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International Journal of Pharmaceutics 261 (2003) 153–158
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Formation and stabilisation of triclosan colloidal suspensions using supersaturated systems

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Received 11 February 2003; received in revised form 14 May 2003; accepted 16 May 2003

Abstract

The aim of this paper is to prepare and stabilise, in situ, colloidal microsuspensions of triclosan using the polymer, hydroxypropyl methylcellulose (HPMC). The suspensions were prepared from supersaturated solutions of triclosan. The cosolvent technique was used to create supersaturation. Propylene glycol and water were used as the cosolvents. The triclosan particles had a large needle-shaped morphology, when grown in the absence of the polymer. Moreover, the particles grew rapidly to sizes greater than 5 μm over a period of 7 h. When HPMC was added, the particle sizes were in the range 90–250 nm depending on the amount of polymer present in the solutions. The stability of the solutions was evaluated over a period of 40 days during which the particle sizes did not vary. The results were consistent with the mechanism proposed by Raghavan et al. [Int. J. Pharm. 212 (2001b) 213].

Keywords: Triclosan; Hydroxypropyl methylcellulose; Cosolvent technique; Colloidal dispersions; Crystal growth; Supersaturation

1. Introduction

Colloidal dispersions are often used in the formulation of drugs to increase their therapeutic properties. One of the main problems associated with dispersions is particle growth due to either temperature fluctuations or Ostwald ripening. This leads to sedimentation and also to undesirable changes in bioavailability, efficacy and appearance. Particle growth is especially important when the solubility of the drug is strongly dependent on temperature. If there are temperature fluctuations during storage, an increase of temperature will result in dissolution and a subsequent decrease of the temperature will lead to recrystallisation. This eventually changes the nature of the suspensions.

Control of crystal growth processes is crucial in ensuring stability of colloidal systems. This is often achieved by control of different crystallisation parameters such as degree of supersaturation, temperature, additives and solvents. Small amounts of impurities can drastically change the growth properties. Polymers such as hydroxypropyl methylcellulose (HPMC) and polyvinyl pyrrolidone were found to inhibit crystallisation of several drugs (Simonelli et al., 1970; Sekikawa et al., 1978; Ziller and Rupprecht, 1988; Davis and Hadgraft, 1991; Pellett et al., 1994, 1997; Raghavan et al., 2000, 2001a,b; Iervolino et al., 2000). Their use has found recent applications in (trans)dermal drug delivery where permeation

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0378-5173/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0378-5173(03)00299-0

enhancement was achieved using supersaturated systems (Davis and Hadgraft, 1991; Pellett et al., 1994, 1997; Raghavan et al., 2000, 2001a; Iervolino et al., 2000, 2001). The cosolvent technique was used to produce supersaturated systems, a technique that can create very highly supersaturated systems instantaneously (Davis and Hadgraft, 1991). Polymers were used as anti-nucleant additives to stabilise the thermodynamically unstable supersaturated states. Polymers have also been used as additives to inhibit crystallisation in transdermal patches to prevent crystallisation during storage (Lipp, 1998; Ma et al., 1996).

In this paper, the preparation and stabilisation of colloidal dispersions of triclosan, an antimicrobial agent used up to 2% in cosmetic and detergent formulations for disinfection of the skin, are reported. The suspensions were prepared from supersaturated solutions of triclosan either in the absence or presence of the polymer, HPMC. The particles produced were analysed for particle morphology and size. The crystallisation inhibition of triclosan by HPMC is discussed.

2. Materials and methods

2.1. Materials

Triclosan was obtained from GlaxoSmithKline Consumer Healthcare, Surrey, UK. HPMC was obtained from Shin-Etsu, Japan. Propylene glycol (PG) and acetonitrile were purchased from Sigma, UK.

2.2. Methods

2.2.1. Solubility studies

Triclosan was added to a series of PG–water mixtures varying from 0 to 60% PG (100 to 40% water) and stirred in a water bath maintained at 25 °C for 48 h. Double distilled or de-ionised water was used for all the studies. After ensuring that the solute–solvent equilibrium had been reached, the solution was centrifuged and the supernatant solution diluted and assayed using HPLC.

