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RESEARCH PAPER

Phase Behavior of Resveratrol Solid Dispersions Upon Addition to Aqueous media

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ABSTRACT

Purpose To investigate the phase behavior of resveratrol amorphous solid dispersions upon addition to aqueous media.

Methods Polymers with different crystallization inhibitor properties were used to form amorphous solid dispersions of resveratrol. Resveratrol crystallization in aqueous environments was monitored over time using Raman spectroscopy, and solution concentrations were determined by ultraviolet (UV) spectroscopy.

Results The crystallization behavior varied depending on the type and amount of polymer present in the dispersion. Polyvinylpyrrolidone (PVP) and Eudragit E100 (E100) dispersions did not crystallize for 24 h when slurried in pH 6.8 buffer at 37°C. Even though no crystallization occurred, a supersaturated solution was not achieved, most likely because of resveratrol-polymer complexation. Dispersions formed with cellulose derivatives crystallized rapidly, and the extent of crystallization varied depending on the amount of polymer in the dispersion. The solution concentration achieved in the slurries varied considerably between the various solid dispersions and depended on several factors including the extent of resveratrol crystallization, the nature of the resveratrol-polymer interactions, and the concentration of solid dispersion added to the slurry.

Conclusions It was found that the extent of supersaturation was limited not only by crystallization, but also by soluble and insoluble complex formation between resveratrol and the polymer.

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KEY WORDS amorphous solid dispersion · complexation · resveratrol · solubility · supersaturated solution

ABBREVIATIONS

CMCAB	Carboxymethyl cellulose acetate butyrate
E100	Eudragit® E100
HAS	Hydrochloric acid solution
HPLC	High performance liquid chromatography
HPMC	Hydroxypropyl methyl cellulose
HPMCAS	Hydroxypropyl methyl cellulose acetate succinate
PB	Phosphate buffer
PVP	Poly(vinylpyrrolidone)
RH	Relative humidity
UV	Ultraviolet spectroscopy

INTRODUCTION

Amorphous solid dispersions have become a common formulation strategy for poorly water soluble compounds since, upon dissolution, supersaturated solutions can be generated which subsequently enhance bioavailability (1,2). This approach is only effective if the matrix remains amorphous during dissolution and the enhanced solution concentrations are maintained long enough to improve absorption *in vivo*. However, due to the strong driving force for crystallization, these formulations may fail, as recrystallization to the more stable crystalline counterpart leads to a decrease in the solubility advantage gained from the amorphous solid (3). The presence of a polymeric additive can have a substantial impact on crystallization kinetics from supersaturated solutions, for example, studies have shown that the polymer can influence the nucleation and/or crystal growth rates, as well as the crystal morphology (4–6). Additionally, it is well known that the polymer used to formulate the amorphous solid dispersion has a dramatic impact on the physical stability of the formulation

during storage (7–10). Clearly the polymer employed must be effective at preventing crystallization in several environments including from the solid matrix, hydrated matrix, and from supersaturated solutions. In addition to evaluating the polymeric impact on supersaturation duration, potential solubilization by the polymer also needs to be considered. If the polymer increases the thermodynamic solubility of the crystal form, the extent of supersaturation may be reduced, which in turn will decrease the driving force for membrane transport. Solubilization of poorly water soluble compounds by polymers typically used in amorphous solid dispersions may sometimes occur (11), but has not been widely reported.

The goal of the current study was to determine the crystallization tendency of a compound formulated as an amorphous solid dispersion with different polymers, and to better understand the link between crystallization, drug-polymer complexation, and solution concentration. The model compound used in this study was the phytochemical resveratrol (CAS Registry Number CAS 501-36-0) (12). Resveratrol is found in nature as both cis and trans isomers; however the trans-isomer is thought to be the most abundant and biologically active form (Fig. 1). Resveratrol has been suggested to possess antiplatelet, antioxidative, antifungal, anticancer and cardio protective properties (13). Resveratrol is moderately hydrophobic (logP 3.1) and has poor aqueous solubility (38.8 μg/ml at 25°C and pH 6) (14), in large part due to its high melting point of 262°C (15) and strong crystal lattice energy. Consequently, considerable improvements in the dissolution rate and apparent solubility should be attained by formulating this compound as an amorphous solid dispersion.

In a study of the solid state stability of amorphous resveratrol solid dispersions formulated with different polymers (7), the manufacturability and physical stability of the resveratrol amorphous solid dispersions were found to be strongly related to the specific interactions that were formed between resveratrol and the polymer (7). Polymers forming weaker hydrogen bond interactions with resveratrol were less effective crystallization inhibitors, while polymers that formed strong intermolecular interactions were extremely effective (7). Some of the amorphous solid dispersions were stable upon exposure to elevated relative humidities (RHs) and/or temperatures, whereas resveratrol underwent rapid crystallization when dispersed with other polymers and exposed to high RH (7,16,17). These less stable dispersions might also be expected to rapidly crystallize from the matrix upon exposure to dissolution media, compromising the formation of supersaturated solutions. Hence, the benefits of formulating resveratrol as an amorphous solid would be negated. The hypothesis tested herein is that the amorphous resveratrol-polymer dispersions that exhibited intermolecular interactions that led to enhanced physical stability upon exposure to elevated RH will also be more resistant to crystallization during dissolution, resulting in higher solution concentrations of resveratrol compared to dispersions that undergo rapid crystallization

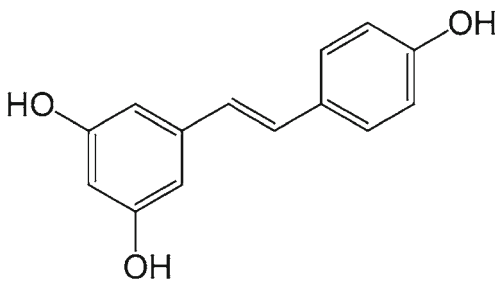

Fig. 1 Chemical structure of resveratrol.

MATERIALS AND METHODS

Materials

Resveratrol was obtained from AK Scientific, Union City, CA, USA. PVP (K29/32, M_w 58,000) was purchased from Sigma-Aldrich (St. Louis, MO, USA). HPMC (grade 606, hypromellose United States Pharmacopeia substitution type 2910, M_w 35,600) and HPMCAS (grade AS-MF, M_w 17,000) were provided by Shin-Etsu Chemical Co., Ltd., Tokyo, Japan. Eudragit E100 (M_w 47,000) was obtained from Rohm GmbH, Darmstadt, Germany. CMCAB (641-0.5, M_w 35,000) was obtained from Eastman Chemical Co., Kingsport, TN, USA. The polymers were kept in desiccators with P_2O_5 for at least 1 week prior to use to remove any residual moisture. Dichloromethane (ChromAR grade) and acetone (ChromAR grade) were obtained from Mallinckrodt Baker, Inc., Paris, KY, USA. Ethanol (200 proof) was obtained from Pharmaco Products, Inc., Brookfield, CT, USA and Aaper, Shelbyville, KY, USA. Formic acid (>95%, reagent grade) and high performance liquid chromatography (HPLC) grade acetonitrile were purchased from Sigma-Aldrich (St. Louis, MO, USA). Two solutions were prepared according to USP30-NF25 standard method: 50 mM phosphate buffer at pH 6.8 (PB 6.8) and hydrochloric acid solution at pH 1.2 (HAS 1.2).

