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Note

Solubilisation of griseofulvin, quercetin and rutin in micellar formulations of triblock copolymers $E_{62}P_{39}E_{62}$ and $E_{137}S_{18}E_{137}$

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ABSTRACT

The solubilisation of two poorly soluble flavonoids, quercetin and rutin, in micellar solutions of mixtures of a block copolymer of ethylene oxide and styrene oxide ($E_{137}S_{18}E_{137}$) with one of ethylene oxide and propylene oxide ($E_{62}P_{39}E_{62}$) has been studied at 25 and 37 °C. Solubilisation capacities were higher than those for the model poorly water-soluble drug griseofulvin and comparable with published values for the solubilisation of rutin by β-cyclodextrin.

1. Introduction

Flavonoids are a group of polyphenolics which are common constituents in plants, being found, for example, in many fruits, seeds, vegetables, leaves, herbs, olive oil, and wine. They are among the 10 most important pharmaceutical products exported by Brazil (ABIQUIF, 2008). The pharmacological activity of the flavonoids, and in particular the two flavonoids of this current investigation, rutin and quercetin (see Scheme 1), arises from their ability to inhibit certain enzymes, and from their antioxidant properties (De Groot and Rauen, 1998; Middleton et al., 2000; Kessler et al., 2003). In vitro and animal studies have demonstrated that flavonoids may inhibit cancer cell growth by binding to type II receptors, which are over-expressed in a wide range of tumour tissues, e.g. breast, ovarian, colon and lung (Cipak et al., 2003). Interest in flavonoid drugs has also increased because of their antioxidant activity: flavonoids such as quercetin may delay oxidant injury and cell death by scavenging oxygen radicals, protecting against lipid peroxidation, and chelating metal ions (Inal and Kahraman, 2000). Experimental evidence of their antioxidant activity in vitro (Takahama, 1985; Limasset et al., 1993), especially their ability to inhibit LDL oxidation by macrophages (De Whalley et al., 1990) has led to the suggestion that they may help to reduce the incidence of coronary heart disease. Flavonoids have also been shown to have an anti-thrombotic action in vivo (Gryglewski et al., 1987). Clinical evidence of the importance of flavonoids in reducing mortality from coronary heart disease was provided by the Zutphen Elderly Study (Hertog et al., 1993). Topical administration of antioxidants has been examined as a method of enriching the endogenous cutaneous protection system and has been proposed as a strategy for inhibiting ultraviolet radiation-induced cutaneous oxidative stress and inflammation (Saija et al., 1998; Röpke et al., 2002; Casagrande et al., 2007).

Vehicles investigated for the oral delivery of flavonoids have included liposomes (Gordon and Roedig-Penman, 1998); cyclodextrins (Miyake et al., 2000; Calabro et al., 2005); and pectin/HPMC matrix tablets (Monteiro et al., 2007). Several drug carrier systems have been investigated for topical delivery of flavonoids including liquid crystalline dispersions (Vicentini et al., 2007); gels, emulgels and microemulsion gels (Ibrahim et al., 2007); water-in-oil microemulsions (Vicentini et al., 2008); and deoxycholate-hydrogels (Valenta et al., 1999).

Low aqueous solubility is a problem in the formulation of many drugs, including those of this study, with solubilities ($mg\,l^{-1}$) in water at 25 °C of 45 and 7.7 reported for rutin and quercetin, respectively (Lauro et al., 2002). A useful response has been the

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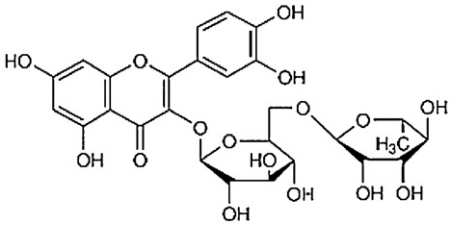
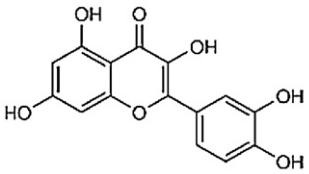
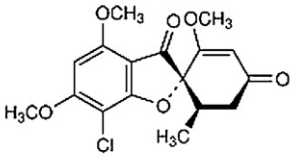


rutin



quercetin



griseofulvin

Scheme 1. Drug structures.

