International Journal of Pharmaceutics, 40 (1987) 93–99
Elsevier

IJP 01346

The behaviour of Pluronic F127 in aqueous solution studied using fluorescent probes

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(Received 20 May 1987)
(Accepted 5 June 1987)

Key words: Pluronic; Poloxamer; Sustained release; Fluorescent probe; Polymeric gel; In vitro diffusion; Viscosity

Summary

Gels formed from solutions of Poloxamer 407 (Pluronic F127 or PF127) are of interest as possible controlled release drug delivery systems. The hydration and microviscosity in the solution and gel form of PF127 have been studied using the fluorescent probe molecules pyrene and 8-anilino-1-naphthalene sulphonic acid (ANS). The results indicate that the microviscosity of the aqueous PF127 decreases with increasing temperature, which confirms earlier studies on drug release from PF127 gels. The addition of polyethylene glycols had little effect on the microscopic diffusion coefficients or polymer hydration, despite producing changes in the gel–solution transition temperature.

Introduction

Pluronic F127 (PF127 or Poloxamer 407) is one of the series of Poloxamer ABA block copolymers produced by the condensation of ethylene oxide and propylene oxide subunits. It contains 70–79% of the polyoxyethylene unit and has a nominal molecular weight of 12,500, and a general formula:

$$H(O\text{-}CH_2\text{-}CH_2)_A\text{-}(O\text{-}CH(CH_3)\text{-}CH_2)_B$$

$$\text{-}(O\text{-}CH_2\text{-}CH_2)_A\text{-}OH$$

Several of the series, most notably PF127, display the phenomenon of reverse thermal gelation in aqueous solution (above 20% w/w), forming a liquid at 5°C that increases in viscosity with increasing temperature until a gel matrix is formed. The gel is thought to be micellar in nature, possibly constructed from a cubic array of micellar subunits (Chen-Chow, 1980). Gelation is thought to occur as a result of polymer dehydration leading to increased chain friction and entanglement (Rassing et al., 1984; Vadnere et al., 1984). PF127 is non-toxic and has a high solubilizing capacity; consequently PF127 gels have been widely studied as potential controlled release drug delivery systems. (Chen-Chow and Frank, 1981; Miller and Donovan, 1982; Miyazaki et al., 1984; Collett et al., 1985; Gilbert et al., 1986).

We have used fluorescent probe techniques to study the hydration and diffusion within aqueous PF127 solutions of intermediate and high concentrations (1–30% polymer), and the changes in environment with temperature. The hydrophobic probe pyrene is capable of providing information on the microviscosity and consequent microscopic

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diffusion coefficient in the hydrophobic regions of the gel, using the classical excimer fluorescence technique (Birks et al., 1963), whereas the polarity-sensitive probe 8-anilino-1-naphthalene sulphonic acid (ANS; Azzi, 1974), which is hydrophilic in character, demonstrates the changes in polarity within the hydrophilic palisade layer. Additionally the effect of adding polyethylene glycol (mol. wt. 10,000; PEG 10,000) to the polymer solutions was studied, PEG having previously been shown to alter the gel–solution transition temperature (Gilbert et al., 1987b).

Materials and Methods

Fully corrected fluorescence spectra were recorded using a Perkin-Elmer model 3000 spectrofluorimeter with temperature-controlled sample-holder. The samples were contained in standard 1 cm fluorescence cells and were presaturated with nitrogen. Excitation and emission slit widths were 5 and 2.5 nm, respectively. Pyrene (Sigma) was dissolved in dimethylformamide (BDH, Analar) and added to the polymer solutions to a final concentration of 0.5 mM. The excitation wavelength was 290 nm, and excimer and monomer emission intensities were measured at 475 and 398 nm, respectively. ANS (Sigma) was dissolved in acetone (M + B Proanalysar) and added to the polymer solution at a final concentration of 4 μM. It was excited at 376 nm (15 nm slits) and emission was measured at 474 nm (2.5 nm slits). The macroscopic gelation temperatures of PF127 solutions were determined by the previously described method of heating the gel in a water bath until it did not flow on inversion (Gilbert et al., 1987b).