Effect of Polymer on Resveratrol Solubility

The equilibrium solubility of crystalline resveratrol was measured at 37°C in PB 6.8 in the presence and absence of the polymers. Crystalline resveratrol (20 mg) was dispersed in 20 ml of PB 6.8, in which HPMC and HPMCAS had been previously dissolved leading to final polymer concentrations of 5, 10, and 20 mg/ml. For E100 and CMCAB, saturated solutions of polymer in PB 6.8 were used as these polymers have a low solubility at pH 6.8. Due to evidence of complexation between PVP and resveratrol, a constant solution

concentration of 20 mg/ml of PVP in PB 6.8 was used and the amount of crystalline resveratrol was varied from approximately 650 μg/ml to 11,000 μg/ml in order to explore this phenomenon. All of the solutions were allowed to equilibrate at 37°C for 24 h. Following equilibration, the solutions were centrifuged at 40,000*g* for 15 min to remove insoluble material. The supernatant was removed and analyzed by ultraviolet–visible (UV–Vis) spectroscopy using the method described below.

UV–Vis Spectroscopy

Solution concentrations of resveratrol following dissolution were determined using ultraviolet spectroscopy. Light absorption was detected by monitoring the extinction at 305 nm using a SI Photonics UV/vis spectrometer (Tuscon, Arizona), fiber optically coupled with a 1 cm path length dip probe. A calibration curve for resveratrol from 1.5 to 6.5 μg/ml was produced in PB 6.8. The supernatant from the crystalline solubility and solid dispersion studies was diluted in PB 6.8 so that the absorbance was below 1 absorbance unit and in the linear region of the calibration curve. The influence of polymers on the UV spectrum was evaluated for solutions containing polymers. In the case of PVP-containing solutions, the UV spectral pattern shifted as the concentration of resveratrol and PVP changed. Because of this, a dedicated calibration curve for solutions containing PVP was created whereby the wavelength monitored for the calibration was at peak trough (258 nm). The calibration dataset was obtained by varying both PVP and resveratrol concentrations. HPMC and HPMCAS did not influence the UV spectrum.

Preparation of Amorphous Solid Dispersions

Solutions were prepared by dissolving resveratrol and polymer at different dry weight ratios in a 1:1 (by weight) mixture of dichloromethane and ethanol. All mixtures were visually inspected to confirm that the resveratrol and polymers were fully dissolved, and that the systems formed uniform one-phase solutions. The solvent was removed using a rotary evaporator (Brinkman Instruments, Westbury, NY, USA) with reduced pressure, heated in a water bath maintained at 60°C. The samples were then placed under vacuum for 24 h to remove any residual solvents. The obtained material was subsequently cryo-milled in a 6750 freezer mill (Spex Sampleprep, Metuchen, New Jersey, USA) in a liquid nitrogen bath for a total milling time of 4 min and then sieved to ensure the particle size was between 53 and 250 μm. This approach was successfully used in a previous study to create amorphous solid dispersions of resveratrol in the selected polymer (7).

X-ray Diffraction

Resveratrol-polymer dispersions were analyzed to confirm that they were amorphous using a Shimadzu XRD-6000 instrument (Shimadzu Corporation, Kyoto, Japan) equipped with a Cu-Kα source and set in Bragg-Brentano geometry between 5 and 35° 2θ at a scan rate of 8°/min with a 0.04° step size. Before each day of measurement, the accuracy of the 2θ angle was checked by verifying that the [111] peak of a Si-standard sample was between 28.423 and 28.463°. Samples were confirmed to be X-ray amorphous and were used for dissolution experiments.

Raman Spectroscopy

Crystallization kinetics of the resveratrol solid dispersions in an aqueous environment were studied using Raman spectroscopy. Phase transformations of slurried resveratrol amorphous solid dispersions were monitored using a RamanRxn2P^{h}AT-785 Raman Spectrometer (Kaiser Optical Systems, Inc., Ann Arbor, MI) with a laser wavelength of 785 nm. Slurries consisted of approximately 300 mg to 500 mg of solid added to 5 ml of either PB pH 6.8 or HAS pH 1.2. The spectra were collected using a fiber-optic P^{h}AT system probehead. iC Raman software (Version 4.1.910, Kaiser Optical Systems, Inc., Ann Arbor, MI) was used to control the Raman spectrometer. The probe was placed above the slurry of the solid dispersion and the temperature was controlled at 37°C. Spectra were collected every 5 min for the first hour, every 15 min for the next 2 h, every 30 min for the next 5 h, and then every hour until 24 or 48 h was reached. The data were analyzed by determining the ratio of the peak intensities at 1630 and 1605 cm^{-1}, which arise from stretching of the aromatic rings (18) and are sensitive to the crystallinity of resveratrol, and plotting the ratio *vs.* time. The residual solid material was dried and analyzed by XRPD to qualitatively analyze crystallinity and results were in agreement with the Raman data. The remaining solutions were then centrifuged at 40,000*g* for 15 min to remove any insoluble material. The supernatant was removed and analyzed by UV–Vis spectroscopy as described above to determine resveratrol solution concentrations.

High Performance Liquid Chromatography

The supernatant solutions obtained at the end of the slurry experiments for resveratrol dispersions in HPMCAS and HPMC were analyzed by HPLC to determine the polymer concentrations in solution. The instrument used in this study was an Agilent 1100 high performance liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, CA) and the detector was a NQAD QT-500 (Quant Technologies, Blaine, MN). The chromatographic conditions used are summarized in Table I. The calibration set was prepared by

Table I HPLC Method for Determining HPMC and HPMCAS Concentration

Mobile phase	Solvent A = 1000 ml water + 1 ml formic acid		
	Solvent B = 1000 ml Acetonitrile + 1 ml formic acid		
Gradient time (min)	Time (min)	% Solvent A	% Solvent B
	0	100	0
	20	20	80
	30	20	80
	35	100	0
Flow rate	0.5 ml/min		
Injection volume	25 μL		
Column polymer	Polymer X RP-1 (5 μm, 150 mm × 4.6 mm), at 30°C		
Detection	NQAD QT-500		
	Evaporator temperature 35°C		
Run Time	45 min		

weighing an appropriate amount of HPMC or HPMCAS in a 20 ml volumetric flask, followed by addition of PB 6.8 to volume. The solutions were stirred for at least an hour, thus ensuring complete dissolution. The standards and samples were analyzed in triplicate. The standard curve exhibited good linearity ($r^2 > 0.99$) over the concentration range analyzed. For the HPMC calibration, the range was from 2 to 10 mg/ml with an LOD of 0.9 mg/ml and an LOQ was 2.6 mg/ml. For the HPMCAS calibration the range was from 4 to 10 mg/ml and the LOD was 1.1 mg/ml and the LOQ was 3.4 mg/ml.

Polarized Light Microscopy

Polarized light microscopy images were obtained using a Nikon Eclipse E600 Pol microscope (Nikon Co., Tokyo, Japan) coupled with a DS-Fi1 camera (Nikon Corporation, Tokyo, Japan). Samples containing 25 wt.% resveratrol:HPMCAS solid dispersions following slurrying in a dissolution medium of either pH 6.8 or pH 1.2 were evaluated after 24 h using polarized light to aid in the detection of birefringent, crystalline material.

