Solubilisation of Drugs in Micellar Systems Studied by Eluent Gel Permeation Chromatography

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Purpose. The purpose of the study was to investigate the potential of a chromatographic method which is based on elution gel chromatography (EGPC) in the study of solubilisation of drugs in micellar solutions. ***Methods.*** The EGPC method differs from conventional GPC in the use of a solution of the associating surfactant as eluent (rather than solvent) and the injection of a small volume of solution of different concentration (or alternatively injection of solvent alone) to probe the association equilibrium in the eluent. The technique was applied to a study of the solubilisation of selected drugs in aqueous micellar solutions of a triblock copolymer (Synperonic-PE F127) composed of oxyethylene [E, OCH_2CH_2] and oxypropylene [P, $OCH_2CH(CH_3)$] units with nominal molecular formula $E_{98}P_{67}E_{98}$. ***Results.*** EGPC curves were obtained showing vacancy peaks at the elution volumes of the drugs, clearly demonstrating their solubilisation. In addition, the micelle-molecule equilibrium of the copolymer surfactant could be monitored at all times. Quantitative determination of the partition of solute between micelles and solvent phase was not possible due to the incomplete conversion of molecules to micelles in solutions of the selected copolymer. ***Conclusions.*** The EGPC technique provides evidence for the solubilisation of the drugs in aqueous solutions of Synperonic F127; a more thorough assessment of its potential for quantitative measurement of solubilisation requires the use of a surfactant which is wholly (or at least mainly) in the micellar state under the conditions of use.

KEY WORDS: solubilisation; eluent gel permeation chromatography; micelles; copolymers.

INTRODUCTION

Gel permeation chromatography (GPC) is a technique based on porous, rigid polymer packings which was introduced by Moore (1) in 1964 and applied initially to the separation of synthetic polymers. For systems of biological interest and aqueous solvents, GPC was preceded by gel filtration chromatography (GFC), a technique introduced by Porath and Flodin (2) in 1958, originally based on soft, highly-swollen dextran gels and nowadays associated with the Pharmacia range of gels (Sephadex, Sepharose, etc.). Rigid packings (porous glass) were first used in biochemical applications by Haller (3) in 1966. Advances in technology have brought these techniques together, particularly because of the water-compatible rigid polymer packings now available, one of which (TSK gel-PW) is used in work to be described below.

Ideally the separation mechanism in GPC is size exclusion, and the technique is sometimes called size exclusion chromatography (SEC). However, separation in GPC often includes contributions from partition and adsorption. The eluent-solid interface of a polymeric GPC packing is in reality a concentrated polymer solution, which allows partition of a solute between the stationary surface phase and the mobile phase, with particular effect if the solvent is poor for the solute. Polymer chains and large particles are effectively excluded from the surface layer, but partition of small molecules generally affects their elution. Adsorption occurs when solute and packing interact through specific bonding. The many identical chain units of a polymeric solute allow cooperative binding, which significantly affects their elution. The temperature dependences of the effects differ, size exclusion being essentially an entropic (i.e., athermal) effect and independent of T, while adsorption and partition are thermal effects, either exothermic or endothermic depending on circumstances, and therefore dependent on T.

The application of GPC to a micellar solution is complicated by the micelle-molecule equilibrium. The conditions necessary for successful elution of micelles in conventional GPC were first demonstrated theoretically by Coll (4) and verified experimentally for aqueous solutions of poly(oxyethylene) n-alkyl ethers (5,6). Micelles elute at a faster rate than molecules, so the separation process in GPC with water as eluent inevitably leads to their dissociation. Conditions can be defined under which the micellar peak can be detected (4–6), the important factor being a high concentration of surfactant in the injected solution relative to its critical micelle concentration. GPC carried out under these conditions has been used to determine the average hydrodynamic volumes of micelles of oxyethylene/oxypropylene (7,8) and oxyethylene/oxybutylene (9–11) block copolymers, and the values so obtained have been shown to be consistent with independent measurements of hydrodynamic radius by dynamic light scattering (7,9–11). The recent experiments (7–11), carried out with rigid packings, gave satisfactory evidence that adsorption of micelles was not a problem under the conditions investigated. Unassociated block copolymer molecules under poor solvent conditions did exhibit delayed elution, which was attributed (11) to adsorption on the packing *via* the hydrophobic block (an endothermic effect, giving efficient adsorption at high temperatures).