PF127 (Batch No. F1T5N40) was kindly donated by Upjohn, Crawley. Polyethylene glycol (mol. wt. 10,000) was purchased from BDH.

Results

Fig. 1 shows the excimer/monomer fluorescence ratio of pyrene (0.5 mM) as a function of temperature in PF127 solutions of 26%, 30% and 34% polymer. In all cases the excimer fluorescence increased as the temperature was increased, with no break or discontinuity being visible at the macroscopic gelation temperature in any case. The macroscopic gelation temperatures of these polymer solutions are shown in Table 1. As predicted, they decreased with increasing polymer concentra-

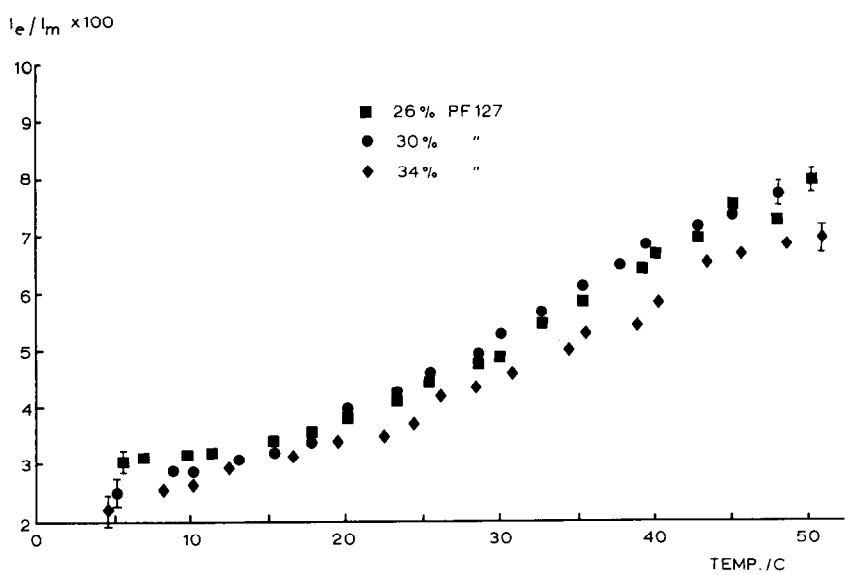

Fig. 1. Pyrene (0.5 mM) excimer/monomer fluorescence emission ratios as a function of temperature in 26–34% solutions of PF127.

TABLE 1

Macroscopic gelation temperatures of PF127 solutions

Concn. PF127 (%)	Gelation temp. (°C)	+1% PEG 10,000
26	43	> 56
28	35	> 56
30	26	39
32	18	23
34	15	15.5

tion from 43°C at 26% PF127, to 15°C at 34% PF127.

No significant difference in the excimer/monomer emission ratio was produced by the addition of PEG 10,000 (1%) to any of the PF127 solutions as typified by Fig. 2 (30% PF127). However, the addition of 1% PEG 10,000 decreased the gelation temperature as shown in Table 1.

The fluorescence intensity of ANS (4 μM) in PF127 (30%) as a function of temperature is shown in Fig. 3. The intensity decreased as the temperature was increased from 4 to 48°C. Again no discontinuity was visible in the curve. The addition of 1% PEG 10,000 had no significant effect on the fluorescence intensity.

The behaviour of the fluorescent probes in more dilute polymer solutions was considerably different. Pyrene (0.5 mM) in PF127 (10%) displayed a high excimer/monomer emission ratio at 5°C, which rapidly fell to a minimum at 15°C as the temperature was increased (Fig. 4). Further increases in temperature caused only a small increase in the excimer/monomer emission ratio. Similar behaviour was displayed in a 5% PF127 solution, but with the fluorescence minimum shifted to 23°C (Fig. 5).