2.2.2. Creation of supersaturation

Supersaturation was produced using the cosolvent method described previously by Davis and Hadgraft (1991). The cosolvents used were PG and water. Supersaturated systems were formed by mixing a solution of triclosan in PG with either water or HPMC solution. The degrees of saturation were calculated from the cosolvent solubility plot (Fig. 1) by dividing the concentration of the drug in the solution by its saturated solubility in the cosolvent mixture.

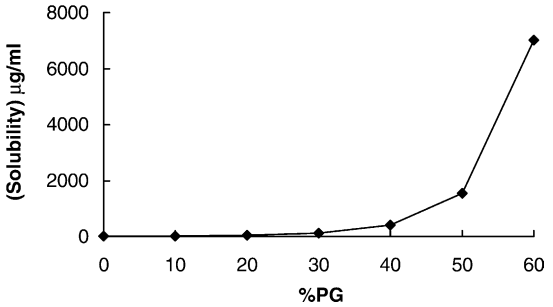

Fig. 1. Solubility plot of triclosan in the PG-water cosolvent system at 25 °C.

2.2.3. HPLC analysis

HPLC analysis of triclosan was performed using a Spectra Series P100 isocratic pump (Thermoseparation Products, Riviera Beach, FL) set at a flow rate of 1.5 ml/min, with an AS100 auto-sampler, a Spectra Series UV 100 detector set at 280 nm and a Spectra Series SP4400 Integrator.

The stationary phase was an Apex reverse phase ODS 5 μm packed column (250 mm × 4.6 mm). The mobile phase was acetonitrile:water (30:70% v/v). Calibration curves were constructed on the basis of the peak area measurements using standard solutions of known concentrations. The retention time was ∼5.3 min.

2.2.4. Particle size analysis

The particle size distribution was measured at 20 °C using a Coulter N4 Plus Mastersizer. The samples for the measurements were prepared by diluting them in water. Each measurement was carried out three times and the run time was at least 200 s.

The samples were subjected to sonication for half an hour before analysis to disperse any aggregates. However, it is possible that sonication may induce crystallisation and hence may affect the particle size measurements especially if the suspension is in a metastable state. Particle size measurements were

hence carried out before and after sonication but no significant differences were observed.

3. Results and discussion

3.1. Solubility

Fig. 1 shows the saturated solubility plot of triclosan in the cosolvent system of PG and water at 25 °C. The solubility values were found to increase exponentially with increasing amounts of PG. The solubility of triclosan in water is extremely low (11 μg/ml). As the proportion of PG was increased, the solubility showed a sharp increase beyond 50%.

3.2. Creation of supersaturation

Supersaturation was created by the cosolvent technique. This technique, described by Davis and Hadgraft (1991) and subsequently used effectively to enhance permeation of drugs through both model silicone membranes and skin (Davis and Hadgraft, 1991; Pellett et al., 1994, 1997; Raghavan et al., 2000; Iervolino et al., 2000), involves mixing a solution of the drug in solvent A in which the drug is soluble with solvent B in which the drug has much lower or no solubility. Using this method, very high degrees of supersaturation (DS) can be created instantaneously. This method has advantages compared to the evaporation or heating–cooling method. In the evaporation method, even though high DS can be achieved by increasing the evaporation rates, crystallisation is uncontrolled. More often than not, the crystallisation process may involve lengthy times unless highly volatile solvents are used. In the heating–cooling method, the DS that can be achieved are limited by the temperature dependence of the solubility. In the cosolvent technique, extremely high DS can be easily achieved by mixing the cosolvents. The maximum DS that can be achieved depends on the solubility of the drug in the two cosolvents. The higher the difference in the solubilities, the higher the DS that can be achieved. In the case of triclosan, the DS that are achievable by mixing 0.5, 1 and 2% triclosan in PG with water in different ratios are given in Fig. 2. The data show that the maximum DS that can be achieved are 23×, 46× and 92× for 0.5, 1 and 2% triclosan, respectively.