It has not proved possible under any conditions to quantitatively probe the micelle-molecule equilibrium by conventional GPC with water as the eluent. This was first achieved in 1977 by injection of a very large sample of solution of high concentration into water eluent and analysis of the effect of micellar dissociation on the "tail" of the GPC signal. The method, pioneered by Sasaki *et al.* (12) and Goto *et al.* (13), has been developed by Funasaki and coworkers (14) and applied to a number of non-ionic surfactant systems. More recently a new approach to the GPC of micellar solutions has been reported (15,16). The essentials of this method, referred to as eluent gel permeation chromatography (EGPC), are the use of a solution of the associating surfactant as

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eluent (instead of the solvent alone as previous work) and the injection of a small volume of solution of different concentration (or alternatively injection of solvent alone) to probe the association equilibrium in the eluent. An important feature, which underpins the viability of the method as applied to surfactants, is that the EGPC curve is characteristic of the equilibrium state of the surfactant in the eluent and is insensitive (within limits) to the concentration of surfactant in the injected solution (16). In other words, when using a refractive index detector the response signals (peak areas) attributable to micelles and molecules are in much the same proportion by weight as those which pertain at equilibrium in the eluent. The reason is that the probe is diluted as it passes through the EGPC system, typically from an injection volume of 0.1 cm^3 (i.e. 100 μl) to an emergence volume of 1–2 cm^3, which means that an initial 100% excess concentration over eluent on injection is only a 5–10% excess on emergence.

Another development in GPC relates to the detection and evaluation of preferential absorption of a low-molar-mass substance (solute) by a polymer (17–19). In this procedure, the eluent is a solution of the solute, and the injected solution is polymer dissolved in the eluent. The preferential absorption of the solute by the polymer is assessed from the peak area of either the solvated polymer peak (compared with the area of the unsolvated peak) or the negative solute peak (vacancy peak), or both. Under carefully controlled conditions the method is quantitative (19).

We have combined these two methods to study solubilisation in micellar solutions, and here report an exploration of the potential of this technique in the study of the solubilisation of drugs. The surfactant was a triblock copolymer (Synperonic-PE F127) composed of oxyethylene [E, OCH_2CH_2] and oxypropylene [P, $OCH_2CH(CH_3)$] units with nominal molecular formula $E_{98}P_{67}E_{98}$. Aqueous solutions of the copolymer containing solubilised drug were used as eluent. The probe was a solution of polymer in eluent, i.e. a solution with the same drug concentration but an excess copolymer concentration compared to eluent.

As discussed above, the elution of the low-molar-mass drug solute may be delayed by adsorption, but is more likely to be delayed by partition. The present experiments were not designed to distinguish between these possibilities. In what follows, in an arbitrary though logical way we attribute delayed elution of the copolymer to adsorption and that of the drug to partition.

MATERIALS AND METHODS

Materials

The sample of F127 (Synperonic PE/F127) was kindly donated by ICI Specialities Ltd., Wilton, UK. This sample differed in molecular composition from that used in previous studies in our laboratory (16,20). Analytical gel permeation chromatography using tetrahydrofuran (THF) as a good solvent for both components (details given below) gave a GPC curve with a subsidiary peak (*ca.* 25% of the total signal) at a higher elution volume than that of the predominant narrow peak. Based on a poly(oxyethylene) calibration, the molar masses corresponding to the two peaks were $M_{pk} \approx 15000$ and 6000 g mol^{-1}, which when combined gave fair agreement with the expected number-average molar mass for F127 for $M_n \approx 12000$ g mol^{-1}. As described below, the evidence from EGPC confirmed this distribution, and gave evidence of further diversity of composition in the sample, including a small fraction of copolymer which did not associated even at 67°C, the highest temperature used in this work.

The solutes used in the work were acetaminophen (BDH), sodium phenobarbitone (BDH), diphenhydramine hydrochloride (Sigma), and atenolol (Sigma). Their purity conformed to the requirements of the British Pharmacopoeia (not less than 98%).

The poly(oxyethylene) standards were either from Polymer Laboratories (molar mass > 10000) or were commercial samples of poly(ethylene) glycols with molar masses checked by end group analysis using NMR.

Gel Permeation Chromatography

The EGPC system comprised two columns, each 30 cm long packed with TSKgel-PW (G4000 and G3000), and a differential refractometer detector (Waters Assoc. Model R401). Eluent, prepared by dissolving F127 and solute in distilled water followed by filtration, was pumped at 0.5 cm^3 min^{-1}. The column temperature (range 20–70°C) was controlled by means of an aluminium-block thermostat. Probe solutions were prepared by dissolving F127 in eluent abstracted from the solvent reservoir and heated to the column temperature by placing them on the block prior to injection *via* a 0.1 cm^3 loop. The system was calibrated at appropriate temperatures with poly(oxyethylene) standards. The exclusion limit of the system was *ca.* 10 cm^3, which was well below any elution volumes encountered in the work. The permeation limit, taken to be the elution volume of ethylene glycol, was 20.1 cm^3.