The fluorescence intensity of ANS (4 μM) in more dilute PF127 solutions also displayed unusual behaviour (Fig. 6). Fluorescence in a 5% PF127 solution was initially weak, but increased rapidly to a peak at 22°C, and decreased with increasing temperature thereafter in a similar fashion to the more concentrated solutions. Similar behaviour was displayed with 10% PF127 solutions, the peak being shifted to 16°C at the higher polymer concentration. Above 20% PF127 the peak in intensity could not be seen, probably since it occurred at too low a temperature for fluorescence measurements to be obtained without cell misting. The addition of 1% PEG 10,000 made no significant difference to the fluorescence intensities at any temperature (Fig. 7).

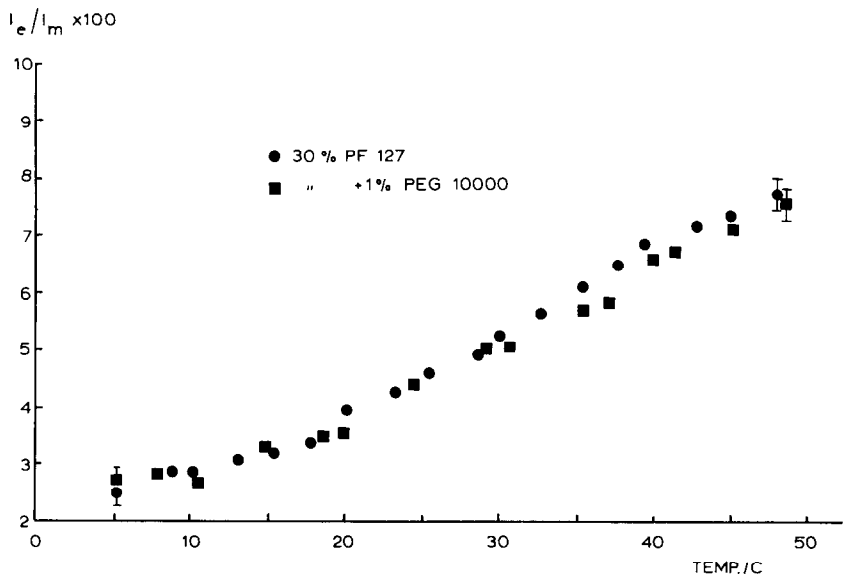

Fig. 2. The effect of PEG 10,000 (1%) on pyrene (0.5 mM) excimer/monomer fluorescence in 30% PF127 as a function of temperature.

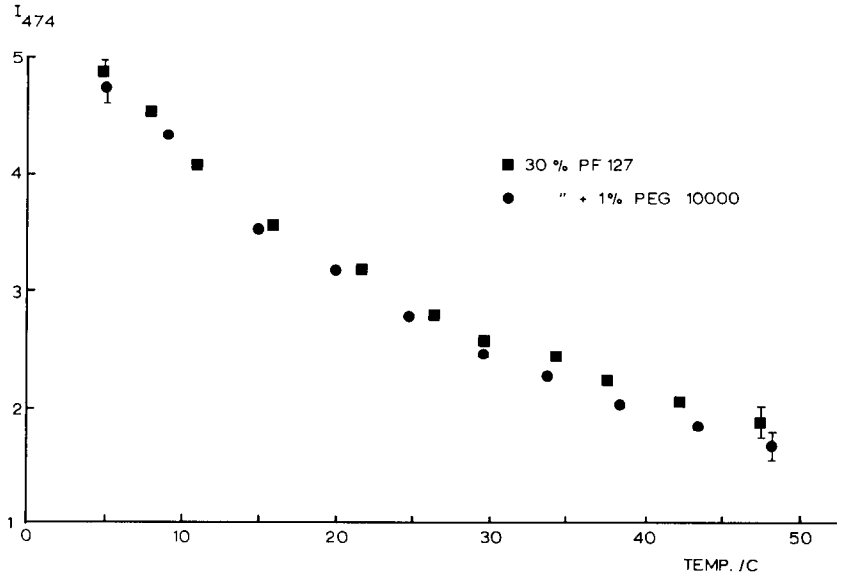

Fig. 3. The fluorescence intensity of ANS (4 μM) in 30% PF127 solutions with and without 1% PEG 10,000 as a function of temperature.