development of colloidal delivery systems based on amphiphilic block copolymers, the micelles of which can accommodate poorly soluble guest molecules (Attwood et al., 2007; Attwood and Booth, 2007; Chiappetta and Sosnik, 2007; Gaucher et al., 2005; Kabanov et al., 2002). Attention has been focused on copolymers with poly(oxyethylene) hydrophilic blocks and poly(oxyalkylene) hydrophobic blocks formed from poly(propylene oxide), poly(1,2-butylene oxide), poly(styrene oxide) and poly(phenyl glycidyl ether) (Attwood and Booth, 2007), taking advantage of the 'stealth' properties of the poly(oxyethylene) corona of their micelles (Kataoka et al., 1993; Adams et al., 2003). Copolymers of type $E_nP_mE_n$, with hydrophilic poly(oxyethylene) and hydrophobic poly(oxypropylene) blocks, have been commercially available for several decades. Here E denotes oxyethylene OCH_2CH_2, P denotes oxypropylene $OCH_2CH(CH_3)$, and subscripts *m* and *n* indicate chain length in P or E units. Concentrated solutions of certain $E_nP_mE_n$ copolymers display thermally reversible gelation in the temperature range required for in situ gelling, either in topical use (e.g. Schmolka, 1972; Rodheaver et al., 1980) or following subcutaneous injection (e.g. Chen-Chow and Frank, 1981; Miyazaki et al., 1986), but they have not found wide application for drug solubilisation primarily because of their low solubilisation capacity.

We have investigated a number of micellar solutions and gels in which the core-forming blocks have been designed to be more hydrophobic and more compatible with the drug to be solubilised (Attwood and Booth, 2007; Crothers et al., 2005; Ribeiro et al., 2009). Recently we have used mixtures of copolymer $E_{62}P_{39}E_{62}$ (commercial notation F87) with copolymer $E_{137}S_{18}E_{137}$ to obtain a system with useful gelation characteristics combined with a satisfactory drug-loading capacity for griseofulvin (see Scheme 1) (Pinho et al., 2007). Here S denotes $OCH_2CH(C_6H_5)$ from the copolymerisation of styrene oxide. The sol–gel diagram obtained for solutions of an 80/20 wt.% mixture (ESE/EPE) of the two copolymers resembles that of $E_{62}P_{39}E_{62}$, whereas no sol–gel transition is observed on heating solutions of $E_{137}S_{18}E_{137}$ alone (see Fig. 1). It is seen, for example, that a 26 wt.% solution of the 80/20 wt.% mixture is a mobile fluid

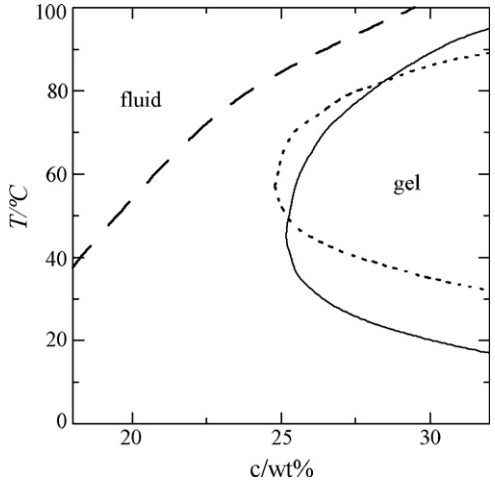


Fig. 1. Sol–gel boundaries for aqueous solutions of (dashed curve) $E_{137}S_{18}E_{137}$, (dotted curve) $E_{62}P_{39}E_{62}$, and (full curve) an 80/20 mixture of the two (%w/w ESE/EPE). The results are taken from Pinho et al. (2007).

at 20 °C which, on heating, passes through a sol–gel transition at 30 °C and forms a stable gel at body temperature.

The hydrophobicities of P and S units are very different: as judged from values of the critical micelle concentration (cmc, molar concentrations) they are in approximate ratio P:S = 1:10 (Yang et al., 2003). On this basis, the hydrophobicities of the central blocks of the two copolymers are in ratio $P_{39}:S_{18} \approx 1:5$. Accordingly, copolymer $E_{137}S_{18}E_{137}$ micellises much more readily than copolymer $E_{62}P_{39}E_{62}$ and will be fully micellised at a low temperature. The gel state in solutions of the mixed copolymers is achieved when the proportion of $E_{62}P_{39}E_{62}$ molecules which micellise on heating the solution is sufficient to increase the overall volume fraction of micelles in solution to a value at which they pack. Consequently, the lower boundary is particularly sensitive to the presence of $E_{62}P_{39}E_{62}$. Even though the two copolymers form separate micelles, investigation by small-angle X-ray scattering has shown that gels of $E_{62}P_{39}E_{62}$ alone, of $E_{137}S_{18}E_{137}$ alone, and of a 50/50 wt.% mixture of the two, all have similar body-centred cubic (bcc) structures with similar lattice dimensions (Hamley et al., 2007), which points to gels of high elastic modulus across the composition range.

A preliminary to any investigation of the use of micellar gels as vehicles for drug delivery is a determination of the solubilisation capacity of the micellar solutions. Our purpose in the work reported in this note was to investigate the applicability of the $E_{62}P_{39}E_{62}/E_{137}S_{18}E_{137}$ system to the solubilisation of the two important flavonoid drugs, rutin and quercetin, using results for griseofulvin as control.