The analytical GPC system used with organic eluent for characterising the sample of F127 comprised four columns, each 30 cm long packed with μ-Styragel (500–10^5 Å nominal pore size), and a differential refractometer detector (Waters Assoc. Model R401). THF eluent at room temperature (*ca.* 20°C) was pumped at 1 cm^3 min^{-1}. Sample solutions (concentration 5 g dm^{-3}) were injected *via* a 0.1 cm^3 loop. The system was calibrated with poly(oxyethylene) standards.

RESULTS AND DISCUSSION

General Observations

With the exception of sodium phenobarbitone, the drug solutes were found to elute beyond the permeation limit of the column set (*ca.* 20 cm^3), with some of them well beyond, e.g. acetaminophen at 54 cm^3: see Table I. Details of these experiments are given below. This delayed elution indicated partition of the solutes into the surface layer of the packing, which might be expected since water is a poor solvent for the drugs. As described below, we also found effects of adsorption in the elution of the block copolymer molecules (though not the micelles) as the eluent temperature was increased above ambient. These effects meant that the elution volumes of copolymer molecules and drug solutes could not be related to their hydrodynamic volumes *via* the poly(oxyethylene) calibration, whereas the elution volumes of the micelles

were consistent with their hydrodynamic volumes (see below). This complication does not in itself invalidate the present method, where the paramount need is for separation (by whatever means) of the elution peaks of copolymer molecules, copolymer micelles, and solutes. However, partition of the drugs does bring a second equilibrium into consideration, since the concentration of drug in the eluent is then determined by its distribution between the surface of the packing, the micelles and the solution phase. In the present experiments the quantitative implications of partition were not explored. Instead, attention was directed towards establishing the potential of the EGPC method for detecting drug solubilisation, with particular emphasis on the role of the micelle-molecule equilibrium in the process.

EGPC of F127

The experiments described in this section were carried out with an eluent containing 0.77 ± 0.01 g dm^{-3} of F127. The temperature dependence of the micelle-molecule equilibrium in this solution was explored in some detail. The probe contained 2.0 g dm^{-3} excess concentration of F127 over that in the eluent. As shown previously (15,16) and discussed above, within limits the exact choice of probe concentration was not important.

Examples of EGPC curves are shown in Figure 1. At the lowest temperature used, 23°C, the EGPC curve mirrored the GPC curve found using the THF system, except for evidence of a small fraction eluting on the low-volume side of the main peak. Taken together, these peaks in the range 13–17 cm^3 were assigned to the sample in its molecular state. As temperature was increased, the molecule peaks broadened and moved to higher elution volumes, the peak at lower elution volume (presumably of higher P content) moving faster than that at higher elution volume so that the two peaks eventually overlapped. A similar effect has been found previously (12,13) for a related sample. As it is known (16) that the elution volumes of poly(oxyethylene) standards in water are essentially independent of temperature, this effect was attributed to an increased contribution of adsorption of the copolymer (*via* the oxypropylene block) onto the surface of the TSK-gel packing as the solvent power of the aqueous eluent decreased with increase in temperature.

At a critical temperature of 33–34°C micelles formed in the eluent, as evidenced by the appearance of a peak at *ca.* 11–12 cm^{-3}. As the temperature was increased above its critical value, the micelle peak increased in area relative to the molecular peak until at the highest temperatures studied (60–67°C) the eluent was almost completely micellar except for a residual fraction which was assigned to a poly(oxyethylene)-rich component of the sample, possibly to be identified with the low-elution-volume tail of the distribution detected in EGPC at low temperatures. The elution volume of the micelle peak decreased towards a limiting value of *ca.* 11.5 cm^3 as temperature was increased to *ca.* 50°C, and increased with increasing temperature thereafter. Making use of the 'universal calibration' as described previously (16), an elution volume of 11.5 cm^3 was found to correspond to a hydrodynamic radius of *ca.* 11 nm, in fair agreement with a value of *ca.* 10 nm found previously (18) by dynamic light scattering for micelles of copolymer F127. The change of r_h with temperature is subtle. At low temperatures the micelles are growing; this is a region which cannot be probed by dynamic light scattering due to the mixture of molecules and micelles (16). When micellisation is complete, the temperature dependence of r_h is small or negative. This is a well-known effect attributable to the balance of micellar growth and the shrinkage of the poly(oxyethylene) fringe (21).

Given the proportionality between mass concentration and refractive index difference, the weight fractions of micelles (w_{mic}) were calculated from

$$w_{mic} = A_{mic}/A_{total}$$

where A_{mic} is the area under the micelle peak, and A_{total} is the area under both peaks. A plot of w_{mic} against temperature, containing results from Figure 1 and other similar curves, is shown in Figure 2. The scatter of results at high temperatures was traced to inadequate temperature control at temperatures higher than 40–50°C. In view of this limitation, the results reported below for F127 + drug systems are restricted to temperatures below 50°C.