Discussion

Pyrene excimer fluorescence

The pyrene excimer technique is described in detail in most standard photochemistry texts (see e.g. Birks et al., 1963). Briefly, excimer formation is a second-order process whose rate is dependent on competition between excited monomer decay and collision with a ground-state species to form the excimer. At a given pyrene concentration, the collision rate is dependent on the diffusion coefficient of the pyrene excited monomer; hence excimer formation is favoured in systems of high diffusion coefficient. High excimer emission im-

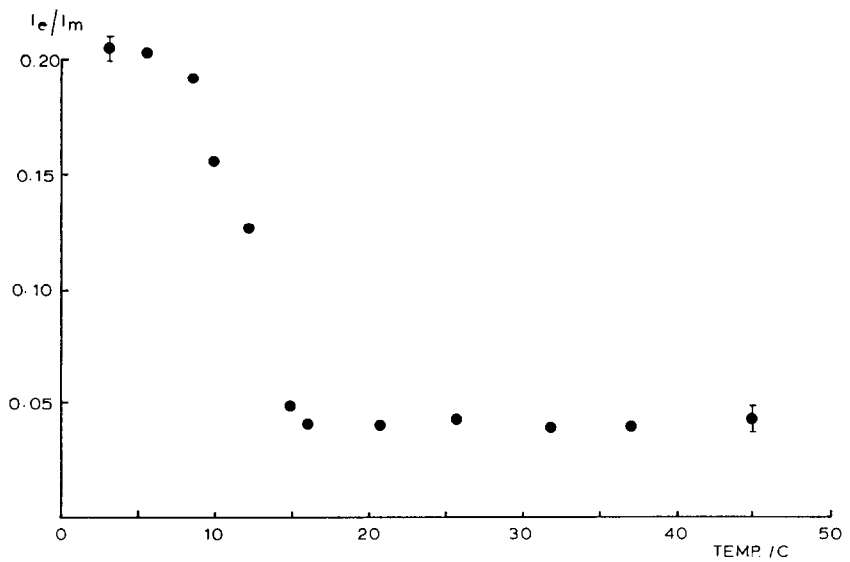

Fig. 4. Pyrene (0.5 mM) excimer/monomer fluorescence emission ratios as a function of temperature in a 10% solution of PF127.