Diffusion Rate Measurements

A side-by-side diffusion cell (PermeGear, Inc. Hellertown, Pennsylvania) was used to evaluate the thermodynamic properties of the resveratrol in solution (19). The donor and receiver chambers were separated by a regenerated cellulose membrane with a MWCO of 6–8 KDa. The donor and receiver cells had a capacity of 34 ml. The orifice (opening connecting the donor and receiver compartments) measured 30 mm in diameter (surface area of 7.065 cm^2). The temperature was maintained at 37°C using a circulating water bath. The receiver compartment was filled with 32 ml of PB 6.8 while the donor compartment was filled with 32 ml of dissolution mixture. Dissolution mixture was prepared by weighing out different amounts of solid dispersions containing 15% resveratrol and 85% HPMCAS. The samples were added to 32 ml of PB 6.8 and allowed to stir for 24 h at 37°C. A saturated solution of crystalline resveratrol was obtained by adding excess resveratrol to PB 6.8 and allowed to stir for 24 h at 37°C. Dip probes with a 1 cm path length coupled to the UV–Vis spectrometer were inserted into each compartment *via* sample ports in the upper part of the diffusion cell. This allowed for continuous *in situ* monitoring of solution concentrations without the need to withdraw samples. Resveratrol concentrations in the receiver cell never reached the equilibrium crystalline solubility and sink conditions (maximum solution concentration in the receiver cell was less than one-third of the crystalline solubility) were maintained in the receiver cell. Concentration *versus* time plots were generated, and the slope of the linear region was estimated using linear regression. This value was multiplied by the volume to give the flux.

RESULTS

Impact of Polymer on Resveratrol Solubility

The solubility of crystalline resveratrol in PB 6.8 was measured as 42±6 μg/ml at 37°C. However, this value can be influenced by the presence of polymers; a polymeric additive in solution may change the equilibrium solubility of the crystalline drug (11). Therefore, it is important to understand the effect of the polymer on the equilibrium solubility of resveratrol when investigating the supersaturation generated by dissolving an amorphous solid dispersion. This is because increases in solution concentration achieved by dissolving a solid dispersion may be due to supersaturation, solubilization by the polymer, or a combination of the two effects.

The presence of different polymers resulted in different resveratrol concentrations in solution. Two highly soluble

cellulosic polymers, HPMC and HPMCAS, were found to substantially increase the equilibrium solubility of crystalline resveratrol (Fig. 2) above a threshold polymer concentration. HPMCAS had a very large impact on the equilibrium solubility whereby a concentration of 10 mg/ml of HPMCAS increased resveratrol solubility almost 10 fold to 414 μg/ml. The increases in resveratrol solubility in the presence of HPMC were more modest, with a 10 mg/ml polymer concentration increasing the solubility by approximately 25% to around 51 μg/ml. CMCAB and E100 have limited solubility at pH 6.8, and no increase in resveratrol solubility was observed in the presence of these polymers.

In the case of PVP, initial studies found no clear trends in resveratrol solubility with increasing PVP concentration but there appeared to be a gel-like precipitate forming in the solution as the polymer concentration was increased suggesting the formation of an insoluble complex. Therefore, a different approach was taken to understand the solution behavior of resveratrol in the presence of PVP in which the PVP solution concentration was held constant at 20 mg/ml (PB 6.8), and the amount of crystalline resveratrol added was varied. An interesting trend was observed in terms of the amount of resveratrol that dissolved in the presence of PVP as a function of added crystalline resveratrol (Fig. 3). For small amounts of added resveratrol, all the resveratrol was completely dissolved, *e.g.*, adding approximately 650 μg/ml resveratrol to a 20 mg/ml solution of PVP in PB 6.8 resulted in complete dissolution (Table II). Increasing the resveratrol amount to about 1800 μg/ml produced a small amount of a gelatinous precipitate, but high solution concentrations were again obtained. Nevertheless, upon further increasing the amount of crystalline resveratrol added to the solution, a drastic drop in dissolved resveratrol concentration was observed, along with an

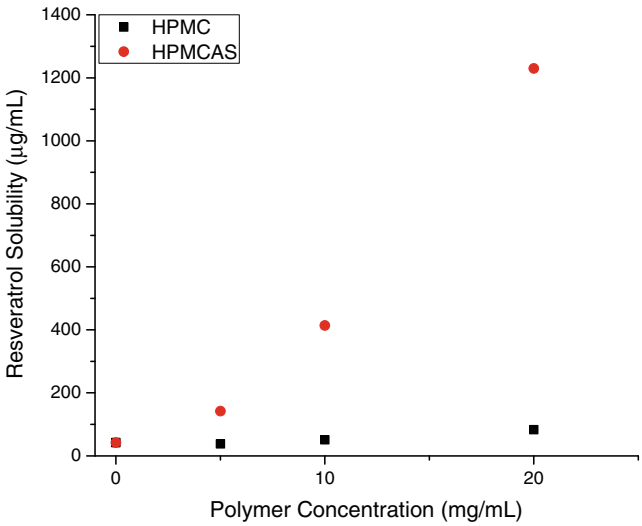

Fig. 2 Crystalline resveratrol solubility in 50 mM phosphate pH 6.8 buffer at 37°C as a function of HPMC (*Black Squares*) and HPMCAS (*Red Circles*) concentration as determined by UV–Vis spectroscopy.

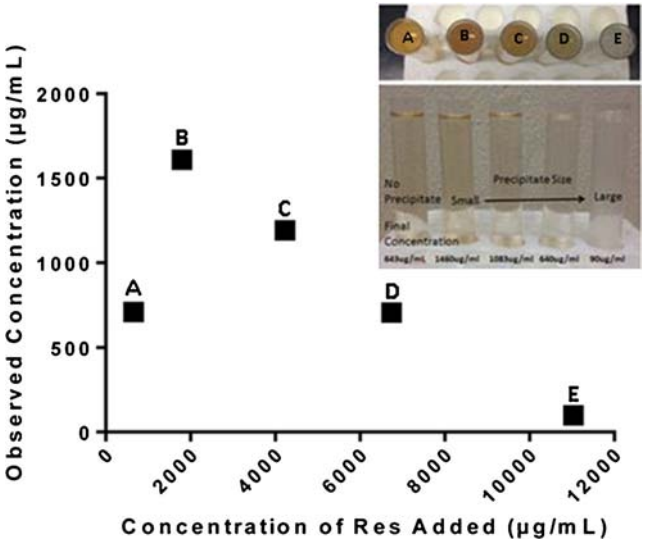

Fig. 3 Evidence for resveratrol complexation with PVP. Measured resveratrol solution concentration in 50 mM phosphate pH 6.8 buffer plotted as a function of concentration of crystalline resveratrol added. Solutions contained 20 mg/ml of PVP. The images show precipitate formation.

increase in the amount of the gelatinous precipitate seen (Fig. 3). The precipitate was analyzed by powder X-ray diffraction and was found to be non-crystalline. Infrared analysis of the isolated and dried precipitate showed that it contained both resveratrol and PVP (data not shown).