The onset of micellisation in the solution is clearly defined in Figure 2 at 33.5 ± 0.5°C. This is the critical micelle temperature (*c.m.T.*) of the solution of our sample of F127 at

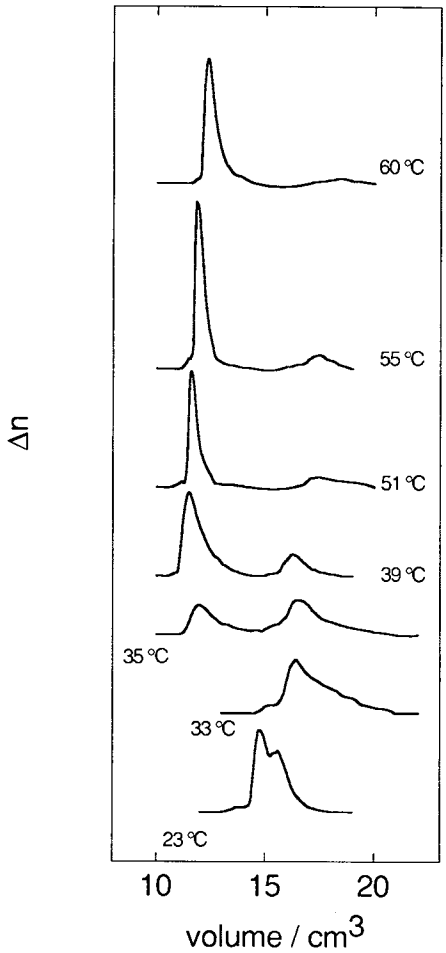

Fig. 1. EGPC curves (refractive index difference, Δn, versus elution volume) of a 0.77 g dm^{-3} aqueous solution of copolymer F127 at various temperatures (as indicated). The probe was a 2 g dm^{-3} excess of copolymer over the eluent.

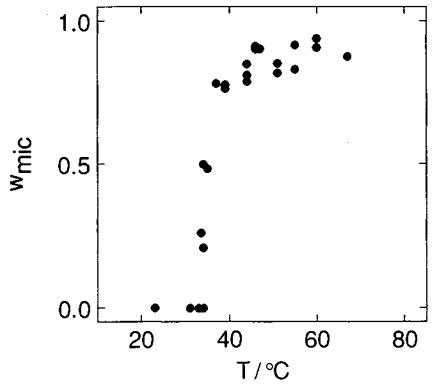

Fig. 2. Weight fraction of micelles in equilibrium with molecules in 0.77 g dm^{-3} aqueous solution of copolymer F127.

concentration c = 0.77 g dm^{-3}. Similar values of the *c.m.T.* have been found previously for other samples of F127 at similar concentrations in water: e.g. by light scattering (20) (35°C, c = 0.77 g dm^{-3} by interpolation); by EGPC (16) (34°C, $c \approx 0.62$ g dm^{-3}), and by dye solubilisation (22) (32°C, $c \approx 0.77$ g dm^{-3}). Exact agreement between the various measurements would not be expected since the molecular composition of F127, as for other commercial $E_mP_nE_m$ copolymers, varies somewhat from batch to batch.

As can be seen in Figure 2, the extent of micellisation of our sample of F127 in aqueous solution rose rapidly to $w_{mic} \approx 0.8$ at 40°C, and to 0.90–0.95 at 50–60°C. Even at the highest temperature used, i.e. 67°C, a small fraction (<5 wt-%) of non-associated copolymer molecules remained.

Effects of Drug Solutes on the Micellisation of F127

Eluent was prepared containing 0.77 ± 0.01 g dm^{-3} of F127 and 7.95 ± 0.01 g dm^{-3} of drug. As for the EGPC of F127 alone, the probe contained 2.0 g dm^{-3} excess concentration of copolymer. The elution volumes of the drugs in the eluent at 25°C are noted in Table I. These values were obtained by injecting a solution with an excess concentration of drug over that in the eluent. The elution volume of sodium phenobarbitone was less than that of ethylene glycol. The other drugs eluted at much larger volumes, giving evidence of retention by partition. The elution volumes of the two electrolytes (sodium phenobarbitone and diphenhydramine hydrochloride) were not entirely reproducible, which was attributable to a variable electrical exclusion effect (double layer effect) in the unbuffered solution of low ionic strength.