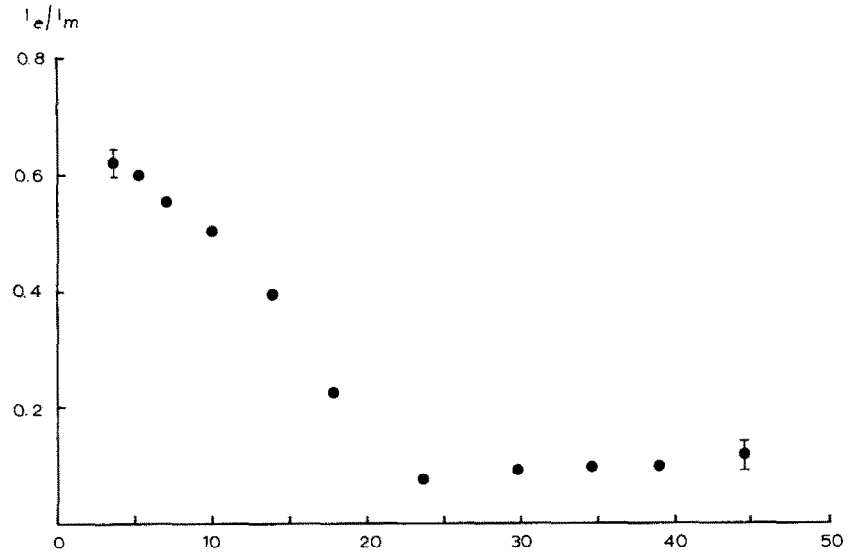

Fig. 5. Pyrene (0.5 mM) excimer/monomer fluorescence emission ratios as a function of temperature in a 5% solution of PF127.

plies a large diffusion coefficient and low microviscosity.

In solutions of PF127 of concentration 26–34%, pyrene excimer fluorescence increased monotonically with increasing temperature. Consequently the diffusion coefficient of pyrene (which would be expected to be localised in the hydrophobic polypropylene chain region of the polymer) increased with increasing temperature. This is in contrast to the increase in macroscopic viscosity with temperature (Gilbert, 1987c), and confirms that the gelation process is primarily due to chain entanglement processes as suggested by previous workers (Rassing et al., 1984). Chain entangle-

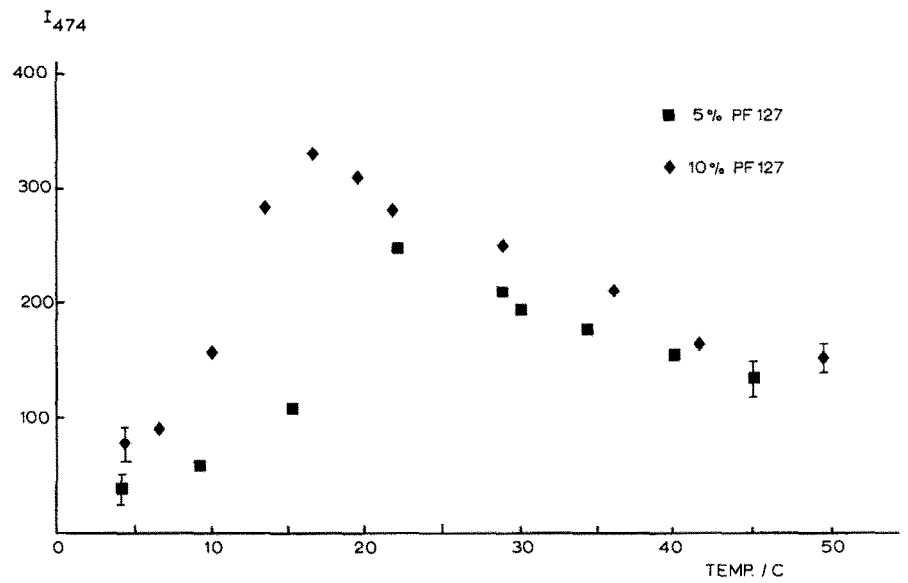

Fig. 6. The fluorescence intensity of ANS (4 μM) in 5% and 10% solutions of PF127 as a function of temperature.

