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Inhibitory effects of water-soluble polymers on precipitation of RS-8359

Fusao Usui [a,b,*], Kazuko Maeda [b], Akira Kusai [b], Kenji Nishimura [b], Keiji Yamamoto [a]

[a] *Faculty of Pharmaceutical Sciences, Chiba University, 1–33 Yayoicho Inage-ku, Chiba, Japan*
[b] *Product Development Laboratories, Sankyo Co., Ltd., 2–58 Hiromachi 1-chome Shinagawa-ku, Tokyo, Japan*

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Abstract

Hydroxypropylmethylcellulose (HPMC), hydroxypropylcellulose (HPC) and polyvinylpyrrolidone (PVP) were used as water-soluble polymers. Incorporation of small amounts of each of these polymers into a phosphate buffer solution (pH 6.8) showed inhibitory effects on the precipitation of RS-8359 from supersaturated solutions. In the case of both HPMC and HPC, precipitation time lags of the compound were effectively prolonged as the polymer concentration increased. Further, all of the polymers decreased precipitation rates of the compound. The most effective concentration to decrease the precipitation rate existed and it was 0.01 mg/ml for each of HPMC and HPC, and 0.0001 mg/ml for PVP within this experimental range. The precipitates contained the polymers. Further, the incorporation of the polymers in the solutions increased the solubility of the compound. These evidences suggest the interaction of the polymers with the compound. © 1997 Elsevier Science B.V.

Keywords: Hydroxypropylmethylcellulose; Hydroxypropylcellulose; Polyvinylpyrrolidone; RS-8359; Supersaturation; Precipitation

1. Introduction

It is known that dissolution properties can be improved by decreasing the crystallinity of a compound. Some well-known methods to decrease crystallinity or to prepare an amorphous solid are (1) coprecipitation with a polymer (Doherty et al., 1987; Suzuki et al., 1990); (2) preparation of a solid dispersion with carbowax, which is semisolid at room temperature (Stavchansky et al., 1984) and (3) grinding a compound with excipients including polymers (Yonemochi et al., 1994; Otsuka et al., 1995). Oral absorption can be increased by improving dissolution properties using the above

* Corresponding author.

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methods (Goldberg et al., 1966; Chiou et al., 1970).

An amorphous state or crystal imperfection usually makes the solubility higher than the crystalline state, because it is metastable. However, the compound in a supersaturated solution usually precipitate with the lapse of time. Shefter and Watanabe showed that addition of gelatin or casein in a solution sometimes keeps supersaturation or decreases a precipitation rate (Shefter et al., 1963; Watanabe et al., 1990). Water-soluble polymers are commonly used to prepare solid dispersions by either the coprecipitation or grinding method to improve dissolution properties. The solid dispersion, however, is not usually perfectly amorphous and sometimes contains crystalline parts. The existence of crystalline parts may influence the precipitation rate.

In this paper, study was performed on the inhibitory effects of water-soluble polymers at a low concentration on precipitation of a water-insoluble compound in aqueous solutions. The supersaturated conditions were prepared by adding small amounts of methanol solution of the compound into aqueous buffer solution, instead of a solid dispersion, to avoid the influence of the potential existence of crystal parts. RS-8359 (Fig. 1), a MAO-A inhibitor (Yokoyama et al., 1989), was selected as a model compound of a water-insoluble compound, and hydroxypropylmethylcellulose (HPMC), hydroxypropylcellulose (HPC) and polyvinylpyrrolidone (PVP) were selected as water-soluble polymers that are commonly used to prepare an amorphous state by the coprecipitation method or the grinding method.

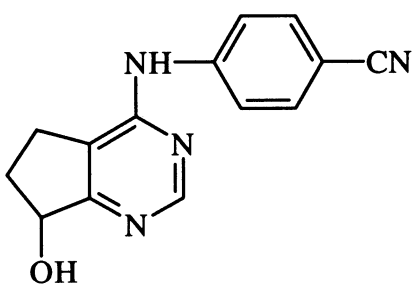

Fig. 1. Chemical structure of RS-8359.

