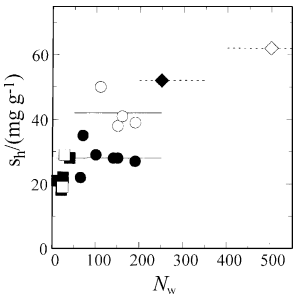

Fig. 2. E/S copolymers: diblock copolymers, spherical micelles, (●) 25 °C; (○) 37 °C; triblock copolymers, spherical micelles, (■) 25 °C; (□) 37 °C; $E_{17}S_8$, cylindrical micelles, (♦) 25 °C; (◊) 37 °C. See Table 2 for details. The lines highlight the maximum extent of solubilisation for the different species and conditions.

for spherical micelles. The almost constant value of s_h found for spherical micelles, very evident for solutions at 25 °C, can be attributed to the same mechanism as that which limits the value of the association number, that is the entropy penalty when the hydrophobic blocks of the core become more stretched by the increase in core radius caused by incorporation of the drug. This increase is small (1–2%), but if the S blocks are already stretched the decrease in entropy may be significant.

3.2.2. Effect of core composition

Values of s_h found for micellar solutions of E/B copolymers are uniformly low compared with those for corresponding solutions of E/S copolymer. This is illustrated in Fig. 3, where the lines, taken from Fig. 2, represent the limiting values of s_h for spherical micelles

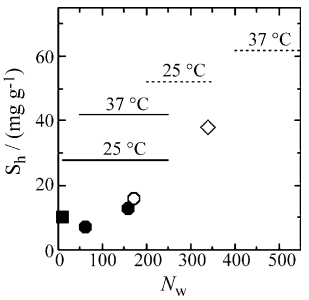

Fig. 3. E/B copolymers: diblock copolymers, spherical micelles, (●) 25 °C; (○) 40 °C; $E_{43}B_{14}E_{43}$, spherical micelles, (■) 25 °C; $E_{11}B_8$, cylindrical micelles, (◊) 40 °C. See Table 2 for details. The lines, taken from Fig. 2, indicate the maximum extent of solubilisation found for (full) spherical and (dashed) cylindrical micelles of E/S copolymers.

of the majority of E/S copolymers, and the dashed lines the results for the cylindrical micelles of $E_{17}S_8$. The solution temperatures for the E/S copolymers are indicated. A high value of s_h is found only for the solutions of copolymer $E_{11}B_8$ at 40 °C, in which the micelles are cylindrical. Considering spherical micelles of high association number, the ratio of values of s_h is in the range B:S = 1:3–1:4 (see Fig. 3), which is a higher ratio than that reported for the values of the cmc in molar units, i.e., B:S = 1:2, presumably reflecting the aromatic nature of both core and solubilisate.

3.2.3. Effect of temperature

For the solutions in which the copolymers form spherical micelles, the micelle association number is not strongly dependent on temperature. Nevertheless, a significant increase in core solubilisation capacity occurs as temperature is increased (see Fig. 2). This increase is attributed to the usual effect of temperature on the solubility of a crystalline compound, i.e., dependent on melting temperature and enthalpy of fusion. Indeed the reported solubilities of griseofulvin in water alone (Yalkowsky and He, 2003) show a similar temperature dependence: a least-squares line through the collected data plotted as log(solubility) versus reciprocal temperature indicates an increase in solubility of ca. 50% in the interval 25–37 °C, much the same as the increase relative to the solubility at 25 °C reported in Fig. 2. The fact that the solubility is greater at 37 °C than at 25 °C means that any drug solubilised at 25 °C stays solubilised under physiological conditions.

3.3. Copolymer solutions: method 2

Values of the excess solubilisation capacity of the copolymer solutions for griseofulvin (s_{cp}) obtained by method 2 are listed in Table 3. As for method 1, results for different copolymer concentration (1 or 2 wt.%) were averaged. Only the UV assay was used. As in Table 2, the results were used to calculate values of the solubilisation capacity of the micelle core, s_h, with correction made for drug solubilised in the micelle corona.