Examples of EGPC curves obtained with drugs in the eluents are shown in Figures 3–6. In all cases the behaviour of the block copolymer followed the general pattern illustrated in Figure 1, i.e. micellisation occurred as temperature

Table I. Elution Volumes of Drugs in Eluent at 25°C

Sample	Elution volume/cm^3
Ethylene glycol (marker)	20.1
Acetaminophen	53.4
Sodium phenobarbitone	17.0
Diphenhydramine hydrochloride	28.5
Atenolol	35.0

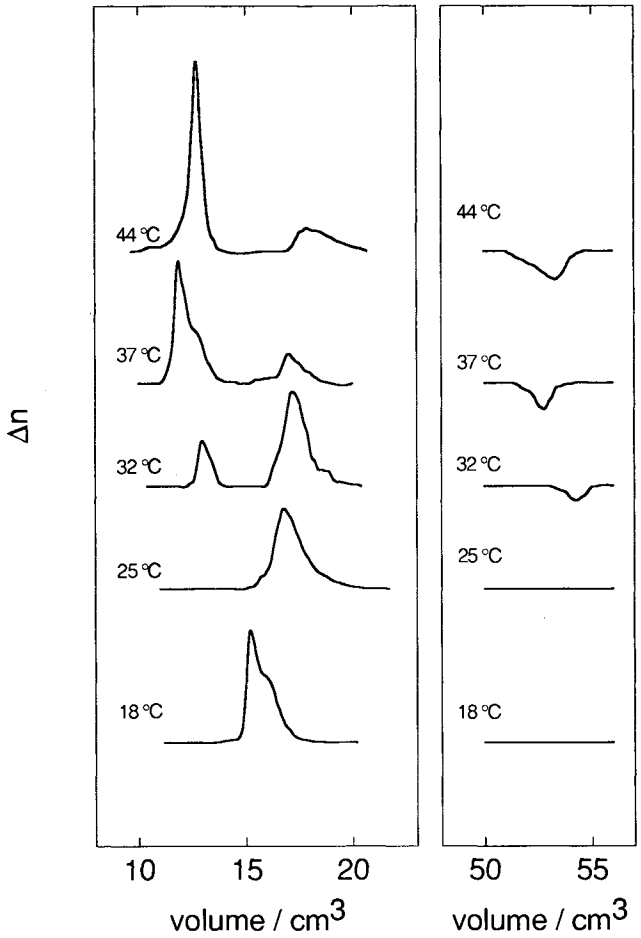

Fig. 3. EGPC curves (refractive index difference, Δn, versus elution volume) of an aqueous solution of 0.77 g dm^{-3} of copolymer F127 plus 7.95 g dm^{-3} acetaminophen. The eluent temperatures are indicated. The probe was a 2 g dm^{-3} excess of copolymer over the eluent.

was increased from ambient. The critical micelle temperatures found for F127 in the presence of the drugs were lower than the value established for F127 alone: plots of the ratio of micelle peak area to total area (see Figure 2 for example), using the illustrated results and similar curves, gave the critical temperatures listed in Table II. The depression of *c.m.T.* by the solute correlates well with the drug elution volume (i.e. the partition effect), both being larger the more hydrophobic the solute. Compared with what happens in water, the effect of lowering the *c.m.T.* is to increase the weight fraction of F127 in micellar form at a given temperature in the temperature range just above the *c.m.T.* For example, in the presence of acetaminophen the weight fraction in micellar form at 30°C is approximately 20% compared to 0% in water.

Apart from this overriding effect on *c.m.T.*, differences between the micelle peaks are apparent on comparing Figures 1 and 3–6. Interpretation of these effects is not attempted here. Presumably they result from the compositional mix of our sample of F127 and variation in absorption/partition of the drugs. In this respect the EGPC method is rich in information, which is very much in contrast with

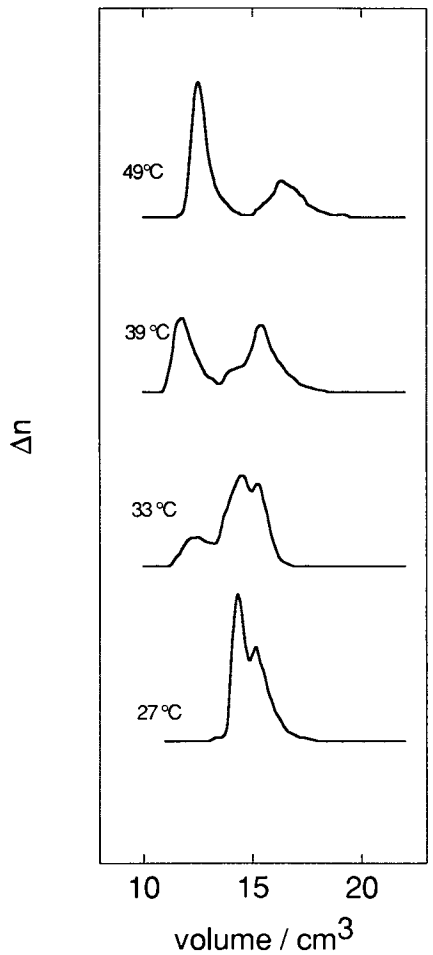

Fig. 4. EGPC curves (refractive index difference, Δn, versus elution volume) of an aqueous solution of 0.77 g dm^{-3} of copolymer F127 plus 7.95 g dm^{-3} sodium phenobarbitone. The eluent temperatures are indicated. The probe was a 2 g dm^{-3} excess of copolymer over the eluent.