2. Materials and methods

2.1. Materials

RS-8359 is (±)-4-(4-cyanoanilino)-5,6-dihydro-7-hydroxy-7H-cyclopenta[d]pyrimidine, and it was provided by Sankyo, HPMC (Shin-Etsu Chemical, TC-5EW), HPC (Nippon Soda, SSL) and PVP (BASF, K-30) were used as received. Reagent grades of hydrochloric acid, potassium phosphate mono basic, sodium hydroxide, adipic acid, hydroiodic acid, *n*-octane, xylene, citric acid, 0.006 N iodine solution and methanol were used.

2.2. Preparation of supersaturation

A total of 40 mg of RS-8359 and proper amounts of a polymer(0-400 mg) were dissolved in a mixture of 0.5 N HCl and methanol (1:4), and then this solution was diluted, to make 20 ml. Then, 2 ml of this solution was added into 38 ml of phosphate buffer solution (pH 6.8) at 20°C. After mixing, this solution was kept at 20°C, and samples were taken out at predetermined time intervals. The sampled solution was filtered through an Ekicrodisc (0.45 μm) (Gelman Sciences Japan, Ltd.) and then diluted 2-fold with a mixed solution of 0.5N HCl and methanol (1:4). High-performance liquid chromatographic analysis was performed on this solution, using a reversed-phase column and a mobile phase of a mixed solution of 0.5% ammonium acetate solution, methanol, and acetonitrile (15:3:2). After 96 h, precipitates were filtered with a millipore filter, washed with purified water three times, and then dried at 50°C for 1 h in a vacuum oven.

2.3. Solubility determination

A total of 50 mg of RS-8359 was put into the phosphate buffer solution (pH 6.8) containing the polymer, and the mixture was stirred using a magnetic stirrer at 20°C for 24 h. Then the solution was filtrated through an Ekicrodisc (0.45 μm), and the concentration of RS-8359 in the polymer solutions was determined by high-performance liquid chromatographic analysis using the same method mentioned above. Solubility was calculated from the concentration.

2.4. Assay of HPMC or HPC in precipitates

Assay for HPMC and HPC in precipitates of RS-8359 was performed in accordance with the Japanese Pharmacopoeia XII. Then 25 mg of the precipitate was mixed with the solution that contained adipic acid, hydroiodic acid, *n*-octane and xylene, and then the solution was shaken for 30 s. Then, the solution was heated at 150°C for 60 min, with shaking at 5-min intervals. After cooling, the supernatant was analyzed by a gas chromatographic method.

2.5. Assay of PVP in precipitates

Precipitate of RS-8359 (70 mg) from the phosphate buffer solution containing PVP was dissolved in methanol and made to be 50 ml. Of this solution 10ml was mixed with 8% aqueous solution of citric acid, to make 50 ml. Then, 10 ml of this solution was mixed with 4 ml of 0.006N iodine solution, and this was kept at 20°C for 30 min. Absorbance at 500 nm was determined for this solution and a reference which was made by the same method without the precipitate.

2.6. Viscosity determination

Viscosity was determined at 20°C using an Ubbelohde-type viscometer.

2.7. X-ray diffraction profiles

X-ray diffraction profiles were obtained with a Geiger Flex Rint 2200V (Rigaku Denki) with Cu-K alpha radiation.

2.8. Scanning electron microscope (sem).

The scanning electron microscope used was ABT-55 (Topcon).

3. Results

The chemical structure of RS-8359 is shown in Fig. 1. Because it is a basic compound, the solubility of the compound is higher in an acidic solution than in a neutral or an alkaline solution, as shown in Table 1. When the compound is in an acidic condition, such as in the stomach, it may be soluble. However, when the compound is put into a neutral pH condition such as in the intestine, it may precipitate because of low solubility. Such a neutral pH of 6.8 was selected for this experiment.