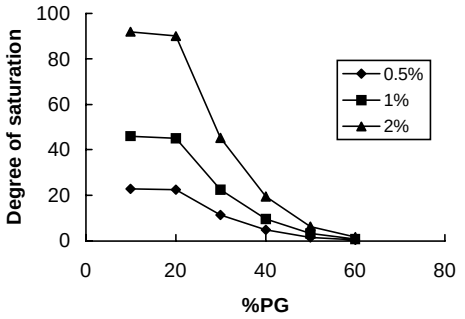

Fig. 2. Degrees of saturation that are achievable by mixing 0.5, 1 and 2% triclosan in PG with water.

3.3. Preparation of colloidal microsuspensions

In order to choose suitable concentration ranges of triclosan and HPMC, supersaturated solutions of triclosan were prepared by mixing 0.5, 1 and 2% triclosan solutions in PG with either water or HPMC solutions containing different amounts of the polymer. The HPMC content was varied between 0 and 2%. The ratio of PG to water (or HPMC solutions) was kept constant at 20:80% v/v. The DS for these mixtures were 22.5×, 45× and 90×. The solutions precipitated immediately on mixing of the two cosolvents both in the absence and presence of HPMC. This is understandable as these DS are extremely high and the solutions are in the labile zone. The nature of the crystals was evaluated by observing them under the microscope or by visual examination and the results are given in Table 1.

In the absence of the polymer, well-formed, needle-shaped crystals were observed under the microscope

Table 1
Stability data of triclosan solutions in the presence of HPMC after 60 days

HPMC (%)	Triclosan (%)			
	0.5	1	2	5
0.05	+	+	Crystals	
0.1	+	+	Crystals	
0.5	+	+	A	
1	A	A	A + S	A + S
2	A	A + S	A + S	

+, A and S refer to stable, agglomeration and sedimentation, respectively.

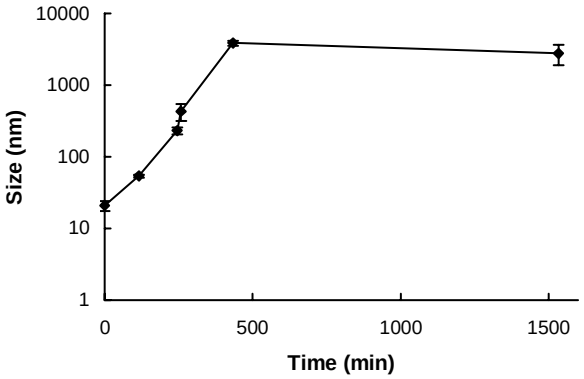

Fig. 3. Particle size of triclosan produced in the absence of HPMC at a degree of saturation of 22.5 as a function of time.

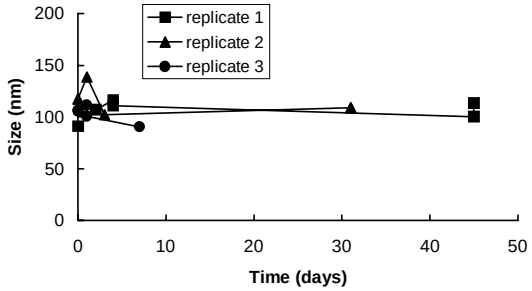

Fig. 4. Particle size of triclosan produced at a degree of saturation of 22.5 using 0.1% HPMC as a function of time.

and grew to large sizes (∼100 μm) in a very short period (few hours). When the polymer was present, the solutions were either translucent or slightly opaque depending on the initial concentrations of the drug and the polymer. At 22.5× and 45× DS, the solutions were translucent but crystals were not observed under the microscope. This indicates that the particles are in the sub-micron colloidal size range. However, at 90× DS, crystals were observed, independent of the presence of HPMC. At this very high DS, HPMC is ineffective in inhibiting nucleation as well as growth. At high HPMC concentrations (1 and 2%), agglomeration was observed at all DS. At these polymer concentrations, the particles tend to aggregate. At high drug and HPMC concentrations, agglomerated particles sediment to the bottom.

