ELSEVIER
International Journal of Pharmaceutics 112 (1994) 79–85
international journal of pharmaceutics

The controlled crystallisation of a model powder: 2. Investigation into the mechanism of action of poloxamers in changing crystal properties

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Received 28 March 1994; accepted 27 April 1994

Abstract

A model drug (ethyl *p*-hydroxybenzoate) has been crystallised out of a propan-2-ol/water cosolvent by the addition of water. The effect of a range of concentrations of different poly(oxyethylene)-poly(oxypropylene)-poly(oxyethylene) block copolymers on the size and habit of the crystals has been investigated. The surfactants resulted in decreased particle size and a change to a prismatic habit. The decrease in particle size seems to correlate with the molecular weight of the polyoxyethylene chain, if the molecular weight of the polyoxypropylene is kept constant. These effects were concentration dependent, occurring after a certain threshold concentration of surfactant had been added. The effect was also dependent upon the nature of the surfactant. The greater the molecular weight the greater the effect; this was especially true for increases in molecular weight of the polyoxyethylene. These and other data are used to propose that the polyoxyethylene is adsorbed to the surface of hydrophilic faces of the crystal in order to exert this effect on the crystal properties. It has been shown that the surfactant does not exert this effect by changes in solubility or supersaturation profiles.

Keywords: Poloxamer; Pluronic; Crystallization; Habit; Size; Ethyl *p*-hydroxybenzoate

1. Introduction

In the preceding paper (Mackellar et al., 1994), it has been shown that the physicochemical properties of ethyl *p*-hydroxybenzoate (EHB) could be manipulated by a variety of means. It was shown that the rate and extent of supersaturation development and the stirring rate within the crystalliser, as well as the presence of poloxamer 188 added during crystallisation, could affect the size and shape of the crystals produced. It was hypothesised that the poloxamer adsorbed to specific crystal faces and in this way reduced crystal growth rates and changed the crystal habit. The formed crystals were characterised by differential scanning calorimetry (DSC), solution calorimetry and contact angle analysis, with the body of data indicating that the surfactant adsorbed to the hydrophilic regions of the crystal by a reversible adsorption process.

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SSDI 0378-5173(94)00164-Z

Poloxamers are *a-b-a* block copolymers of poly(oxyethylene)-poly(oxypropylene)-poly(oxyethylene). The proportions of polyoxyethylene and polyoxypropylene can be altered in order to change both the total molecular weight, and the relative hydrophilicity of the surfactant. The nomenclature is such that the three figure number defines the structure, as the first two numbers multiplied by 100 approximate to the molecular weight of the hydrophobe, whilst the third number multiplied by 10 gives an estimate of the % content of polyoxyethylene.

In this paper the crystallisation process is investigated in the presence of other poloxamers, to investigate the way in which these poloxamers adsorb to crystal faces. The previous study utilised poloxamer 188, while in this work 184 and 182 are also considered. These poloxamers differ from each other in terms of their molecular weight and hydrophilicity. In order to determine which of these factors dominates, a further series in which the poloxamer molecular weight increased but in which the percentage hydrophilicity remained constant is also investigated (188, 238 and 338).

The effects of poloxamers on solution viscosity and on solute solubility (and therefore supersaturation) were also studied. These studies were undertaken to investigate whether any mechanism, other than that due to poloxamer adsorption, could be responsible for the effect seen by poloxamer 188 (in the preceding paper). Supersaturation is the driving force for crystallisation and as such it controls many crystallisation processes including crystal growth, homogeneous and heterogeneous nucleation (Mullin, 1972). Generally, it is well accepted that surfactants can increase solubility and poloxamers have been shown to increase solute solubility (Collett and Tobin, 1979). Obviously, if the poloxamers used in this work affected the solubility of EHB (and therefore the supersaturation operating during crystallisation) to a different extent then the changes seen in crystal product could be ascribed to this effect.