Amorphous Solid Dispersion Solution Behavior

In order to investigate the phase behavior of the solid dispersions slurried in dissolution media, Raman spectroscopy was used to monitor the evolution of crystallinity as a function of time, and at the end of the run (typically 24 h) the solution concentration was determined by UV spectroscopy. The Raman spectra of crystalline resveratrol and amorphous solid dispersions of 40% resveratrol and 60% PVP are shown in Fig. 4. By monitoring the ring stretching in the 1650–1600 cm^{-1} region, it is apparent that the ratio of the peaks

Table II Amount of Resveratrol Added to 18 ml of a 20 mg/ml Solution of PVP in 50 mM Phosphate Buffer at pH 6.8 at 37°C, Theoretically Calculated Expected Concentration in μg/ml, Resveratrol Concentration as Determined by UV–Vis Spectroscopy, and the Difference Between the Observed and Calculated Values

Amount of res added (mg)	Theoretical concentration (μg/ml)	Observed concentration (μg/ml)	Observed-calculated (μg/ml)
11.84	658	709	51
32.31	1795	1610	−185
76.16	4231	1193	−3038
121.4	6744	706	−6038
198.67	11,037	99	−10,938

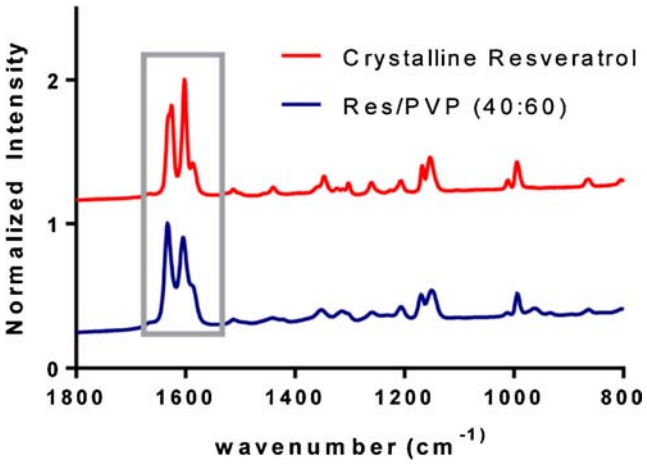

Fig. 4 Raman spectra of crystalline resveratrol and solid dispersion of resveratrol/PVP (40:60).

at 1630 cm^{-1} and 1605 cm^{-1} changes as a function of crystallinity. A fully crystalline sample has a 1630/1605 cm^{-1} peak ratio of 0.76, while the amorphous solid dispersions have a value around 1.1 (note: this value differed slightly for all the solid dispersions and no pure amorphous reference was available). Therefore this peak ratio was used to qualitatively monitor the evolution of crystallinity in the slurried amorphous dispersions.

Effect of Polymer Type and Amount

The transformation kinetics of resveratrol solid dispersions formed with different polymers at a drug level of 35% by weight and at a solid dispersion loading of about 20 mg/ml are compared in Fig. 5, whereby it is shown that the crystallization behavior of resveratrol in slurries was highly dependent on the polymer used to form the dispersion. Both PVP and E100 dispersions remained amorphous over the time scale of the study. HPMC, HPMCAS, and CMCAB dispersions exhibited some crystallization within the first hour, although complete crystallization was never reached. These samples

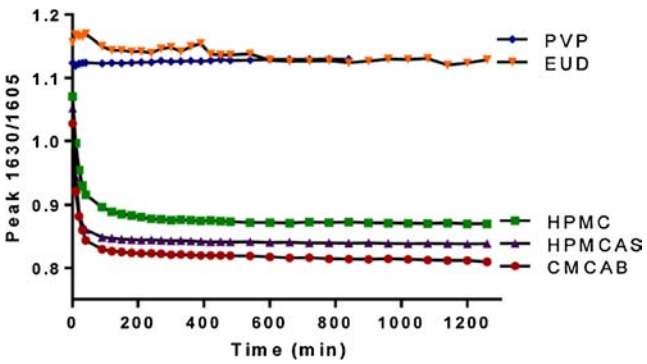

Fig. 5 Crystallization kinetics of various resveratrol solid dispersions (35 wt. % resveratrol) following immersion in 50 mM phosphate pH 6.8 buffer as monitored by the ratio of 1630/1605 cm^{-1} peaks in the Raman spectra, $n = 3$.

crystallized very rapidly to a plateau value and then remained partially crystalline for the duration of the experiment. At the 35% resveratrol level, CMCAB dispersions crystallized the most, followed by HPMCAS and then HPMC dispersions for which the least crystallization was observed.

The solution concentrations of resveratrol determined at the end of the study (~24 h) following separation of the solution from any undissolved material are listed in Table III. No enhancement in resveratrol solubility was found for resveratrol dispersions formed with E100 or PVP, even though these dispersions did not recrystallize, or with CMCAB from which the resveratrol partially recrystallized. Even though the dispersions with the cellulosic polymers partially crystallized, the HPMC and HPMCAS systems resulted in enhanced resveratrol solution concentrations. Since an increase in equilibrium resveratrol solubility was seen for dispersions prepared with HPMC and HPMCAS (Fig. 2), the polymer solution concentration was also measured at the end of the 24 h for these systems. For HPMC, the polymer concentration was below the level at which an increase in the equilibrium solubility of crystalline resveratrol was observed (refer to Fig. 2), but for HPMCAS, the polymer concentration was sufficiently high to solubilize resveratrol. To obtain a measure of the apparent resveratrol supersaturation in the HPMCAS solution, the concentrations were corrected to account for the enhancement in crystalline resveratrol solubility in the presence of the polymer. After accounting for the solubilization of crystalline resveratrol by dissolved HPMCAS (Fig. 2), it is evident that supersaturation exists even at the 24 h time point.

Changing the resveratrol-to-polymer ratio significantly influenced the resveratrol crystallization kinetics profile (Fig. 6). Only the cellulosic polymers were included in this experiment as they were the only dispersions for which resveratrol crystallization was observed. Increasing the percentage of polymer led to a decrease in the extent of crystallization observed, and hence more of the sample remained amorphous. For all the polymers, there was a minimum level of polymer needed to completely inhibit crystallization upon slurrying in aqueous media (Table IV). Upon analyzing the solution concentrations of resveratrol after 24 h, a correlation between the amount of crystallized resveratrol and the level of apparent supersaturation was found: unsurprisingly the larger the extent of crystallization, the less enhanced the solution concentration relative to the crystal solubility (Fig. 7). The degree of solution concentration enhancement from the HPMCAS dispersion was much greater than that from the HPMC dispersion. For example, for a non-crystallizing system, the increase in resveratrol solution concentration above the equilibrium solubility (measured in the presence of dissolved polymer) is 50 μg/ml for HPMC (90% polymer) *vs.* 1166 μg/ml for HPMCAS (85% polymer). This is a noteworthy increase in solution concentration and will be discussed in detail later.

Table III Summary of Solution Concentrations for Amorphous Solid Dispersions in 50 mM Phosphate Buffer at pH 6.8 at 37°C

Solid dispersion (65% Polymer)	Resveratrol solution concentration (μg/ml)	Polymer solution concentration (mg/ml)	Solubility increase due to polymer (μg/ml)	Excess concentration (μg/ml)	Crystallized
E100	46	NA[a]	None	None	No
PVP	42	NA[a]	None	None	No
HPMCAS	458	4.7	302	155	Partial
HPMC	62	3.7	None	20	Partial
CMCAB	39	NA[a]	None	None	Partial

Apparent supersaturation can be evaluated by calculating the excess resveratrol concentration above the crystalline solubility resveratrol in a medium containing the appropriate amount of polymer. In the absence of a polymer, the resveratrol solution concentration was 42 ± 6 μg/ml

[a] Not assessed due to the insoluble polymer

Effect of pH

Transformation kinetics of the HPMCAS dispersions at a resveratrol level of 25% were monitored at two different pH conditions (pH 6.8 and pH 1.2, Fig. 8). It is clear that there was no significant difference in the crystallization rate and extent of the sample between pH conditions that model gastric and small intestinal environments. However, it should be noted that at pH 6.8 HPMCAS is soluble, while at pH 1.2 HPMCAS is essentially insoluble. This is evident from the polarized light microscopy images (Fig. 9) which show that the solid dispersion particles at pH 6.8 disperse and dissolve with evidence of the formation of small particles of crystalline material, while the dispersion particles in pH 1.2 media remain intact and crystallization is seen within the matrix of the dispersion. Since the crystallization kinetics are comparable, these data suggest that the route of crystallization may also be analogous. While the crystals in the pH 1.2 sample clearly are a result of crystallization from the matrix, it can be inferred that those in the pH 6.8 sample also most likely resulted from crystallization from the matrix, rather than first dissolving and then crystallizing from a supersaturated solution.