These studies show that low concentrations of the drug and the polymer form colloidal suspensions with a uniform size range. Hence, triclosan concentrations of 0.5 and 1% and HPMC concentrations of 0.05, 0.1 and 0.5% were chosen for further studies.

3.4. Particle size analysis

3.4.1. Without HPMC

The particle sizes of the triclosan in colloidal suspensions were measured using the Malvern Mastersizer. The sizes were measured as soon as the solutions were prepared and further monitored as function of time to observe growth of these particles. Fig. 3 shows the average particle size of triclosan produced from 22.5× saturated solution. When no HPMC was present, the particles were ∼20 nm in size immediately on preparation but grew rapidly to greater than ∼4000 nm in about 7 h. After this period, the particle size remained constant or decreased slightly. This slight decrease is likely to be an artefact caused by sedimentation of larger particles. The cells were not stirred, as this might induce nucleation and other changes in the crystal growth.

3.4.2. In the presence of HPMC

Triclosan suspensions were prepared in the presence of different amounts of HPMC by mixing either 0.5 or 1% triclosan in PG with polymer solutions in the ratio 20:80% corresponding to 22.5× and 45× DS. All the solutions precipitated immediately on mixing due to the very high DS used. Fig. 4 shows three replicates of particle sizes of triclosan prepared from a 22.5× saturated solution and using 0.1% HPMC solution. The sizes were measured immediately after preparation and monitored as function of time. The particles were in the range 90–120 nm and remained constant over a period of 45 days. Moreover, with this composition, the sizes were highly reproducible and did not show significant size variations.

Fig. 5 shows the particle sizes obtained from 22.5× and 45× saturated solutions using 0.05, 0.1 and 0.5% HPMC as the anti-nucleant additive. The particles grown in the presence of HPMC were much smaller compared to those grown without HPMC. At a given polymer concentration, the particle sizes were not significantly different at the two different degrees of saturation. One would expect the growth rates of the particles to be different at different degrees of saturation. This suggests that the polymers inhibit the growth of the particles.

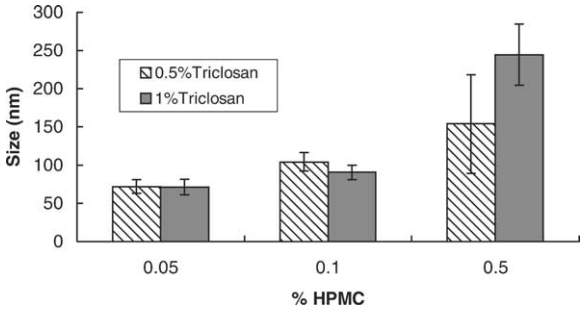

Fig. 5. Particle size of triclosan produced at a degree of saturation of 22.5 and 45 as function of polymer concentration.

The particle sizes increased with increasing polymer concentration from ~70 nm at 0.05% HPMC to ~250 nm at 0.5% HPMC for 45× saturation. Moreover, the standard deviation was very high at high polymer concentrations (≥0.5% HPMC). Large standard deviations are probably caused by the scattering of light by the polymer at these concentrations and not by the particles themselves. The increase in the size with increasing polymer concentration is surprising but could be a result of agglomeration of triclosan particles in the presence of a high concentration of HPMC. The solutions of the precipitate were translucent but agglomerates were not observable under the optical microscope due to its low resolution. Hence, it was difficult to conclude if this was the case.

The experimental observations can be understood based on the mechanism proposed by Raghavan et al. (2001b) that describes the influence of polymers on the crystallisation of drug materials. According to this mechanism, the crystal growth is inhibited

(i) by the adsorption of polymers onto the growing crystal surface due to hydrogen bonding and
(ii) by the formation of a hydrodynamic boundary layer around the growing crystal in which the polymer molecules accumulate.