Table 1
Solubility of RS-8359 in various pH solutions at 20°C

pH	Solubility (μg/ml)
1.2	4750
2.3	366
4.1	12.7
5.1	6.7
6.8	5.0
7.5	7.2

Precipitation profiles of this compound from the supersaturated solutions containing HPMC are shown in Fig. 2(a). Higher solubilities were observed in the phosphate buffer solutions containing HPMC than in a polymer-free solution, even after 96 h, except for a phosphate buffer solution containing 0.0001 mg/ml HPMC. Precipitation time lags were also observed in these solutions. As the HPMC concentration increased, the time lag increased and it reached about 2 h at an HPMC concentration of 1 mg/ml. After the time lag, the compound precipitated and the concentration approached the intrinsic solubility. We defined precipitation half-time as a yardstick of the precipitation rate, as the time to reach the average concentration of the initial concentration and the intrinsic solubility of the compound in the solution excluding the time lag. Precipitation half-times are shown in Fig. 3. The precipitation half-time of the compound was the longest at the HPMC concentration of 0.01 mg/ml and it increased to over ten times as long as the half-time in the polymer-free solution.

Precipitation profiles from the supersaturated solutions containing HPC are shown in Fig. 2b. The precipitation profiles from the phosphate buffer solutions containing HPC are very similar to those from the phosphate buffer solutions containing HPMC. Higher solubilities were observed in the phosphate buffer solutions containing HPC

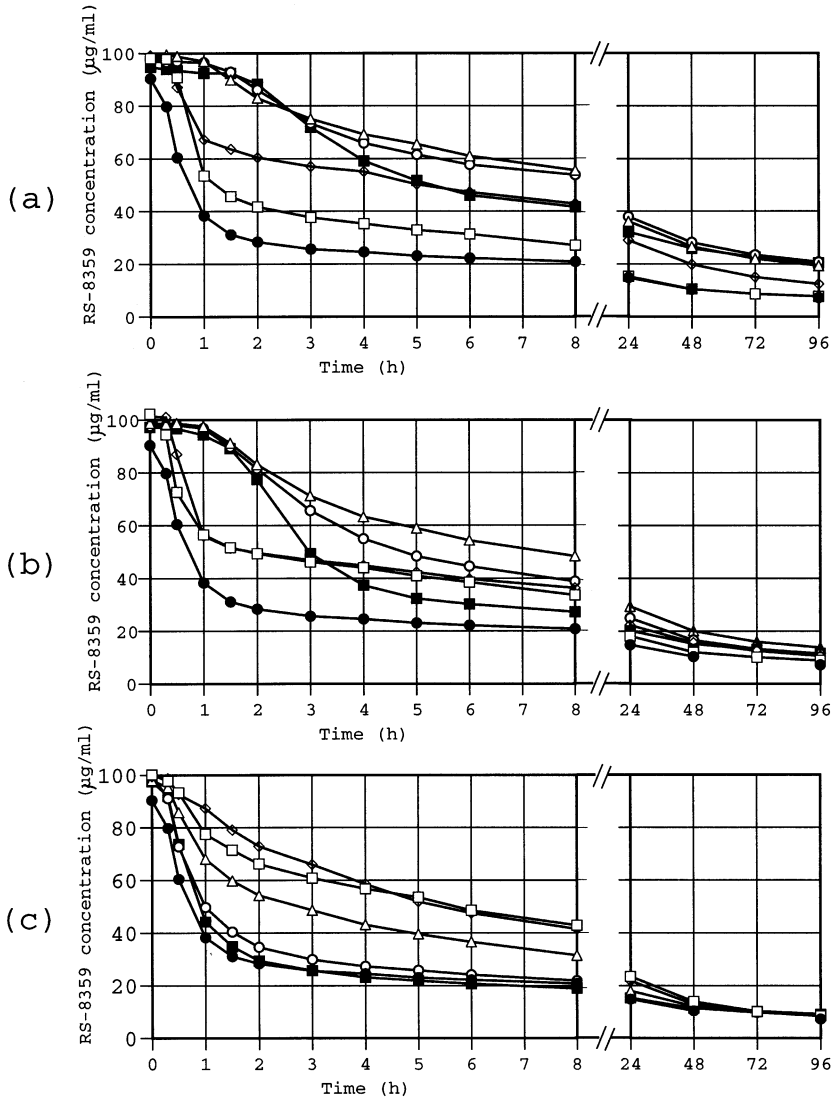

Fig. 2. Precipitation profiles of RS-8359 in the phosphate buffer solution (phosphate buffer solution (pH 6.8) containing 4% methanol) containing various polymers. (a) HPMC (b) HPC (c) PVP Polymer concentration: – ■ –, 1 mg/ml; – ◦ –, 0.1 mg/ml: – ▲ –, 0.01 mg/ml: – ◇ –, 0.001 mg/ml; – □ –, 0.0001 mg/ml; – ● –, polymer-free.

than in the polymer-free solution after 96 h. The longest time lag was about 1 h at 1 mg/ml, and the longest precipitation half-time was observed at 0.01 mg/ml.