Comparison of results obtained by the two methods is made in Fig. 4. The scatter is large, but on average the solubilisation capacity is approximately doubled by incorporating griseofulvin into the melt before dissolving the copolymer. The effect is similar for both E/S and E/B copolymers, although at a lower level for the E/B copolymers. The limiting conditions for microphase separation in E/B copolymer melts are well known, and the melts of $E_{96}B_{18}$ and $E_{43}B_{14}E_{43}$ are certainly disordered (Ryan et al., 2001). Microphase separation has not been detected in the melts of E/S copolymers, no doubt because the charge transfer interaction between ether oxygen and the phenyl ring greatly reduces the value of the Flory-Huggins χ parameter, much as observed for block copolymers of poly(ethylene oxide) and styrene (Frielinghaus et al., 2001). So the marked effect seen in Fig. 4 is not a result of griseofulvin favouring less polar domains in an ordered melt, but rather a rapid and irreversible transfer to the micelle cores from the disordered melt at the point of micellisation when the drug-loaded melt is transferred to the aqueous phase at 65 °C.

The effect of transferring the drug-loaded melt at 65 °C into water at 25 °C was investigated. In this procedure the solubilisation capacity was only marginally

Table 3
Solubilisation of griseofulvin in block copolymer solutions (method 2) at 25 °C, expressed as solubilisation capacity per gram of copolymer (s_{cp}) and per gram of hydrophobic block (s_h)

Copolymer	s_{cp} (mg g^{-1})	s_h (mg g^{-1})
$E_{45}S_8$	10.7	32
$S_{10}E_{135}$	9.6	56
$E_{82}S_8E_{82}$	5.8	48
$E_{20}S_{10}E_{20}$	31.4	77
$E_{96}B_{18}$	6.9	29
$E_{43}B_{14}E_{43}$	6.1	19

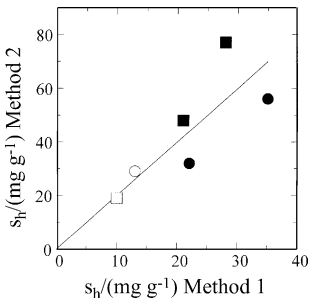

Fig. 4. The solubilisation capacity per gram of hydrophobic block determined by micellisation of drug-loaded melt (method 2) compared with that determined by loading the drug into the pre-micellised solution (method 1). All solutions are at 25 °C: (●) $E_{45}S_8$ and $S_{10}E_{135}$; (■) $E_{82}S_8E_{82}$ and $E_{20}S_{10}E_{20}$; (○) $E_{96}B_{18}$; (□) $E_{43}B_{14}E_{43}$. The least-squares straight line through the points is shown.

improved compared with the conventional procedure, no doubt because the loaded melt crystallised on cooling with consequent separation of its components. The time allowed for equilibration after mixing the drug with the melt was also investigated, with little benefit found for prolonging the time beyond 3 h. We were, of course, conscious that we were heating a poly(ether) to 65 °C in air, and were anxious to limit any degradation of the copolymer caused by heating in air.

The effect of dissolving a drug-loaded melt on micelle shape was not explored. Probably the procedure facilitates the formation of larger micelles, possibly cylindrical micelles in some cases. A change in micelle shape from spherical to cylindrical caused by solubilisation of a core-compatible substance has been predicted by Nagarajan (1999). Light scattering measurements on the resulting solutions should be revealing. Also, it would be interesting to use drug solubilisation by method 2 with copolymers $E_{17}S_8$ and $E_{11}B_8$, copolymers, which, at appropriate temperatures, readily form cylindrical micelles in water. Because synthesis of the copolymers in the laboratory was limited in scale, and because their micellisation and micelle properties had been investigated in detail (Yang et al., 2003a; Chaibundit et al., 2002), our supply of these materials had been used before the results described in this section were known.