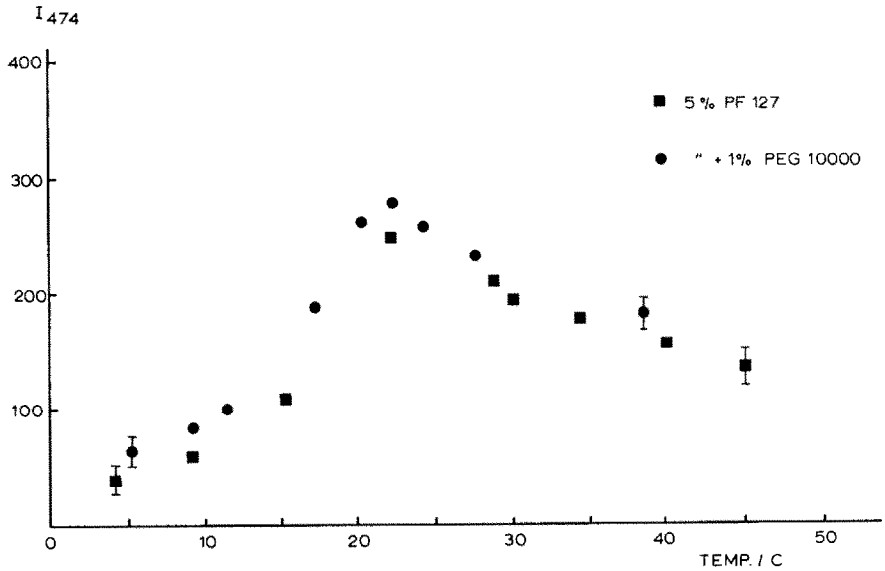

Fig. 7. The fluorescence intensity of ANS (4 μM) in 5% solutions of PF127, with and without 1% added PEG 10,000, as a function of temperature.

ment is essentially a long-range process and does not appear to impede the diffusion of smaller molecules, such as pyrene, within the gel network. The release of model drugs (hydroxybenzoate esters) from PF127 gels in vitro also increased with increasing temperature (Gilbert et al., 1986) confirming the increase in diffusion coefficient within the gel with increasing temperature, despite the increase in macroscopic viscosity.

The very high pyrene excimer fluorescence observed in PF127 gels of 5% and 10% concentration at low temperatures (< 20 °C) suggests that the microviscosity is low until a critical temperature is reached, when a sudden increase occurs. The reasons for this are uncertain, but it cannot be due to entanglement processes since the results at higher concentrations indicate that this process only influences the macroviscosity.

ANS fluorescence and hydration

The fluorescence quantum yield of ANS is sensitive to the polarity of its environment, changing from 0.004 in water to 0.98 in hexane (Azzi, 1974). In a 30% solution of PF127 the fluorescence intensity falls by a factor of 2 over the temperature range 4–48 °C. ANS is a polar species which would be localised in the hydrophilic polyoxyethylene region of the polymer, and the decrease in fluorescence intensity indicates that the polarity of its microenvironment is increasing with temperature. This correlates with the suggestion of Rassing et al. (1984) that the polymer dehydrates as the temperature is increased, so that more unbound water is available in the hydrophilic regions of the gel.

ANS dissolved in lower concentrations of PF127 (5–10%) shows a maximum in fluorescence intensity with increasing temperature, which implies a minimum in environment polarity. The intensity maximum occurs at the same temperature as the decrease in pyrene excimer fluorescence, confirming that the changes in fluorescent probe behaviour are indicative of changes in the polymer environment, and are not simply characteristics of a single fluorescent probe. The reasons for the changes in fluorescence at these temperatures in the more dilute PF127 solutions are unclear, but Rassing et al. (1984) have observed changes in the ^{13}C-NMR spectra at similar temperatures, which they attribute to changes in the conformation of the methyl group side chains of the polypropylene oxide chain.

In all cases the addition of 1% PEG 10,000 produced no significant changes in the fluorescent behaviour of either probe, indicating that PEG has no significant effect on probe microenvironment at the concentrations studied. PEG 10,000 has previously been shown to produce marked changes in the gel–solution transition temperature of PF127 aqueous solutions (Gilbert et al., 1987b). These findings suggest that although PEG causes changes in the macroviscosity of the PF127 solutions it has little effect on microscopic diffusion coefficients within the gel structure. This is in agreement with recent work indicating that the incorporation of PEG 10,000 into aqueous PF127 gels does not significantly affect the in vitro release of a range of model drugs (Gilbert et al., 1987a).

Acknowledgements

The authors would like to thank SERC and Upjohn Ltd. for financial support, and for the provision of a CASE award for J.C.G.

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