Effect of Solid Dispersion Solids Loading on Resultant Solution Concentration

The amount of solid dispersion added to solution had an effect on the observed solution concentration of resveratrol, as shown for the 15% resveratrol:85% HPMCAS solid dispersion in Fig. 10a. As the solid dispersion loading was increased, the resveratrol solution concentration increased; however, the solution concentrations were only approximately half of the maximum resveratrol concentration achievable if all the solid dispersion added dissolved. This increase with solids loading continued until a maximum resveratrol solution concentration of 1600 μg/ml was achieved. Further increases in the solid dispersion loading resulted in decreases in the measured solution concentration and the formation of a gelatinous precipitate. The polymer solution concentration followed the same trend; however, after the maximum concentration was attained, the decrease in solution polymer concentrations was not as prominent as for the resveratrol concentrations and the data were more scattered.

In order to assess the thermodynamic state of the resveratrol molecules in solution (complexed or free), diffusion experiments across a semipermeable membrane were performed. The effects of the solid dispersion loading and resveratrol solution concentrations on the diffusion rate are reported in Fig. 10b. Faster flux rates are consistent with higher concentrations of free drug in solution; therefore, these data suggest that at lower solids loadings, dissolution of the solid dispersion leads to more free resveratrol in solution. As the amount of solid dispersion increased, the measured solution concentration of resveratrol increased, and the flux decreased, indicating that the amount of free resveratrol was reduced. This scenario continued until the point where the maximum resveratrol solution concentration was achieved (~1600 μg/ml). At this point the free resveratrol reaches a minimum and then appears to become relatively constant with continued increases in concentration. The flux of the saturated crystalline solution (suspension of crystalline drug in the absence of a polymer) was 2.0 μg/min, which was lower than for any of the solid dispersion solutions indicating that some extent of supersaturation was generated by dissolution of the solid dispersions. Interestingly, the degree of supersaturation, which is directly proportional to the flux, varied with the solid dispersion loading but did not increase as a function of the amount of resveratrol released into solution, in fact, showing the opposite trend.

DISCUSSION

While an amorphous solid dispersion needs to have acceptable solid state stability during storage, ultimately its effectiveness *in vivo* depends on its ability to generate a supersaturated solution upon dissolution. This means that the polymer must be able to prevent crystallization of the drug upon exposure to

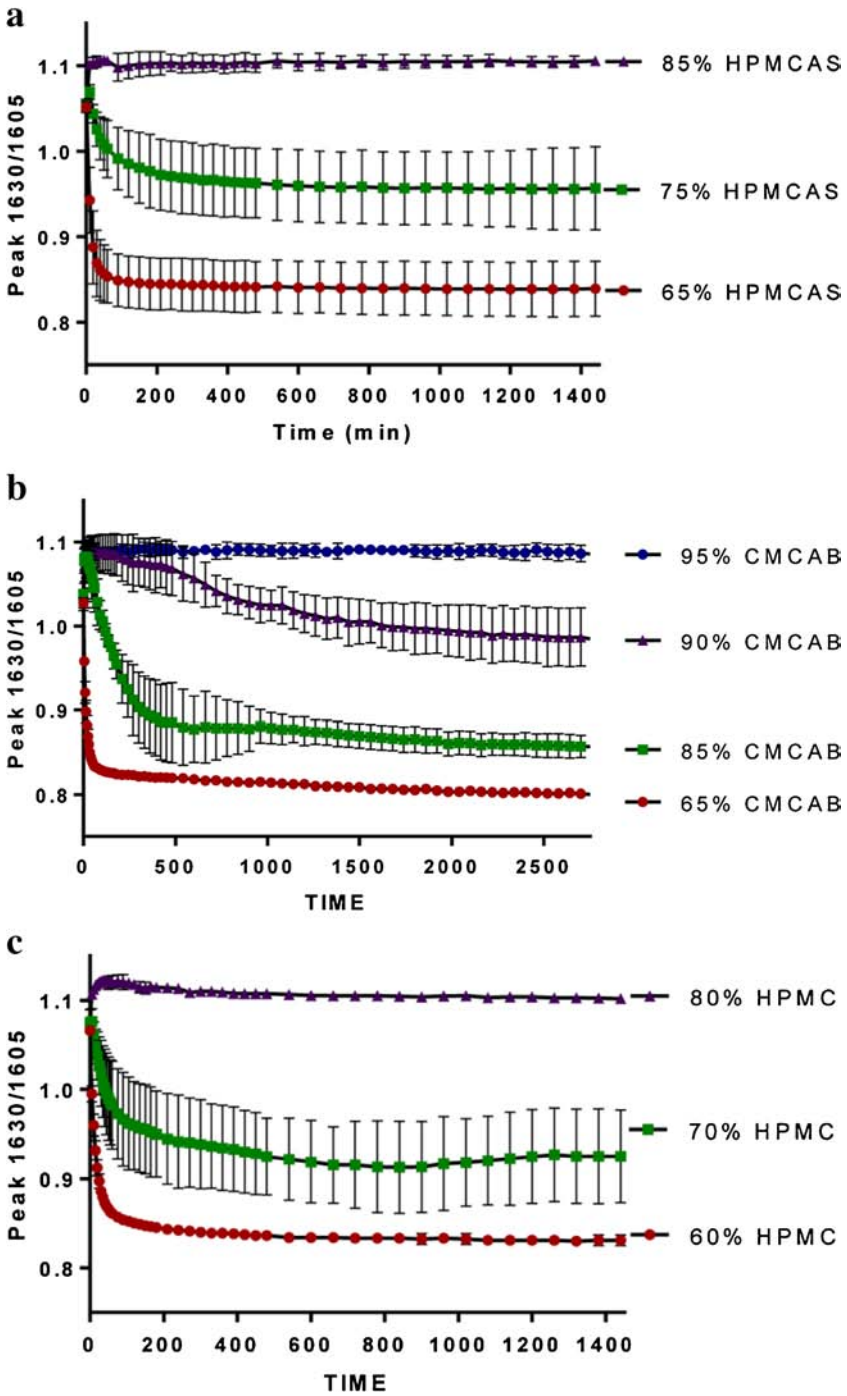

Fig. 6 Crystallization kinetics in 50 mM phosphate pH 6.8 buffer of (**a**) resveratrol:HPMCAS solid dispersions with 85, 75, and 65 wt.% HPMCAS (**b**) resveratrol: CMCAB solid dispersions with 95, 90, 85, and 65 wt.% CMCAB and (**c**) resveratrol:HPMC solid dispersions with 80, 70, and 60 wt.% HPMC as monitored by the ratio of 1630/1605 cm^{-1} peaks in the Raman spectra, $n = 3$.

aqueous media; both from the hydrated matrix and from the supersaturated solution expected to be generated upon dissolution of an amorphous formulation (20). In a supersaturated solution, the chemical potential of the solute is higher than the chemical potential of the corresponding crystal whereby the chemical potential difference provides the thermodynamic driving force for phase transformation (21). The higher chemical potential of the drug in a supersaturated solution is also responsible for the enhanced membrane transport seen for supersaturated solutions (19), and consequently the improved bioavailability (22). In contrast, if complexation occurs between the polymer and drug, although the total solution concentration may be enhanced, the concentration of free drug is not increased and hence faster membrane transport is not observed (23,24). Therefore, when evaluating solubility enhancing formulations, it is important to first determine if the formulation is stable to crystallization when exposed to aqueous media, and then to evaluate whether any observed

Table IV The Minimum wt. % of Polymer Needed to Inhibit Crystallization During Dissolution in 50 mM Phosphate pH 6.8 buffer at 37°C

Polymer	% Needed to inhibit crystallization
CMCAB	95%
HPMCAS	85%
HPMC	80%
E100	65%
PVP	65%

enhancements in solution concentration relative to the reference crystal solubility are due to supersaturation (driving force for crystallization and membrane transport) or solubilization (for polymeric systems, solubilization results from the formation of drug-polymer complexation).