2. Experimental

2.1. Materials

Copolymer $E_{137}S_{18}E_{137}$ ($M_n = 14{,}200\ g\ mol^{-1}$, $M_n/M_w = 1.06$, weight fraction E, $w_E = 0.85$) was prepared in our laboratory. Copolymer $E_{62}P_{39}E_{62}$ (F87, $M_n \approx 7720\ g\ mol^{-1}$, $M_n/M_w \approx 1.09$, $w_E = 0.71$) was a gift from Uniqema Ltd. Values of the cmc observed for the two copolymers in water at 30 °C differ greatly, 23 mg l^{-1} for $E_{137}S_{18}E_{137}$ and 0.81 g l^{-1} for $E_{62}P_{39}E_{62}$, reflecting the much greater stability of the ESE micelles. Details of the methods used to determine values of the cmc can be found elsewhere (Yang et al., 2003; Harrison et al., 2005).

Griseofulvin was supplied by Sigma–Aldrich (Poole Dorset, UK). Rutin and quercetin were supplied by Flora Brasil Ltd. Water was milli-Q quality, methanol was synthesis grade.

2.2. Solubilisation

The two copolymers alone ($E_{62}P_{39}E_{62}$ and $E_{137}S_{18}E_{137}$) and five mixtures of the two with $E_{137}S_{18}E_{137}$ in the range 50–90 wt.%, were used to form 1 wt.% aqueous stock solutions. Finely powdered drug (10 mg) was mixed with stock solution (10 ml), and the mixture stirred at constant temperature (25 or 37 °C) for 4 days before being filtered (0.45 μm Millipore filters) to remove unsolubilised drug. The procedure is equivalent to the standard Shake-Flask method.

The extent of solubilisation was determined by UV spectroscopy as described by Crothers et al. (2005). The drug-loaded solution was diluted quantitatively with sufficient methanol to enable determination of its absorbance at optimum wavelength (griseofulvin 292 nm, rutin 359 nm, quercetin 370 nm) which was then compared with the appropriate Beer's law plot for the drug in methanol. The water content after dilution was low enough to allow the calibration with methanol solutions to be used without correction. Blank experiments (no copolymer) gave the solubility of the drug in water. All measurements were carried out in triplicate and the results averaged; considering all sources of error, we estimate a maximum uncertainty in s_{cp} of ±3 mg ml^{-1}.

3. Results and discussion

Solubilisation capacities measured for the copolymers and their mixtures are listed in Table 1. The quantity s_{cp} records the solubilisation capacity in milligrams of drug solubilised per gram of copolymer after correction for the solubility of the drug in water. Also reported are values of the solubilisation capacity per gram of hydrophobic component in the copolymer or mixture, calculated from values of s_{cp} as $s_h = s_{cp}/w_h$, where w_h is the weight fraction of hydrophobic component (i.e. $w_h = 1 - w_E$, with weight-average values of w_E calculated for the mixtures using the values for the individual copolymers listed in Section 2). The quantity s_h gives a direct measure of the efficiency of solubilisation of a drug in the micelle core, and so is independent of micelle composition. From the values of s_h listed for $E_{62}P_{39}E_{62}$ and $E_{137}S_{18}E_{137}$ in Table 1 it is seen that an ESE micelle solubilises five times more flavonoid than would a EPE micelle of comparable core size. However, in the design of systems for drug delivery the important consideration is the solubilisation capacity.

Values of s_{cp} are plotted against wt.% $E_{137}S_{18}E_{137}$ in Fig. 2. Within the scatter of results the solubilisation capacities are similar for the two flavonoids, and a single straight line serves as the best fit to the two sets of data. The solubilisation capacities measured for griseofulvin are lower, but show a similar straight line dependence on the composition of the copolymer mixture.

Table 1
Solubilisation capacities (mg g^{-1}) of drugs in 1 wt.% solutions of block copolymers and their mixtures.

	T/°C	Griseofulvin		Rutin		Quercetin	
		s_{cp}	s_h	s_{cp}	s_h	s_{cp}	s_h
$E_{62}P_{39}E_{62}$	25	0.5	1.7	2.4	8.3	1.2	4.1
	37	0.8	2.7	3.7	12.9	2.8	9.6
50/50	25	2.1	9.5	4.5	21.4	3.3	15.0
	37	3.0	13.6	4.7	21.3	4.8	21.8
60/40	25	3.5	17.0	4.8	23.3	4.4	21.3
	37	4.9	23.8	6.8	33.0	5.4	26.2
70/30	25	4.0	20.8	5.0	26.0	5.8	30.2
	37	5.8	30.2	7.0	36.4	6.7	34.9
80/20	25	4.6	25.8	5.1	28.6	5.9	33.1
	37	6.1	34.3	7.1	39.9	7.5	42.1
90/10	25	5.0	30.5	5.1	31.1	6.2	37.8
	37	6.7	40.8	7.5	45.7	8.0	48.8
$E_{137}S_{18}E_{137}$	25	4.9	32.7	7.3	48.7	8.0	53.5
	37	5.6	37.3	7.5	50.0	8.2	54.7