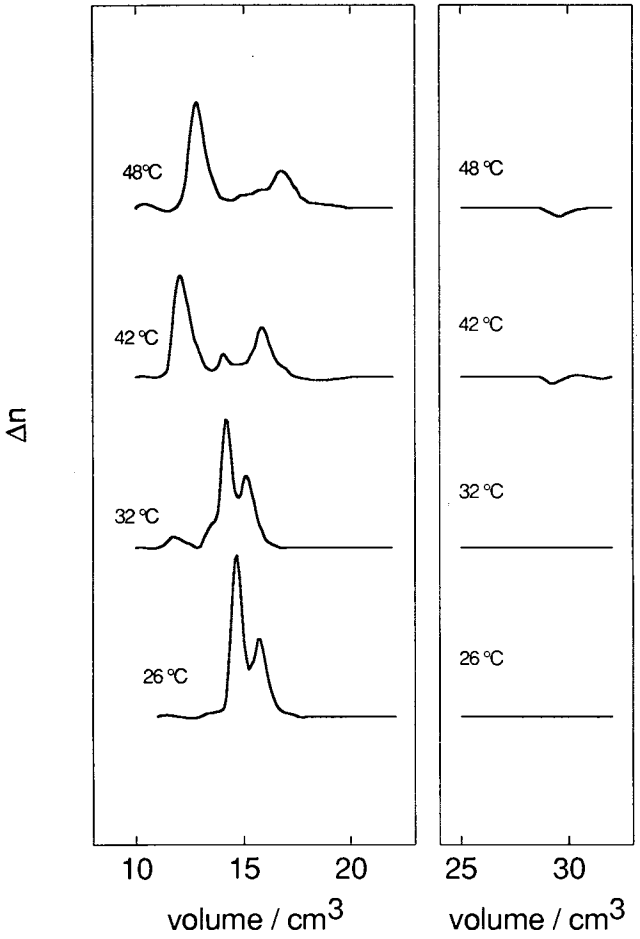

Fig. 5. EGPC curves (refractive index difference, Δn, versus elution volume) of an aqueous solution of 0.77 g dm^{-3} of copolymer F127 plus 7.95 g dm^{-3} diphenylhydramine hydrochloride. The eluent temperatures are indicated. The probe was a 2 g dm^{-3} excess of copolymer over the eluent.

conventional methods of determination of solubilisation wherein micellisation is not monitored.

Solubilisation of Drugs by F127

The EGPC curves shown in Figure 3, 5 and 6 show vacancy peaks at the elution volumes of the drugs, which clearly demonstrate solubilisation (19). The effect was confined to micellar solutions: the molecular solutions of F127 did not solubilise the drugs to any detectable extent. There was no detectable solubilisation of sodium phenobarbitone, which is consistent with its high water solubility.

For the sample of F127 used in this exercise and at any moderate temperature (say 37°C), quantitative measurement of solubilisation was complicated by the incomplete conversion of molecules to micelles. This was the case even when hydrophobic drugs were present in the system. Because of this problem F127 was seen to be a poor choice for quantitative studies, quite apart from the complications introduced by its irregular composition/chain-length distribution. A better choice would be a copolymer with a narrow composition/chain-length distribution and a low *c.m.T.* at low concentration. Assuming that solubilisation of drug solutes is effected mainly in the poly(oxyethylene) fringe of the micelle (23), the requirement is a copolymer with high E content (e.g. > 70 wt-%) and a low *c.m.T.* Considering a copolymer concentration less than 1 g dm^{-3}, the choice among oxyethylene-oxypropylene triblock ($E_mP_nE_m$) copolymers is not encouraging, and the recent survey of *c.m.T.*s of $E_mP_nE_m$ copolymers (22) fails to reveal a likely candidate. Work in our laboratory on oxyethylene-oxybutylene block copolymers allows more optimism, as a number of triblock $E_mB_nE_m$ and (particularly) diblock E_mB_n copolymers have been synthesised and shown to have the required properties. For example, a 0.03 g dm^{-3} solution of block copolymer $E_{53}B_{13}$ has been shown (11) to have a *c.m.T.* of 20°C. Oxyethylene-oxybutylene copolymers have an added appeal over oxyethylene-oxypropylene copolymers in that their preparation by sequential anionic polymerisation is not complicated by the transfer reaction (24,25) enabling the production of samples which give uncomplicated, narrow EGPC curves in both molecular and micellar states.