4. Conclusions

The results discussed in this paper have identified characteristic features of block copolymers that are associated with high solubilisation capacity for the aromatic drug griseofulvin. We have demonstrated a four fold higher solubilisation of this drug (expressed per gram of hydrophobic block) in micellar solutions of copolymers with poly(styrene oxide) hydrophobic blocks compared with those of copolymers with poly(butylene oxide) hydrophobic blocks. Further enhancement of solubilisation capacity can be achieved by inducing the formation of cylindrical (or worm-like) micelles through the careful selection of the relative chain lengths of hydrophilic and hydrophobic blocks to produce micelles with a high aggregation number from copolymers with short hydrophobic blocks. For micelles retaining a spherical structure, the solubilisation capacity per gram of hydrophobic block increases with core volume, with diblock copolymers being more effective solubilisers than triblock copolymers. An alternative to the shake flask method of solubilisation has been explored and higher solubilisation capacities have been achieved using a method in which the drug was incorporated into a copolymer melt and micelles initially formed at an elevated temperature before cooling to 25 °C.

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References

Adams, M.L., Lavasanifar, A., Kwon, G.S., 2003. Amphiphilic block copolymers for drug delivery. J. Pharm. Sci. 92, 1343–1355.

Allen, C., Maysinger, D., Eisenberg, A., 1999. Nano-engineering block copolymer aggregates for drug delivery. Colloids Surf. B: Biointerfaces 16, 3–27.

Allen, G., Booth, C., Hurst, S.J., Price, C., Vernon, F., Warren, R.F., 1967. Effect of the side group upon the properties of polyepoxides. V. Melting points, glass transition temperatures, and dynamic mechanical properties of poly(tert-butylethylene oxide) and poly(styrene oxide). Polymer 8, 406–413.

Anarson, T., Elworthy, P.H., 1981. Effects of structural variations of non-ionic surfactants on micellar properties and solubilization: surfactants containing very long hydrocarbon chains. J. Pharm. Pharmacol. 33, 141–144.

Booth, C., Yu, G.-E., Nace, V.M., 2000. Block copolymers of ethylene oxide and 1,2-butylene oxide. In: Alexandridis, P., Lindman, B. (Eds.), Amphiphilic Block Copolymers: Self-assembly and Applications. Elsevier Science B.V., Amsterdam, Chapter 4.

Booth, C., Attwood, D., 2000. Effects of block architecture and composition on the association properties of poly(oxyalkylene) copolymers in aqueous solution. Macromol. Rapid Commun. 21, 501–527.

Chaibundit, C., Ricardo, N.M.P.S., Booth, C., Crothers, M., 2002. Micellization of diblock(oxyethylene/oxybutylene) copolymer $E_{11}B_8$ in aqueous solution. Micelle size and shape. Drug solubilization. Langmuir 18, 4277–4283.

Chu, B., Zhou, Z.-K., 1996. Physical chemistry of polyoxyalkylene block copolymer surfactants. In: Nace, V.M. (Ed.), Nonionic Sur-

factants, Poly(oxyalkylene) Block Copolymers, Surfactant Science Series, vol. 60. Marcel Dekker, New York, Chapter 3.

Collett, J.H., Tobin, E.A., 1979. Relationships between poloxamer structure and the solubilization of some para-substituted acetanilides. J. Pharm. Pharmacol. 31, 174–177.

Crothers, M., Attwood, D., Collett, J.H., Yang, Z., Booth, C., Taboada, P., Mosquera, V., Ricardo, N.M.P.S., Martini, L., 2002. Micellisation and gelation of diblock copolymers of ethylene oxide and styrene oxide in aqueous solution. Langmuir 18, 8685–8691.

Elworthy, P.H., Patel, M.S., 1982. Demonstration of maximum solubilization in a polyoxyethylene alkyl ether series of non-ionic surfactants. J. Pharm. Pharmacol. 34, 543–546.