The phase behavior of amorphous solid dispersions following addition to aqueous media is extremely complex with many processes taking place simultaneously including hydration of the matrix, phase changes within the matrix, drug and polymer dissolution, drug-polymer solution interactions, and/or crystallization from solution. Resveratrol has a very high melting point and is classified as a rapid crystallizer according to the classification system of Van Eerdenbrugh *et al.*(25), therefore having effective polymeric crystallization inhibitors is essential. It was noted previously that only polymers that

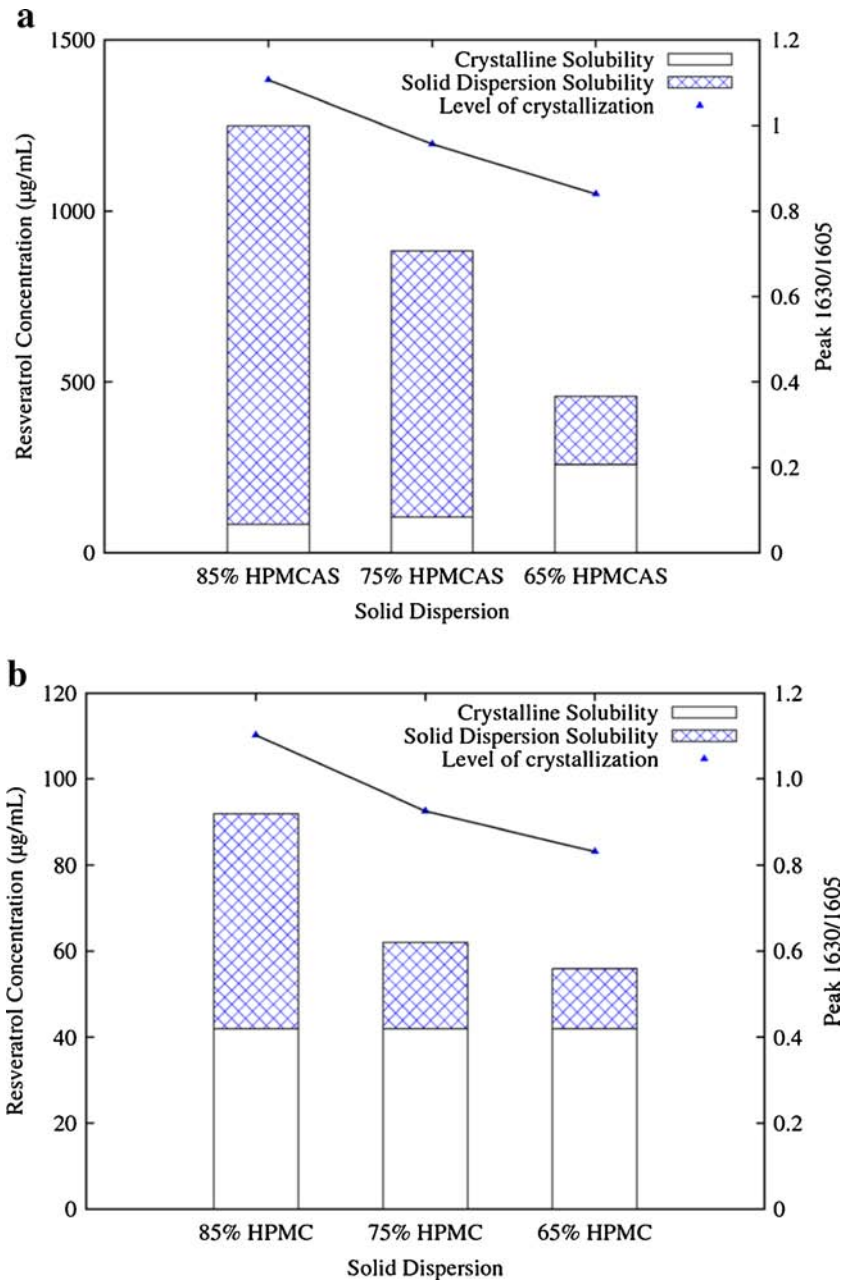

Fig. 7 Bar graph (*left axis*) shows the equilibrium crystalline solubility of resveratrol and (**a**) HPMCAS and (**b**) HPMC solid dispersion solubility after 24 h of equilibration in pH 6.8 buffer, for solid dispersions with different polymer levels. Line graph (*right axis*) is the ratio of 1630/1605 cm^{-1} peaks in the Raman spectra at the end of 24 h.

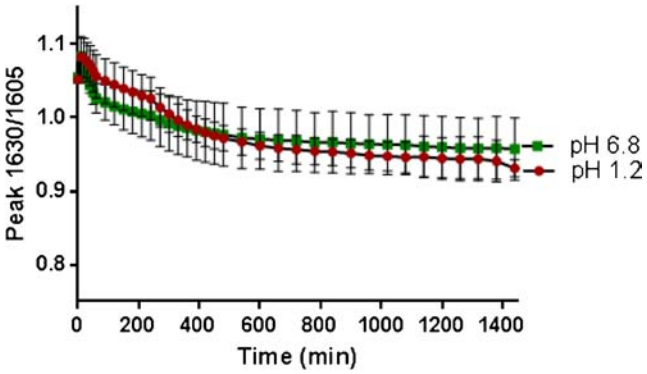

Fig. 8 Crystallization kinetics of resveratrol:HPMCAS solid dispersions with 25 wt.% resveratrol in slurries at pH 6.8 and pH 1.2 as monitored by the ratio of 1630/1605 cm^{-1} peaks in the Raman spectra, $n = 3$.

formed strong intermolecular interactions with resveratrol were effective crystallization inhibitors in powdered amorphous solid dispersions exposed to high RH and temperature (7), Thus PVP which formed strong hydrogen bonds and E100 which participated in an ionic interaction with resveratrol were effective at preventing crystallization (7). In contrast, dispersions with the cellulosic polymers HPMC, HPMCAS, and CMCAB, which formed much weaker hydrogen bonds with resveratrol in the dispersions, crystallized rapidly when exposed to high RHs (7). Based on these observations, it was anticipated that these same intermolecular interactions would be important in delaying crystallization in an aqueous environment.

From the Raman experiments shown in Figs. 5 and 6, it is evident that the polymer does indeed play an important role in determining the crystallization kinetics in solution. PVP and E100 dispersions were resistant to crystallization when immersed in the dissolution medium for up to 24 h, and thus behaved in a similar manner to solid dispersion powders exposed to high RH. In contrast, the dispersions formed with the cellulosic polymers were not stable in the dissolution medium unless there was a large quantity of polymer in the dispersion. Resveratrol crystallization appeared to occur in or at the surface of the matrix based on evaluation by microscopy as well as the fact that rapid crystallization was observed by Raman spectroscopy, even when compound release was prevented as a result of polymer insolubility, as for CMCAB dispersions and HPMCAS dispersions at pH 1.2 (Fig. 9).