CONCLUDING REMARKS

The results presented in this report illustrate several

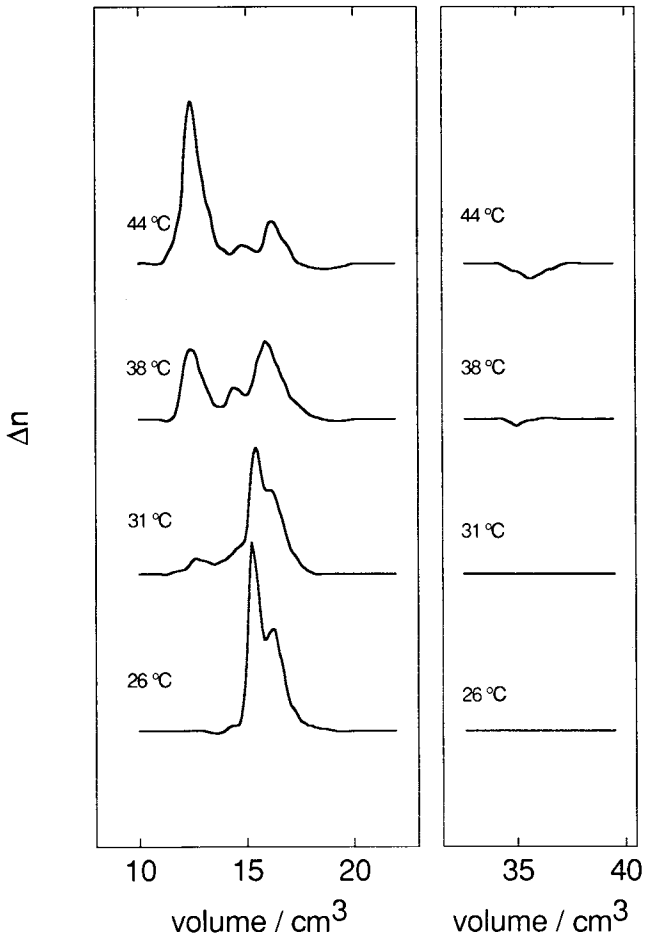

Fig. 6. EGPC curves (refractive index difference, Δn, versus elution volume) of an aqueous solution of 0.77 g dm^{-3} of copolymer F127 plus 7.95 g dm^{-3} atenolol. The eluent temperatures are indicated. The probe was a 2 g dm^{-3} excess of copolymer over the eluent.

features of the EGPC technique which can be used to advantage in studying the solubilisation of drugs. The experiment is straightforward and takes no more than two hours. The state of the copolymer surfactant is monitored at all times, and any effect of interaction with the drug solute is immediately apparent. The locus of solubilisation can be readily determined, as in the present case where it is clear that the copolymer in its molecular state does not solubilise the drug solutes.

The potential of EGPC in quantitative determination of the partition of solute between micelles and solvent phase was not proven in this work. This would require use of a copolymer surfactant which was wholly (or at least mainly) in the micellar state under the conditions of use. In that event two measures of solubilisation would be available, one from the area of the vacancy peak and the other from the increase in area of the micellar peak. The latter should give quantitative results in a straightforward way (19). In this respect, oxyethylene-oxybutylene diblock copolymers promise to be superior to oxyethylene-oxypropylene copolymers. Further insight into the problem of adsorption would require independent measurement of partition between eluent and packing, which may be feasible with a simpler system such as this.

Table II. Critical Micelle Temperatures of F127[a] in Eluent at 25°C[a]

Eluent	c.m.T./°C
Water only	33–34
Acetaminophen	26–27
Sodium phenobarbitone	31–32
Diphenhydramine hydrochloride	30–31
Atenolol	29–30

[a] Critical micelle temperature of 0.77 g dm^{-3} solutions of F127 either in water or in 7.95 g dm^{-3} aqueous drug solution.