A study by El-Bary et al. (1990) into the effect of polysorbate 80 and polyvinylpyrrolidone on the crystallisation of chlorpropamide suggested that the presence of surfactant during crystallisation could increase solution viscosity and so reduce crystal growth rates. If, therefore, the poloxamers affect solution viscosity to significantly different extents this could account for the differences seen in crystal form in the presence of poloxamers.

2. Experimental

2.1. Crystallisation procedure

The crystallisation procedure as described in detail by Mackellar et al. (1994) was followed here with the poloxamer solution (in the concentration range from 0.027 to 0.230% w/w) being added to effect crystallisation. In essence, this involved preparing a saturated solution of the solid in a mixture of propan-2-ol and water, and then causing precipitation by adding water (in this case water with added surfactant). The entire apparatus was maintained at 30°C. Stirring was maintained by use of a magnetic stirrer at 900 rpm, and the precipitating fluid was added rapidly from a pipette. The crystals were collected by filtration, and dried.

Throughout this paper, the concentration of the poloxamer solution used to force precipitation will be used to define the sample. This is neither the concentration that was present in the crystalliser, nor the concentration of poloxamer in the (or associated with) the crystal. However, the concentration in the crystalliser and the amount of surfactant that is available to be associated with the crystal will vary in proportion to the concentration that was present in the solution that was used to induce crystallisation.

2.2. Size and shape analysis

The size of the crystals was measured using a laser diffraction apparatus (Malvern 2600) with a dispersion of the crystals into a saturated solution in water, containing a small amount of surfactant dispersant.

The shape of the crystals were defined by use of an image analysis system. A video camera was mounted on top of a microscope, and the image transferred to an IBM XT computer. After the creation of a binary image, particles were selected individually and analysed. Shape factors were produced for each individual particle from moments of inertia. The crystals were defined in terms of elongation and aspect ratio, where elongation is the difference between the maximum and minimum moments of inertia, divided by the sum of the moments. Aspect ratio is the ratio of the square root of the maximum and minimum moments of inertia. At least 200 particles were studied for each crystallisation batch.

2.3. *Viscosity*

Dynamic viscosities were determined at 30°C using method I from the British Pharmacopoeia (1988). The time taken for 5 ml of poloxamer solution to pass through a type B capillary was measured in triplicate and using the equation below a value of viscosity (in Pa s) was calculated:

$$\eta = Kt\rho \qquad (1)$$

where η is the dynamic viscosity, ρ denotes the solution density and t is the time for the solution to pass through the capillary.

2.4. *Effect of poloxamer on EHB supersaturation*

The relative supersaturation (σ) was calculated using the formula shown below:

$$\sigma = (c - c_{eq})/c_{eq} \qquad (2)$$

where c is the concentration of EHB immediately prior to crystallisation and c_{eq} represents the solubility of EHB in the propan-2-ol/water/poloxamer system. It was not possible, however, to make a direct measurement of the solubility of EHB in a propan-2-ol/water/poloxamer system because when an excess of EHB was stirred with a propan-2-ol/water/surfactant system a thick, milky-white solution formed which was difficult to filter. Estimations of the values of EHB solubility in poloxamer solutions were therefore made from the yields retrieved during crystallisation. This seems to be an appropriate approach if the case of the control type crystallisation is considered. The solubility of EHB in a propan-2-ol/water solvent mixture at a propan-2-ol mole fraction of 0.04 (the mole fraction at which crystallisation takes place) is 0.427% w/w. From this figure a theoretical yield of 338.4 mg should be recovered. The average yield of a control type crystallisation is 316.1 mg. It seems reasonable to assume that this discrepancy (6.6% of the theoretical yield) arises as a result of losses during filtering and collecting. Using a figure of 6.6% for losses, the theoretical yield can be calculated and therefore the supersaturation under which crystallisation took place can be estimated.