Interestingly, even though the PVP and E100 solid dispersions were physically stable, elevated resveratrol solution concentrations relative to the crystal solubility were not observed (Table III). For E100, this observation can be rationalized on the basis that the polymer does not dissolve at pH 6.8, and therefore the release of the drug from the matrix is restricted. However, PVP is extremely soluble in pH 6.8 buffer so another explanation must be sought. It is proposed that the low resveratrol solution concentrations observed from this system result from the formation of an insoluble resveratrol-PVP complex. Indeed, it was observed that the solid dispersion did not readily dissolve, suggesting that the dispersion was not soluble. Furthermore, an amorphous precipitate was observed when crystalline resveratrol was added to a solution containing PVP (Fig. 3) supporting the formation of an insoluble complex. Therefore, it appears that the insolubility of the dispersion arises as a result of resveratrol-polymer complexation.

It has been reported that PVP forms insoluble complexes with phenolic compounds (26–28). Resveratrol contains 3 phenol groups, thus the complexation can be readily rationalized. Previous studies with phenolic compounds and PVP have suggested that there is a critical concentration at which the precipitate is formed (26,27). At low concentrations of phenol, the amount of phenol-polymer binding is low, and the complex remains in solution (27). As the ratio of phenol to PVP is increased, phenol interactions with the polymer will displace some of the hydrogen bonded water molecules (27). At this point the complex becomes insoluble due to desolvation effects, and precipitates out of solution (27). Evidence of this type of complex formation was seen when measuring resveratrol crystalline solubility in a solution of PVP whereby when low amounts of resveratrol were added no precipitate was seen, but once a critical concentration was achieved, a precipitate formed (Fig. 3). This precipitate contained resveratrol and PVP, and was non-crystalline. Therefore, it is clear that both soluble and insoluble complexes are formed. Thus it can be inferred that the solution concentration increase reported in Fig. 3 and Table II is due

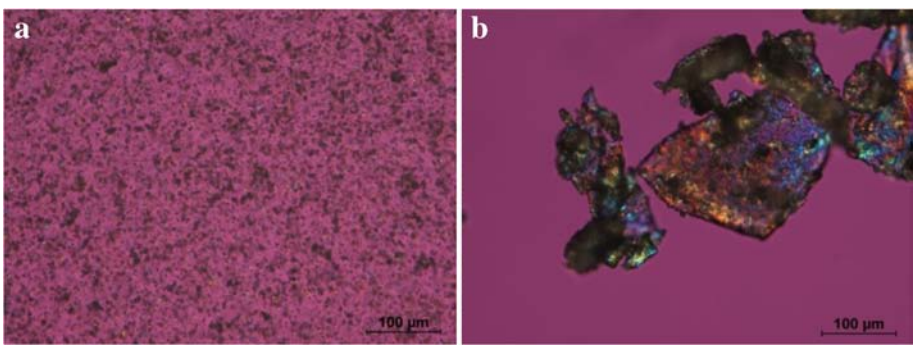

Fig. 9 Polarized light microscopy image of the dissolution slurry after 24 h for the resveratrol:HPMCAS solid dispersions at 25 wt.% resveratrol in a dissolution medium of (**a**) pH 6.8 and (**b**) pH 1.2.

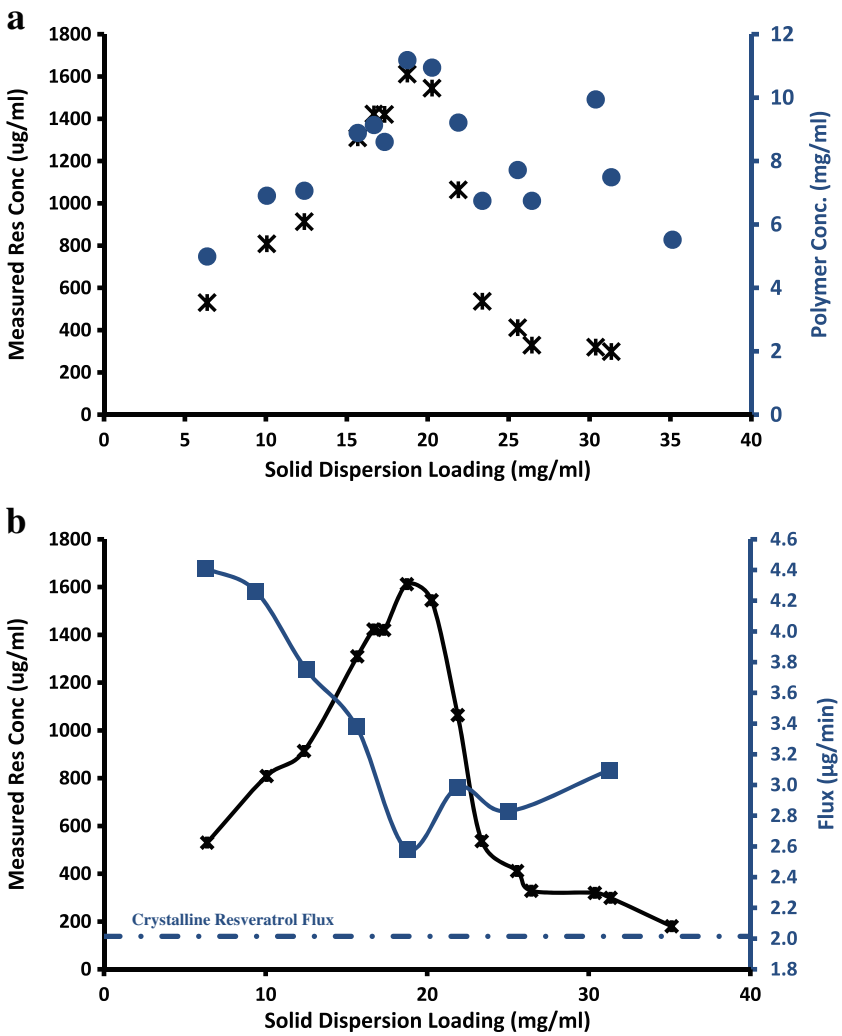

Fig. 10 Relationship between solid dispersion loading (15% resveratrol/85% HPMCAS) in 50 mM phosphate pH 6.8 buffers and (**a**) measured solution resveratrol (*left* y-axis *cross symbols*) and polymer concentration (*right* y-axis *circle symbols*); and (**b**) the measured resveratrol concentration (*left* y-axis *cross symbols*) and the membrane flux (*right* y-axis *square symbols*).

to an increase in soluble complex at certain resveratrol:PVP ratios. As more resveratrol is added to the solution, an insoluble complex forms and resveratrol is removed from solution. This leads to an improved understanding of why no resveratrol crystallization occurs in the PVP dispersion system: the formation of an insoluble amorphous complex in an aqueous environment would be expected to hinder crystallization. Thus the formation of strong compound-polymer interactions in the matrix may be favorable in terms of preventing crystallization during storage of solid formulations, but may be detrimental in terms of compound release upon dissolution.