They also observed that the change in morphology in the presence of the polymer is due to the different levels of hydrogen bonding at the various faces of morphological importance. The growth inhibition depends on the hydrogen bonding groups of the drug and the polymer. Both triclosan and HPMC have functional groups (Fig. 6) that are capable of hydrogen bonding

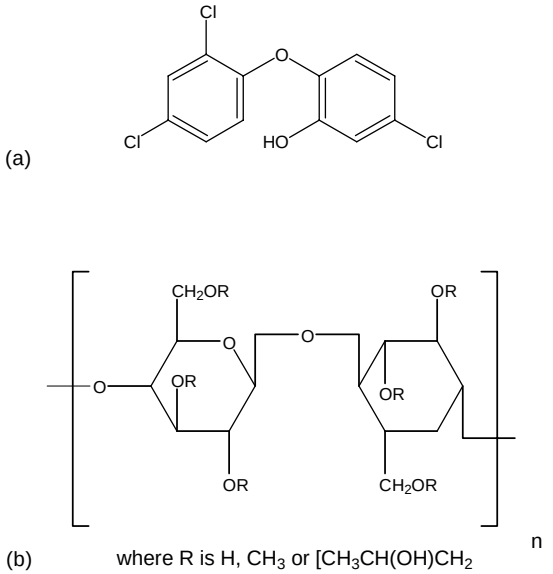

Fig. 6. Molecular structures of triclosan (a) and HPMC (b).

and hence the mechanism of growth inhibition could be expected to be similar.

In the present studies, the crystals grown in the absence of HPMC were found to have long needle-shaped morphology. The translucent nature of the solutions prevented the identification of morphology of the crystals grown in the presence of HPMC. However, the particle size analysis shows that the growth of triclosan was inhibited in the presence of

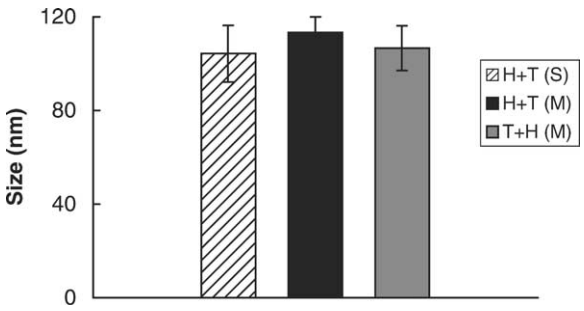

Fig. 7. Particle size of triclosan prepared by mixing 0.5% triclosan in PG with 0.1% HPMC solution in the ratio 1:4. H + T (S)—HPMC solution mixed with triclosan solution and stirred; H + T (M)—HPMC solution mixed with triclosan solution and mixed by shaking manually; T + H (M)—triclosan solution mixed with HPMC solution and mixed by shaking manually.

HPMC. In addition, HPMC also stabilises the colloidal particles over prolonged periods of time, the stability, however, depending on the concentration of the drug as well as the polymer.

The stability of the samples was also analysed to check if there was an influence of mixing on the particle size, the samples were mixed manually, either the triclosan in PG with the polymer solution or vice versa. The solutions were then shaken by hand. The solutions were also mixed using a magnetic stirrer. Fig. 7 shows the results obtained for triclosan prepared by mixing 0.5% triclosan in PG with 0.1% HPMC solution in the ratio 20:80% v/v (DS = 22.5). There was no change in the data obtained with any of these treatments.

4. Conclusions

Colloidal microsuspensions of triclosan were produced from supersaturated solutions and stabilised using HPMC as the additive. The cosolvent technique, using PG and water, was used to produce supersaturated solutions. In the absence of the additive, rapid growth to sizes greater than 5 μm was found to occur and the crystals had a long needle-like morphology. With HPMC as the additive, the particles were in the range 90–250 nm immediately after preparation but the sizes remained constant over a period of 46 days. The results demonstrate that crystal growth of triclosan is inhibited by the presence of HPMC and support the model proposed by Raghavan et al. (2001b). In addition, HPMC is found to be a robust polymer to control nucleation as well as growth of triclosan.

Acknowledgements

The authors would like to acknowledge Engineering and Physical Science Research Council (EPSRC) and GlaxoSmithKline Consumer Healthcare, Weybridge, UK for the financial support to carry out this work. One of the authors (K.S.) wishes to acknowledge a grant to conduct this work under the SOCRATES programme.

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