3. Results

3.1. *The effect of poloxamer 182, 184 and 188 on the size and shape of the crystals*

The sizes of the particles produced by adding various concentration of the poloxamers are presented in Tables 1–3. By comparison with the sizes obtained when no surfactant was present (control crystallisation), it can be seen that the surfactant decreases the size of the crystals formed. For each surfactant there seems to be a concentration threshold below which no effect is seen on particle size. For poloxamer 182, a high concentration is needed before substantial size reduction is observed (approx. 0.230%, Table 1), for 184 the threshold is at 0.09% (Table 2) and for 188 it is at a concentration lower than any investigated (i.e., below 0.046%, Table 3). There is an obvious structure-activity relationship be-

Table 1
Size analysis of crystals produced using aqueous solutions of poloxamer 182, of different concentration, to produce precipitation (all sizes in μm, means ± SD)

[Poloxamer] (% w/w)	50% undersize	90% undersize	10% undersize
0.230	41.0 ± 1.6	74.7 ± 1.3	16.5 ± 1.2
0.150	65.2 ± 3.6	125.4 ± 7.6	24.3 ± 1.6
0.130	59.1 ± 4.3	112.4 ± 3.7	20.1 ± 0.4
0.090	60.5 ± 7.0	115.0 ± 4.6	19.6 ± 0.7
0.061	58.1 ± 8.0	108.6 ± 10.2	22.2 ± 1.2
0.046	64.1 ± 7.8	116.6 ± 7.7	23.3 ± 1.5
0.0 (control)	66.2 ± 4.2	119.2 ± 8.8	24.6 ± 1.6

Table 2
Size analysis of crystals produced using aqueous solutions of poloxamer 184, of different concentration, to produce precipitation (all sizes in μm, means ± SD)

[Poloxamer] (% w/w)	50% undersize	90% undersize	10% undersize
0.230	34.1 ± 0.7	62.5 ± 2.0	14.7 ± 1.3
0.150	47.9 ± 1.4	86.2 ± 3.2	19.6 ± 1.1
0.130	45.7 ± 2.8	92.8 ± 3.4	16.7 ± 0.9
0.090	53.2 ± 1.8	107.3 ± 6.8	18.4 ± 0.6
0.061	56.0 ± 3.2	105.5 ± 6.1	20.3 ± 1.4
0.046	56.6 ± 4.1	101.8 ± 4.3	20.9 ± 0.5
0.0 (control)	66.2 ± 4.2	119.2 ± 4.2	24.6 ± 1.6

tween the surfactants and the effect on size, with a reduction in size being more pronounced with increasing hydrophilicity of the surfactant.

It was postulated before (Mackellar et al., 1994) that the effect of poloxamer 188 on the crystals was due to adsorption to the hydrophilic faces of the growing crystal. The data presented here can be argued to support that case, with increase in effect being observed with the increased length of the hydrophilic chain. The 182 surfactant is an extreme case, as the polyoxyethylene is a very small part of the molecule, and will have a minimal effect. (Any further reduction (i.e., 181) would result in a poloxamer which would certainly not dissolve in water, due to its lack of hydrophilicity.) It is appropriate when considering possible structure activity relationships to represent the surfactant concentrations in terms of molar, rather than %, concentration. It must be stressed that these concentrations relate to the concentration of surfactant in the solution used to induce precipitation, and as such are only proportional to the amounts available for association with the crystals. The 50% undersize data have been plotted as a function of the molar concentrations of surfactant in Fig. 1. The spread in the experimental data in Fig. 1 is large in the region of concentration overlap, but the relative effect of poloxamer 182 (minimal), 184 and 188 (maximal) is very clear. As a guide, at 3×10^{-3} molality, median particle sizes are approx. 60 μm for 182, 50 μm for 184, and about 30 μm for 188. Whilst it is accepted that there is some scatter in the data, this response approximates to a 5 μm reduction in median size for each 10% increase in polyoxyethylene content of the molecule.