Frielinghaus, H., Hermsdorf, N., Sigel, R., Almdal, K., Mortensen, K., Hamley, I.W., Messe, L., Corvazier, L., Ryan, A.J., van Dusschoten, D., Wilhelm, M., Floudas, G., Fytas, G., 2001. Blends of AB/BC diblock copolymers with a large interaction parameter χ. Macromolecules 34, 4907–4916.

Gadelle, F., Koros, W., Schechter, R., 1995. Solubilization of aromatic solutes in block copolymers. Macromolecules 28, 4883–4892.

Hurter, P., Hatton, T., 1992. Solubilization of polycyclic aromatic hydrocarbons by poly(ethylene oxide-propylene oxide) block copolymer micelles: effects of polymer structure. Langmuir 8, 1291–1299.

Hurter, P.N., Alexandridis, P., Hatton, T.A., 1995. Solubilization in amphiphilic copolymer solutions. In: Christian, S.D., Scamehorn, J.F. (Eds.), Solubilization in Surfactant Aggregates, Surfactant Science Series, vol. 55. Marcel Dekker, New York, pp. 191–235.

Hurtrez, G., Dumas, P., Riess, G., 1998. Polystyrene-poly(ethylene oxide) diblock copolymer micelles in water. Polym. Bull. 40, 203–210.

Jada, A., Hurtrez, G., Siffert, B., Riess, G., 1996. Structure of polystyrene-block-poly(ethylene oxide) diblock copolymer micelles in water. Macromol. Chem. Phys. 197, 3697–3710.

Jones, M.-C., Leroux, J.-C., 1999. Polymeric micelles: a new generation of colloidal drug carriers. Eur. J. Pharm. Biopharm. 48, 101–111.

Kabanov, A.V., Batrakova, E.V., Alakhov, V.Y., 2002. Pluronic block copolymers as novel polymer therapeutics for drug and gene delivery. J. Control Release 82, 189–212.

Kabanov, A.V., Chekhonin, V.P., Alakhov, V.Y., Batrakova, E.V., Lebedev, A.S., Melik-Nubarov, N.S., Arzhakov, S.A., Levashov, A.V., Morozov, G.V., Severin, E.S., Kabanov, V.A., 1989. The neuroleptic activity of haloperidol increases after its solubilization in surfactant micelles. FEBS Lett. 258, 343–345.

Kataoka, K., Kwon, G., Yokoyama, M., Okano, T., Sakurai, Y., 1992. Block copolymer micelles as vehicles for drug delivery. J. Control Release 24, 119–132.

Kim, S.Y., Shin, I.L.G., Lee, Y.M., Cho, C.S., Sung, Y.K., 1998. Methoxy poly(ethylene glycol) and ε-caprolactone amphiphilic block copolymeric micelle containing indomethacin. II. Micelle formation and drug release. J. Control Release 51, 13–22.

La, S.B., Okano, T., Kataoka, K., 1996. Preparation and characterization of micelle-forming polymeric drug indomethacin-incorporated poly(ethylene oxide)-poly(γ-benzyl L-aspartate) block copolymer micelles. J. Pharm. Sci. 85, 85–90.

Lin, S., Kawashima, Y., 1985. The influence of three polyoxyethylene–polyoxypropylene surface-active block copolymers on the solubility behavior of indomethacin. Pharm. Acta. Helv. 60, 339–344.

Mingvanish, W., Mai, S.-M., Heatley, F., Booth, C., Attwood, D., 1999. Association properties of diblock copolymers of ethylene oxide and 1,2-butylene oxide in aqueous solution. Copolymers with oxyethylene-block lengths in the range 100–400 chain units. J. Phys. Chem. B 103, 11269–11274.

Nagarajan, R., 1999. Solubilization of hydrocarbons and resulting aggregate shape transitions in aqueous solutions of Pluronic (PEO-PPO-PEO) block copolymers. Colloids Surf. B 16, 55–72.

Nagarajan, R., 2001. Solubilization of "guest" molecules into polymeric aggregates. Polym. Adv. Technol. 12, 23–43.