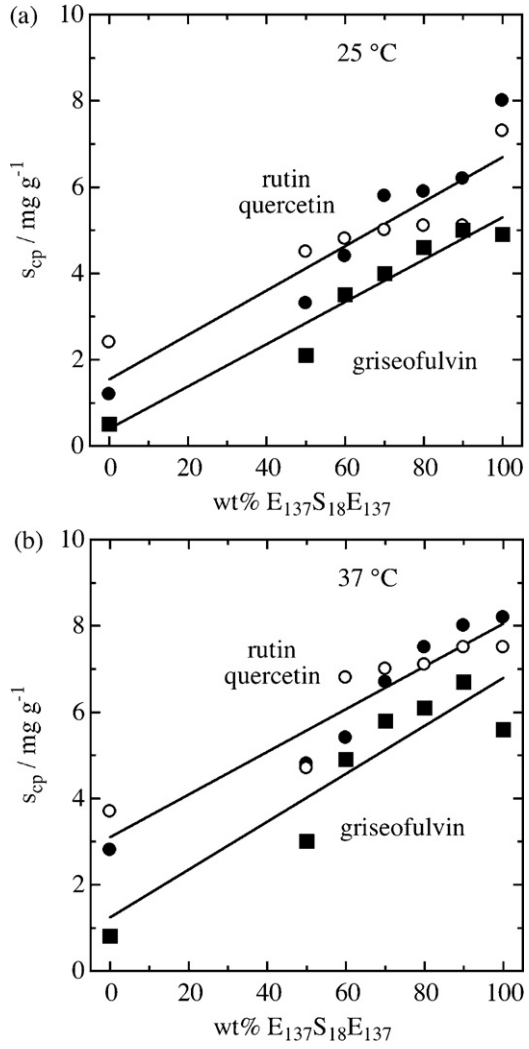


Fig. 2. Dependence of solubilisation capacity (s_{cp}) on wt.% $E_{137}S_{18}E_{137}$ at (a) 25 °C and (b) 37 °C for (○) rutin, (●) quercetin and (■) griseofulvin in 1 wt.% micellar solutions of mixtures of $E_{62}P_{39}E_{62}$ and $E_{137}S_{18}E_{137}$.

The solubilisation capacity of our ESE copolymer system is comparable with that reported previously for β-cyclodextrin (β-CyD). The value of s_{cp} for rutin solubilised in a micellar solution of $E_{137}S_{18}E_{137}$ at 25 °C is 7 mg g^{-1} of copolymer (see Fig. 2a). Calabro et al. (2005) reported 0.05 mM rutin dissolved in 6 mM aqueous β-CyD, which corresponds in our notation to $s_{cp} \approx 5$ mg g^{-1} of β-CyD. The corresponding result for rutin in a micellar solution of an 80/20 wt.% mixture of $E_{137}S_{18}E_{137}$ and $E_{62}P_{39}E_{62}$ is $s_{cp} \approx 5.5$ mg g^{-1} of mixture. We would expect similar results for quercetin and for other poorly soluble hydrophobic drugs.

Finally we note that in this and previous work (Pinho et al., 2007) solubilisation and gelation have been investigated using the same two triblock copolymers, and that the choice of copolymer $E_{137}S_{18}E_{137}$ is far from optimal. For a given value of s_h, the solubilisation capacity measured as s_{cp} will be increased if w_S, the weight fraction of S in the copolymer, is increased, e.g. by replacing the triblock copolymer by a comparable diblock copolymer, and may be greatly increased if the chosen diblock copolymer forms cylindrical micelles (Crothers et al., 2005; Zhou et al., 2008). However, to maintain control over gelation, and to ensure that the gels are coherent with high elastic moduli, it is desirable to work with systems forming cubic structures from packed spherical micelles. Copolymer $E_{137}S_{18}E_{137}$ has $w_S = 0.15$. A copolymer such as $E_{70}S_{18}$ (similar to copolymers described by Crothers et al., 2005) has $w_S \approx 0.4$, and a

value of s_{cp} approaching 20 mg g^{-1} would be expected for a micellar solution of that copolymer alone, with approximately 80% of that value for a micellar solution formed from an 80/20 wt.% mixture of $E_{70}S_{18}$ and $E_{62}P_{39}E_{62}$.

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