Table 3
Size analysis of crystals produced using aqueous solutions of poloxamer 188, of different concentration, to produce precipitation (all sizes in μm, means ± SD)

[Poloxamer] (% w/w)	50% undersize	90% undersize	10% undersize
0.230	32.2 ± 3.0	52.5 ± 3.8	14.6 ± 2.8
0.150	40.3 ± 2.6	71.7 ± 2.3	17.2 ± 0.9
0.130	38.7 ± 1.7	74.3 ± 4.8	17.3 ± 0.4
0.090	38.4 ± 1.3	74.8 ± 3.3	15.7 ± 1.3
0.061	41.7 ± 2.7	73.8 ± 1.8	19.4 ± 3.0
0.046	53.7 ± 1.9	98.8 ± 4.0	23.0 ± 1.7
0.0 (control)	66.2 ± 4.2	119.2 ± 8.8	24.6 ± 1.6

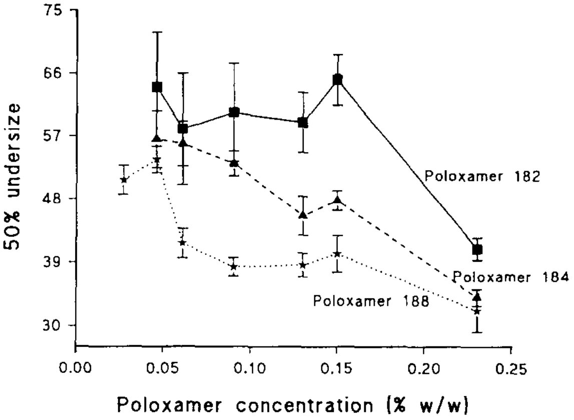

Fig. 1. 50% undersize values for the crystals prepared in the presence of a range of concentrations of the 18 series of poloxamers: (■) 182; (▲) 184; (*) 188.

The concept of threshold concentrations for additives to have an effect on crystallisation was also noted by Fairbrother and Grant (1978, 1979) in their well-cited work on modifications of adipic acid.

As well as having an effect on size, the poloxamers can also be seen to alter the crystal habit of the EHB. This was noted for poloxamer 188 in our previous publication (Mackeller et al., 1994), in which photomicrographs can be seen which show the different habits clearly. A comparison of the effect can be seen by comparing Fig. 2–4, which show the relative proportions of plate and prism habits for the concentration ranges of poloxamer 182, 184 and 188, respectively. The

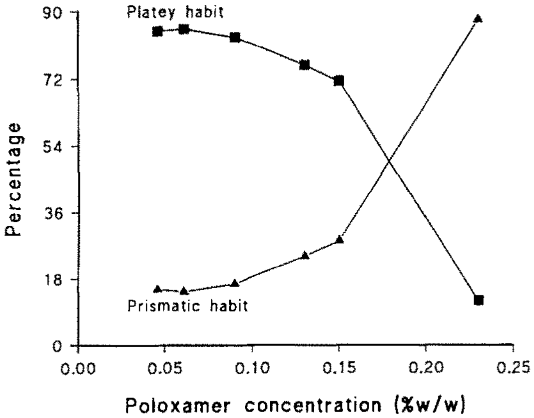

Fig. 2. Proportions of plate and prism habits exhibited after crystallisation was induced with a solution of poloxamer 182, with concentrations as shown.

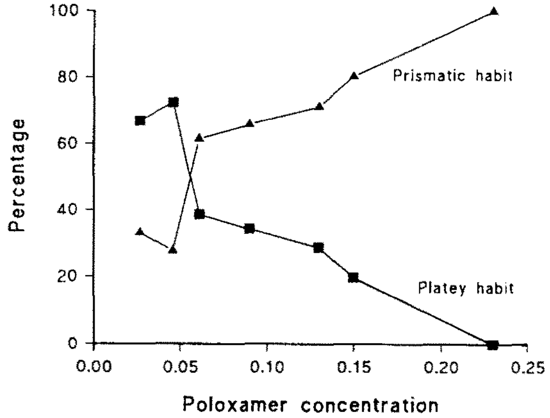

Fig. 4. Proportions of plate and prism habits exhibited after crystallisation was induced with a solution of poloxamer 188, with concentrations as shown.

point of cross-over between predominantly prismatic, and predominantly platey habit is seen to be in the same rank order as the effect on particle size, i.e., 182 requiring to be present in the highest concentration, followed by 184 then 188.