In the case of dispersions prepared with the cellulosic polymers, resveratrol crystallization was observed in a solution environment, with the extent of crystallization increasing as the amount of polymer decreased. The crystallization is thought to be due to the insufficiently strong intermolecular interactions between resveratrol and these polymers (7). However, at low resveratrol loadings, crystallization could be inhibited, even when the solid dispersion was slurried in aqueous media for 24 h. The dependence of the crystallization extent on the amount of polymer most probably reflects the number of functional groups available on the polymer for interaction. The cellulosic polymers do have some functional groups, *e.g.*, hydroxyl groups, that can hydrogen bond with resveratrol, however, these are somewhat limited in number (7). Thus, as the amount of polymer in the dispersion is decreased and the level of resveratrol is increased, the number of functional groups on the polymer capable of interacting with resveratrol decreases, leading to more crystallization. In addition, at low resveratrol loadings, it is more highly dispersed within the polymer matrix and this will also hinder crystallization.

It was interesting that, in spite of the tendency of resveratrol to undergo crystallization, the cellulosic dispersions (with the exception of CMCAB) were the only systems to show any level of enhanced solution concentration over and beyond the solubility of crystalline resveratrol. However, the extent of solubility enhancement was quite different for the HPMC and HPMCAS dispersions. For the dispersions where no crystallization was observed during slurrying (80% HPMC and 85%

HPMCAS, see Fig. 6), the HPMC dispersion had an increase in resveratrol solution concentration of 50 μg/ml over the equilibrium crystal solubility, while the HPMCAS dispersion led to an enhancement in solubility of approximately 1166 μg/ml (Fig. 7). This difference can be explained in part by an increase in thermodynamic solubility of resveratrol by the polymer, which will depend in turn on the amount of polymer released from the dispersion. Hence the HPMC concentration in solution after 24 h was 2.6 mg/ml, which had no effect on the equilibrium solubility of crystalline resveratrol. In contrast, the HPMCAS concentration following dissolution was 4.7 mg/ml which has a substantial impact on the equilibrium solubility of crystalline resveratrol increasing it from 42 to 83 μg/ml (Figs. 2 and 7). Thus it appears that at least some part of the concentration enhancement can be attributed to the formation of a soluble resveratrol-polymer complex.

While it is important to transfer the solid material into the solution phase, the primary goal of using amorphous solid dispersions is to enhanced bioavailability through generation of supersaturated drug solutions. Based on the measurements shown in Fig. 10, the maximum flux across a membrane observed for a 15:85 resveratrol:HPMCAS dispersion was only approximately a factor of two higher than the flux generated from a saturated solution of resveratrol, even though the total solution concentration was much higher than the crystalline solubility. Furthermore, for this particular system, the flux inversely correlated with the concentrations of resveratrol and HPMCAS. These results indicate that the resveratrol concentration enhancement is mainly due to the formation of a soluble resveratrol-HPMCAS complex, and that the maximum amount of free resveratrol is only a factor of two higher than the amount of resveratrol present in a saturated solution (29,30). In other words, the extent of resveratrol supersaturation in the system is very limited and although the concentration that evolves is much higher than its crystal solubility, only a relative modest increase in mass transport is observed. This is in contrast to observations made with felodipine amorphous solid dispersions, where flux rates ten times higher than for the crystalline form were achieved upon dissolution of amorphous solid dispersions (31).

In summary, findings from this study suggest that dissolution of amorphous solid dispersions of resveratrol can lead to elevated solution concentrations through the formation of soluble complexes with the polymer, rather than by generating highly supersaturated solutions as it typically assumed. The formation of soluble complexes might explain the very high concentrations observed to evolve from several polyphenol-polymer dispersions (32–36). The thermodynamic properties of solutions containing complexes are quite different from supersaturated solutions, as shown by flux measurements. Furthermore, when the compound- polymer interactions are extremely favorable, it is also possible to form insoluble

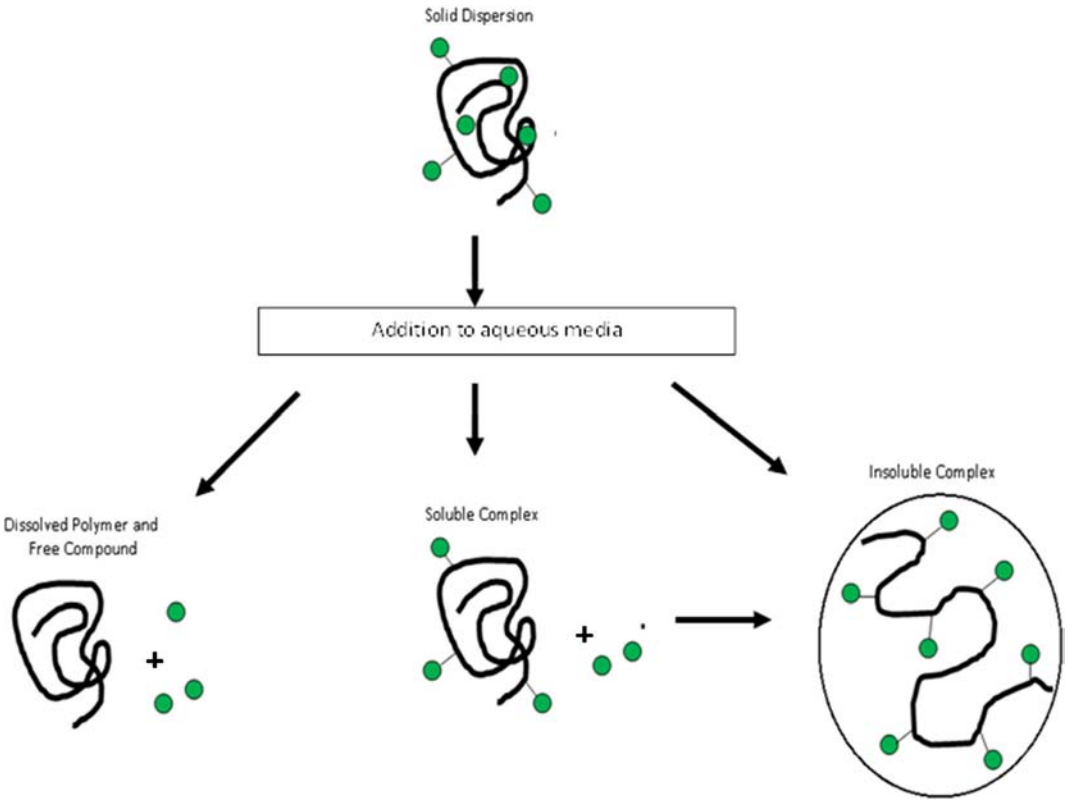

Fig. 11 Cartoon illustration showing the dissolution of the solid dispersion and the potential formation of soluble and insoluble compound-polymer complexes with increasing concentration.

complexes which may limit the amount of dissolution of the formulation even though crystallization is delayed. These various scenarios are summarized in the schematic shown in Fig. 11.

CONCLUSIONS

Preventing crystallization both in the solid state and in solution is an essential component of an amorphous solid dispersion formulation strategy. During dissolution, crystallization can occur from the amorphous matrix thus negating the solubility advantage of the amorphous form. Additionally, the compound must be released from the dispersion in order to achieve elevated solution concentrations; this can be hindered by the formation of an insoluble compound-polymer complex, which was observed for resveratrol-PVP dispersions. Furthermore, soluble complex formation can cause an increase in the solution concentrations observed without leading to enhanced membrane transport. This situation was observed for resveratrol-HPMCAS dispersions. Overall, while strong compound-polymer interactions are favorable for reducing crystallization tendency, they may in certain cases lead to polymer:drug complex formation, which must be assessed when evaluating solid dispersions.

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