Precipitation profiles from the supersaturated solutions containing PVP are shown in Fig. 2c. In the case of the phosphate buffer solutions containing PVP, no significant polymer effect was observed after 96 h. Time lags were about 0.5 h for all the PVP concentration and shorter than in phosphate buffer solutions containing HPMC or HPC. The precipitation rate increased as the polymer concentration increased within this experimental range. The longest precipitation half-time was observed at 0.0001 mg/ml, the lowest concentration of this experiment (Fig. 3).

Solubilities of this compound in the phosphate buffer solutions containing the polymer are shown

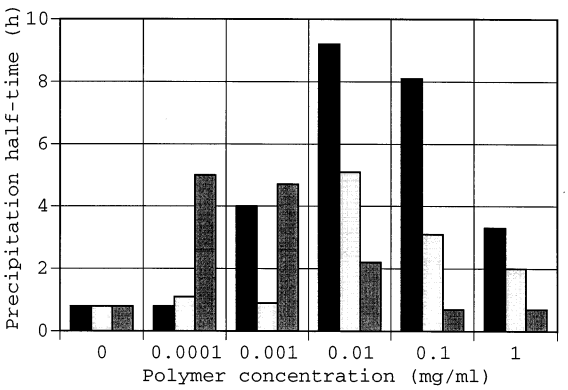

Fig. 3. Effects of polymer on precipitation rates of RS-8359 in phosphate buffer solution (pH 6.8) containing 4% methanol: ■, HPMC □, HPC; ■, PVP.

in Table 2. All of the polymers enhanced the solubility of the compound with an increase in the concentration. The solubilities in the phosphate buffer solutions containing PVP were lower than those in the phosphate buffer solutions containing HPMC or HPC. In the precipitation experiments, the concentration of this compound in the phosphate buffer solutions containing the polymer was still higher than the intrinsic solubility even after 96 h, taking into account the solubility increase in the phosphate buffer solutions containing the polymer.

SEM photographs of the precipitates are shown in Fig. 4. Plate-shaped crystals separated out of the polymer-free solution. On the other hand, agglomerates of small crystals separated out of the HPMC solutions. As the polymer concentration in the solutions increased, the crystals in the agglomerates became small. Precipitates from each of the HPC and PVP solutions showed the same shape as those obtained from the phosphate buffer solutions containing HPMC (photographs are not shown).

Table 2
Solubility of RS-8359 in a phosphate buffer solution (pH 6.8) containing polymer at 20°C (μg/ml)

Polymer concentration in solution (mg/ml)	PVP	HPMC	HPC
No polymer	4.9	4.9	4.9
0.0001	5.0	4.7	4.7
0.001	5.6	5.6	4.9
0.01	7.2	8.5	5.9
0.1	8.5	8.4	6.5
1	8.4	9.7	6.5

[a] 4% of methanol was added in solutions.

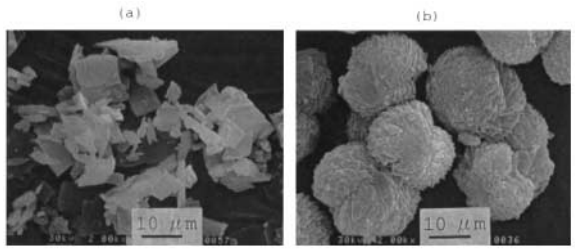

Fig. 4. SEM photographs of the precipitates. (a) Precipitate from a polymer-free solution (phosphate buffer solution (pH 6.8) containing 4% methanol). (b) Precipitate from the phosphate buffer solution* containing 0.1-mg/ml HPMC.