Nagarajan, R., Barry, M., Ruckenstein, E., 1986. Unusual selectivity in solubilization by block copolymer micelles. Langmuir 2, 210–215.

Oh, K.T., Bronich, T.K., Kabanov, A.V., 2004. Micellar formulations for drug delivery based on mixtures of hydrophobic and hydrophilic Pluronic block copolymers. J. Control Release 94, 411–422.

Rekatas, C.J., Mai, S.-M., Crothers, M., Quinn, M., Collett, J.H., Attwood, D., Heatley, F., Martini, L., Booth, C., 2001. The effect of hydrophobe chemical structure and chain length on the solubilizaation of griseofulvin in aqueous micellar solutions of copoly(oxyalkylene)s. Phys. Chem. Chem. Phys. 3, 4769–4773.

Rosler, A., Vandermeulen, G.W., Klok, H.-A., 2001. Advanced drug delivery devices via self-assembly of amphiphilic block copolymers. Adv. Drug Deliv. Rev. 53, 95–108.

Ryan, A.J., Mai, S.-M., Fairclough, J.P.A., Hamley, I.W., Booth, C., 2001. Ordered melts of block copolymers of ethylene oxide and 1,2-butylene oxide. Phys. Chem. Chem. Phys. 3, 2961–2971.

Saettone, M., Giannaccini, B., Delmonte, G., Campigli, V., Tota, G., La Marca, F., 1988. Solubilisation of tropicamide by poloxamers: physicochemical data and activity data in rabbits and humans. Int. J. Pharm. 43, 67–76.

Shin, I.L.G., Kim, S.Y., Lee, Y.M., Cho, C.S., Sung, Y.K., 1998. Methoxy poly(ethylene glycol) and ε-caprolactone amphiphilic block copolymeric micelle containing indomethacin. I. Preparation and characterization. J. Control Release 51, 1–11.

Tian, M., Arca, E., Tuzar, Z., Webber, S.E., Munk, P., 1995. Light scattering study of solubilization of organic molecules by block copolymer micelles in aqueous media. J. Polym. Sci.: Part B Polym. Phys. 33, 1713–1722.

Xing, L., Mattice, L.W.L., 1997. Strong solubilization of small molecules by triblock copolymer micelles in selective solvents. Macromolecules 30, 1711–1717.

Yalkowsky, S.H., He, Y., 2003. Handbook of Aqueous Solubilities. CRC Press, Boca Raton.

Yang, Z., Crothers, M., Attwood, D., Collett, J.H., Ricardo, N.M.P.S., Martini, L.G.A., Booth, C., 2003a. Association properties of ethylene oxide/styrene oxide diblock copolymer $E_{17}S_8$ in aqueous solution. J. Colloids Interface Sci. 263, 312–317.

Yang, Z., Crothers, M., Ricardo, N.M.P.S., Chaibundit, C., Taboada, P., Mosquera, V., Kelarakis, A., Havredaki, V., Martini, L., Valder, C., Collett, J.H., Attwood, D., Heatley, F., Booth, C., 2003b. Micellisation and gelation of triblock copolymers of ethylene oxide and styrene oxide in aqueous solution. Langmuir 19, 943–950.

Yasugi, K., Nagasaki, Y., Kato, M., Kataoka, K., 1999. Preparation and characterization of polymer micelles from poly(ethylene glycol)-poly(D,L-lactide) block copolymers as potential drug carrier. J. Control Release 62, 89–100.

Yokoyama, M., 1992. Block copolymers as drug carriers. Crit. Rev. Ther. Drug Carrier Syst 9, 213–248.

Yu, G.-E., Yang, Y.-W., Yang, Z., Attwood, D., Booth, C., Nace, V.M., 1996. Association of diblock and triblock copolymers of ethylene oxide and butylene oxide in aqueous solution. Langmuir 12, 3404–3412.

Zhang, X., Jackson, J.K., Burt, H.M., 1996. Development of amphiphilic diblock copolymers as micellar carriers of taxol. Int. J. Pharm. 132, 195–206.