REFERENCES

1. J. C. Moore. Gel permeation chromatography. 1. A new method for molecular weight distribution of high polymers. *J. Polym. Sci., Part A,* 2:835–843 (1964).
2. J. Parath and P. Flodin. Gel filtration: A method for desalting and group separation. *Nature (London)* **183**:1657–1659 (1959).
3. W. Haller. Chromatography on glass of controlled pore size. *Nature (London)* **206**:693–696 (1965).
4. H. Coll. Behaviour of micellar solutions in gel permeation chromatography. A theory based on a simple model. *Sep. Sci.* **6**:207–215 (1971).
5. H. H. Teo, M. G. Styring, S. G. Yeates, C. Price, and C. Booth. Size-exclusion chromatography of micellar solutions of poly (oxyethylene) n-alkyl ethers. *J. Colloid Interface Sci.* **114**:416–424 (1986).
6. M. G. Styring, H. H. Teo, C. Price, and C. Booth. Aqueous size-exclusion chromatography of micelles of poly(oxyethylene) methyl-n-alkyl ethers. *J. Chromatogr.* **388**:421–427 (1987).
7. J.-F. Ding, D. Attwood, C. Price, and C. Booth. Use of crown ether in the anionic polymerization of propylene oxide-3. Preparation and micellisation of diblock-copoly (oxypropylene/oxyethylene). *Eur. Polym. J.* **27**:901–905 (1991).
8. M. Malmsten and B. Lindman. Self-assembly in aqueous block copolymer solutions. *Macromolecules* **25**:5440–5445 (1992).
9. W.-B. Sun, J.-F. Ding, R. H. Mobbs, D. Attwood, and C. Booth. Preparation of a diblock copoly(oxybutylene/oxyethylene) and study of its micellisation and surface properties in dilute aqueous solution. *Colloids and Surfaces* **54**:103–111 (1991).
10. C. V. Nicholas, Y.-Z. Luo, N.-J. Deng, D. Attwood, J. H. Collett, C. Price, and C. Booth. Effect of chain length on the micellization and gelation of block copoly(oxyethylene/oxybutylene/oxyethylene) $E_mB_nE_m$. *Polymer* **34**:138–144 (1993).
11. A. D. Bedells, R. M. Arafeh, Z. Yang, D. Attwood, F. Heatley, J. C. Padget, C. Price, and C. Booth. Micellisation of diblock copoly(oxyethylene/oxybutylene) in aqueous solution. *J. Chem. Soc., Faraday Trans.* **89**:1235–1242 (1993).
12. T. Sasaki, M. Yasuoka, and H. Suzuki. Studies of the measurement of the intermicellar concentration of surfactants by gel filtration. *Bull. Chem. Soc. Japan* **50**:2538–2541 (1977).
13. A. Goto, F. Endo, and K. Ito. Gel filtration of solubilized systems. 1. On the gel filtration of aqueous sodium lauryl sulphate solution solubilizing alkyl paraben on Sephadex G-5. *Chem. Pharm. Bull.* **25**:1165–1173 (1977).
14. N. Funasaki, H.-S. Shim, and S. Hada. Micellar growth of octaethylene glycol decyl ether. *J. Chem. Soc., Faraday Trans.* **87**:957–961 (1991).
15. Q.-G. Wang, C. Price, and C. Booth. Eluent gel permeation chromatography: a new method for study of the micellisation of block copolymers in solution. *J. Chem. Soc., Faraday Trans.* **88**:1437–1439 (1992).
16. Q.-G. Wang, C. Price, and C. Booth. Eluent gel permeation chromatography: application to the association of block copolymer F127 in aqueous solution. *Eur. Polym. J.* **29**:665–669 (1993).
17. D. Berek, T. Bleha, and Z. Pevna. Determination of preferential

solvation by gel permeation chromatography. *J. Polym. Sci., Polym. Lett. Edn.* **14**:323–327 (1976).

18. A. Campos, L. Borque, and J. Figueruelo. Preferential solvation of poly(dimethylsiloxane) and poly(methyl methacrylate) in benzene-methanol mixtures by gel permeation chromatography. *J. Chromatogr.* **140**:219–227 (1977).
19. Q.-G. Wang and R.-S. Cheng. Evaluation of preferential solvation by gel permeation chromatography. *Polymer* **33**:3978–3980 (1992).
20. G.-E. Yu, Y.-L. Deng, S. Dalton, Q.-G. Wang, D. Attwood, C. Price, and C. Booth. Micellisation and gelation of triblock copoly(oxyethylene/oxypropylene/oxyethylene) F127 *J. Chem. Soc., Faraday Trans.* **88**:2537–2544 (1992).
21. D. Attwood, J. H. Collett and C. J. Tait. The micellar properties of the poly(oxyethylene)-poly(oxypropylene) copolymer Pluronic F127 in water and electrolyte solution. *Int. J. Pharm.* **26**:25–33 (1985).
22. P. Alexandridis, J. F. Holzwarth, and T. A. Hatton. Micellization of poly(ethylene oxide)-poly(propylene oxide)-poly (ethylene oxide) triblock copolymers in aqueous solutions: thermodynamics of copolymer association. *Macromolecules* **27**:2414–2425 (1994).
23. P. H. Elworthy and M. S. Patel. Demonstration of maximum solubilization in a polyoxyethylene alkyl ether series of nonionic surfactants. *J. Pharm. Pharmacol.* **34**:543–546 (1984).
24. J. F. Ding, F. Heatley, C. Price, and C. Booth. Use of crown ether in the anionic polymerisation of propylene oxide-2. Molecular weight and molecular weight distribution. *Eur. Polym. J.* **27**:895–899 (1991).
25. F. Heatley, G.-E. Yu, W.-B. Sun, E. J. Pywell, R. H. Mobbs, and C. Booth. Triad sequence assignment of the ^{13}C-NMR spectra of copolymers of ethylene oxide and 1,2-butylene oxide. *Eur. Polym. J.* **26**:583–592 (1990).