The X-ray diffraction profiles of the precipitates are shown in Fig. 5. The characteristic peaks of all of the samples appeared at the same diffraction angles, though intensity of the precipitate ob-

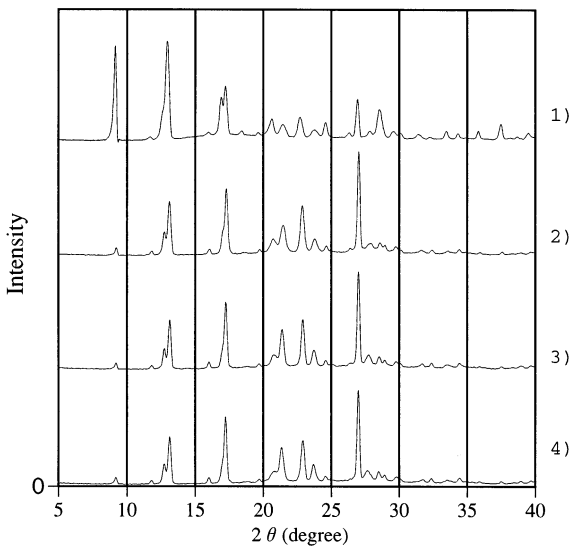

Fig. 5. X-ray diffraction profiles of RS-8359. Precipitate from (1) polymer-free solution; (2) the phosphate buffer solution containing 1-mg/ml PVP; (3) the phosphate buffer solution containing 1-mg/ml HPC; (4) the phosphate buffer solution containing 1-mg/ml HPMC. In all cases the phosphate buffer solution (pH 6.8) contains 4% methanol)

Table 3
Weight composition of the precipitates of RS-8359

	HPMC concentration (mg/ml)			HPC concentration (mg/ml)			PVP concentration (mg/ml)		
	1	0.01	0.0001	1	0.01	0.0001	1	0.01	0.0001
% of RS-8359	99.2	98.5	99.9	98.1	98.0	99.9	98.2	98.9	99.3
% of Polymer	0.8	1.5	0.1	1.9	2.0	0.1	1.8	1.1	0.7
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

tained from the phosphate buffer solutions containing the polymer was different from that obtained from the polymer-free solution.

HPMC, HPC and PVP contents in the precipitates are shown in Table 3. In the cases of HPMC and HPC, no significant differences of the polymer content in the precipitates were observed between those obtained from the polymer concentration of 1 mg/ml and those from that of 0.01 mg/ml. However, the polymer contents in the precipitates from the polymer concentration of 0.0001 mg/ml were less than those from higher concentrations. In the case of PVP, the polymer content in the precipitate increased as the PVP concentration in the solution increased.

4. Discussion

The time lag for the precipitation of the compound increased as the polymer concentration in the solution increased in the case of HPMC or HPC. Crystalline growth occured after the nucleation in the precipitation processes. The prolongation of the time lag by these polymers suggests that the polymers inhibited the nucleation of the compound in the supersaturated solution. In the case of calcium carbonate, existence of two-step precipitation has been reported (Nielsen et al., 1984; Tomson, 1983). In this case that no crystals or no nuclei exist in its supersaturated solution, the concentration of calcium carbonate decreases slightly in the very beginning of the precipitation stage, and then it stay in a certain level during a formation of a metastable precursor or embryo. After that, it decreases again, to the solubility of the stable crystal (calcite). In our experiment, the observed initial concentrations were lower than 100 μg/ml, which is the calculated concentration where all of the added drug was dissolved, and they varied among the experiments. This means that the concentration of the compound in the solution slightly dropped instantly after the methanol solution of this compound was added. Then, this supersaturated state was kept for a while. This is the so-called time lag here. The slightly decreased concentration at the very initial stage suggests the appearance of a precursor stage which was observed in the calcium carbonate as well. The precursor has higher solubility than its crystal form due to the higher surface energy. The solubility of the precursor of the compound varied according to the experimental conditions, depending on its surface energy. The stabilization effect of HPMC and HPC on the precursor stage is greater than that of PVP, and the time lag became longer as the polymer concentration became higher.