Having observed a structure-activity response for the poloxamer series with increasing polyoxypropylene content, but constant polyoxyethylene, it is also worth considering the case in which the polyoxyethylene content is varied, but the polyoxyethylene remains at a set proportion (80%). The particle size data obtained after crystallisation from 238 and 338 are not presented, as on a % w/w basis they were identical to the data obtained for 188, which has already been discussed (Table 3). However, whilst it may seem that these poloxamers have no effect, it must be remembered that they are of significantly different molecular weights and so it is more sensible to discuss concentration in terms of molality. When this conversion is carried out because of the lower molecular concentration of poloxamers 238 and 338 a lower threshold concentration is seen for these two poloxamers. This means that on a molar plot, they will reduce particle size, and produce more prism-like crystals at a lower concentration than 188. It would seem therefore that the molecular property which allows poloxamers to adsorb to crystal faces is their molecular size and that this would explain the structure/activity relationship seen for poloxamers 188, 184 and 182. Fig. 5 shows that a reasonable structure-activity relationship can be plotted based on this hypothesis. However, when the threshold molality concentrations of all five poloxamers are plotted against molecular weight, as in Fig. 5, it is clear that the difference in activity between poloxamers 188, 238 and 338 (the three highest molecular weight points) is much less than the difference between 188, 184 and 182. It remains inconclusive as to whether this is a true exponential relationship for all the poloxamers, or whether

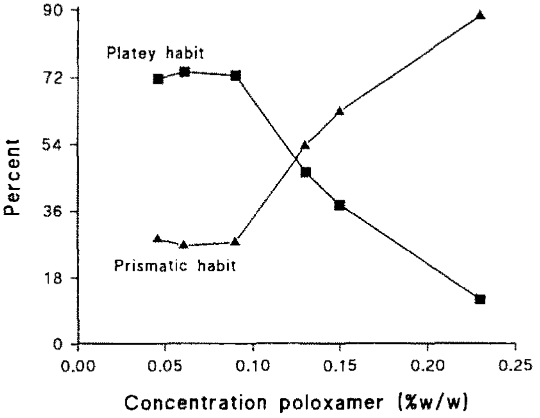

Fig. 3. Proportions of plate and prism habits exhibited after crystallisation was induced with a solution of poloxamer 184, with concentrations as shown.

the more important property is the extent of hydrophilicity.

In our previous study (Mackellar et al., 1994), the surface characteristics of crystals produced in the presence of poloxamer 188 were shown to change (as demonstrated by a lower contact angle with water). It was further suggested that this change was seen because the newly developed crystal faces (conferring an acicular habit) were of a more polar nature and that these faces developed because of the adsorption of poloxamers onto them. This present study indicates that poloxamers that have long hydrophilic chains will alter crystal properties most easily which suggests that poloxamers bind to polar faces of crystals via their hydrophilic chains. This model would explain the structure activity relationship indicated for the 18 series.

It was also shown (Mackellar et al., 1994) that the structure of the surfactants in propanol-water was significantly different from that in water (in that the hydrophobe did not undergo a desolvation with increased temperature in the mixed solvent system, whereas it did in water). It can be concluded that the surfactant adsorption is of this type in this system due to the more favourable interaction between the hydrophobe and the solvent (than exists in water).

Further work would need to be undertaken using other poloxamers if the mechanism, and basis of the structure-activity relationship is to be finally proved.