The other possible factor that affected the time lag was interaction between the compound and the polymer. HPMC or PVP increases the solubility of acetazolamide by a different mechanism from a simple micelle formation (Loftsson et al., 1996). PVP also interacts with sodium salicylate (Higuchi et al., 1954). All of the polymers used in our experiment increased the solubility of the compound. The increased solubility profiles of RS-8359 in the polymer solutions are very different from solubilizing profiles by micelle formation. It suggests that the solubilizing mechanism of the polymers is different from that of surfactants. However, this evidence suggests the interaction of the compound with the polymers. Further, HPMC, HPC or PVP was found in the precipi-

tates obtained from the phosphate buffer solutions containing the polymers. This evidence also suggests the interaction of the compound with the polymers. The interaction affected the stability of the precursor and eventually the duration of the time lag.

After the time lag, this compound precipitated with the lapse of time. Crystal growth is directly proportional to the diffusion rate of a compound in some case. This is called diffusion control (A.E. Nielsen). So, an increase in the precipitation half-time of this compound was also expected as an increase in viscosity of the solution. However, the viscosity of the phosphate buffer solutions containing the polymer increased little until at 0.1 mg/ml (Table 4), and the viscosity increased to only 20% at most at 1 mg/ml, the maximum concentration in this experiment for all of the polymers. So the estimated decrease in the diffusion rate is less than 20% if the diffusion rate of this compound decreases in accordance with the Stokes-Einstein equation. It is obvious that the change in the precipitation half-time was much greater than the change in viscosity. Thus, it is difficult to explain the change in the precipitation half-time based on only the viscosity change.

Not only a diffusion rate but also processes of adsorption and transfer of molecules on its crystal surface also affect the rate of the crystal growth (Nielsen et al., 1984). It is thought that the interaction of the compound with the polymer also affected the process of adsorption or transfer of the molecules of the compound on its crystal surface in the crystal growth processes.

Table 4
Viscosity of the phosphate buffer solutions containing the polymer at 20°C (mm^2/s)

Polymer concentration in solution (mg/ml)	HPMC	HPC	PVP
No polymer	1.104	1.104	1.104
0.0001	1.126	1.136	1.136
0.001	1.120	1.123	1.134
0.01	1.122	1.136	1.136
0.1	1.128	1.140	1.138
1	1.211	1.186	1.157

Each polymer was dissolved in phosphate buffer solution (pH 6.8) containing 4% methanol.

Comparing X-ray diffraction profiles of the precipitates which were obtained from the phosphate buffer solutions containing 1-mg/ml HPMC, HPC or PVP, the characteristic peaks of RS-8359 appeared at the same diffraction angles as that obtained from the polymer-free solution; that is, no change was observed in lattice spacings among them. This suggests that incorporation of the polymers did not change the internal structure of the crystal. Studies by Chow and Duddu showed that additive contents in a crystal influence the crystal habit (Chow et al., 1995; Duddu et al., 1996). The SEM photographs revealed that the precipitate obtained from the phosphate buffer solutions containing the polymers was agglomerates of small crystals, but that obtained from the polymer-free solution was plate-shaped crystals. Further, The peak intensity of X-ray diffraction of the agglomerates was greatly different from the precipitate obtained from the polymer-free solution. These evidences suggest that the crystal habit was different between the precipitates obtained from the polymer-free solution and from the phosphate buffer solution containing the polymer, and that the change in the crystal habit was caused by adhesion of the polymer on the growing plane of the crystal.

5. Conclusions

Small amounts of water-soluble polymers, HPMC, HPC and PVP, have inhibitory effects on the precipitation of RS-8359 from supersaturated solutions. All of the polymers prolonged the precipitation time lags and also decreased the precipitation rates. However, it is difficult to explain the change in the precipitation time lag or rate by only the viscosity change.

The increased solubility of this compound in the polymer solution and the incorporation of the polymer in the precipitates suggest interaction between this compound and the polymers, and this interaction influenced the crystal habit. Based on these results, it was concluded that the inhibitory effects of the polymers on the precipitation of this compound were caused not only by increase in viscosity but also by interaction be-

tween this compound and the polymers. It is expected that incorporation of the polymers into a solid dispersion is useful to maintain higher solubility than the intrinsic solubility, and eventually it dose provide potential to increase oral absorption of this compound.

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