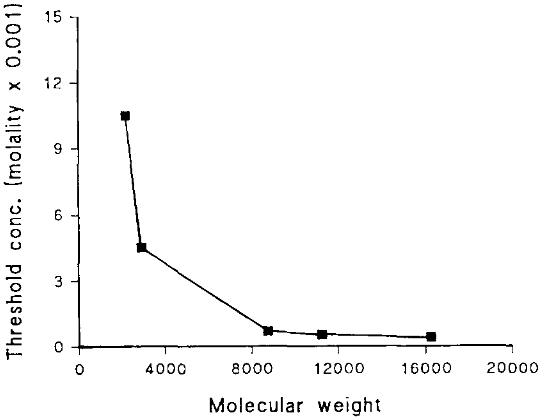

Fig. 5. The relationship between apparent threshold concentration for the onset of action of the surfactants, and the total surfactant molecular weight.

Table 4
Viscosity of various aqueous poloxamer solutions

Solution	Viscosity (mN s m^{-1})
Poloxamer 188 - 0.23% w/w	1.558
Poloxamer 188 - 0.15% w/w	1.537
Poloxamer 188 - 0.09% w/w	1.520
Poloxamer 182 - 0.23% w/w	1.514
Poloxamer 182 - 0.15% w/w	1.511
Poloxamer 182 - 0.09% w/w	1.510

3.2. Viscosity

For completeness, it is necessary to consider other physical properties, and the possibility that they have influenced the crystallisation process, rather than it being due to the surfactant properties of the additives. The surfactant polymers can be expected to affect the solution viscosity, and that in turn can affect both nucleation, and the access of solute molecules to the growing crystal surface.

The viscosities of solutions of poloxamers 188 and 182 at concentrations of 0.23, 0.15 and 0.09% w/w were calculated. These results are listed in Table 4. From these results it can be seen that poloxamer 188 has at all concentrations a greater viscosity than poloxamer 182 suggesting that the effect of poloxamer on viscosity could indeed explain the difference in effectiveness of the two different poloxamers. However, the results for the three concentrations of poloxamer 182 are virtually the same. This would suggest that these poloxamer solutions would have an equal effect on crystal size. However, it was seen that at the highest strength of poloxamer 182 an effect seen on crystal properties that was not apparent at lower concentrations. If viscosity were to be the critical factor, therefore, the viscosity of a 0.23% w/w solution would have to be significantly greater than the viscosities of the other concentrations. Since this is not the case it seems that viscosity is not vital in determining the effect of poloxamer on crystal properties. Furthermore, the effect of viscosity is seen via an effect on the

Table 5
Estimated solubilities (c_{eq}) and relative supersaturations

Concentration (% w/w)	188		184		182	
	c_{eq}	σ	c_{eq}	σ	c_{eq}	σ
0.230	0.52	0.79	0.66	0.39	0.73	0.20
0.090	0.46	1.02	0.49	0.88	0.52	0.76
0.046	0.45	1.02	0.46	0.99	0.48	0.90

diffusion of solute molecules to the growing crystal face (Reynolds, 1963). In this crystallisation procedure stirring is vigorous thereby facilitating molecular diffusion and so it would be unlikely that the diffusion of solute molecules would be susceptible to changes in viscosity.

3.3. Supersaturation

Using the method described above the solubility and therefore relative supersaturation of EHB in three different concentrations of poloxamer 188, 184 and 182 were calculated. Maximum relative supersaturation under which a control type crystallisation is carried out is 1.16. These results are shown in Table 5, and as can be seen all three poloxamers increase the solubility of EHB and in effect, cause the crystallisation to be carried out in conditions of lowered supersaturation in comparison to a control type crystallisation.

From the results it is also apparent, however, that poloxamer 182 has the greatest effect on relative supersaturation. As mentioned above, 182 has the least significant effect on crystal appearance. Therefore, although the poloxamers do change the effective relative supersaturation during crystallisation it seems this is not the way in which they produce their effect on crystal properties.

From these studies it would appear that although poloxamers do affect solution viscosity and the relative supersaturation operating during crystallisation these factors do not cause any effect on crystal appearance. It can therefore be accepted with confidence that poloxamers exert their effect, as discussed above, through adsorption at crystal faces causing a subsequent inhibition of crystal growth.

Acknowledgements

The SERC and SmithKline Beecham are acknowledged for a CASE award to A.J.M.

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