Pharm Res (2014) 31:500–515
DOI 10.1007/s11095-013-1178-1

RESEARCH PAPER

Correlation of Inhibitory Effects of Polymers on Indomethacin Precipitation in Solution and Amorphous Solid Crystallization Based on Molecular Interaction

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Received: 13 December 2012 / Accepted: 9 August 2013 / Published online: 12 October 2013

ABSTRACT

Purpose To correlate the polymer's degree of precipitation inhibition of indomethacin in solution to the amorphous stabilization in solid state.

Methods Precipitation of indomethacin (IMC) in presence of polymers was continuously monitored by a UV spectrophotometer. Precipitates were characterized by PXRD, IR and SEM. Solid dispersions with different polymer to drug ratios were prepared using solvent evaporation. Crystallization of the solid dispersion was monitored using PXRD. Modulated differential scanning calorimetry (MDSC), IR, Raman and solid state NMR were used to explore the possible interactions between IMC and polymers.

Results PVP K90, HPMC and Eudragit E100 showed precipitation inhibitory effects in solution whereas Eudragit L100, Eudragit S100 and PEG 8000 showed no effect on IMC precipitation. The rank order of precipitation inhibitory effect on IMC was found to be PVP K90 > Eudragit E100 > HPMC. In the solid state, polymers showing precipitation inhibitory effect also exhibited amorphous stabilization of IMC with the same rank order of effectiveness. IR, Raman and solid state NMR studies showed that rank order of crystallization inhibition correlates with strength of molecular interaction between IMC and polymers.

Conclusions Correlation is observed in the polymers ability to inhibit precipitation in solution and amorphous stabilization in the solid state for IMC and can be explained by the strength of drug polymer interactions.

KEY WORDS molecular interactions · polymers · poorly soluble drugs · precipitation inhibition · solid dispersion

INTRODUCTION

Supersaturation based drug delivery technologies are used to improve the solubility and bioavailability of poorly soluble compounds. The principle of this technology is to deliver drug as supersaturated solutions, amorphous or metastable crystalline forms to take the advantage of their higher apparent solubility (1). The challenge of this technology is to maintain the stability of the metastable form in both solution and solid state, both *in vitro* and *in vivo*. Precipitation inhibition and maintenance of drugs concentration is needed in the gastrointestinal (GI) tract for increased exposure in the case of oral delivery (2,3). Polymeric excipients are widely used to maintain the metastable state by preventing precipitation in solution and by inhibiting crystallization of amorphous drugs (2–6). The polymer effect on crystallization inhibition is often

Electronic supplementary material The online version of this article (doi:10.1007/s11095-013-1178-1) contains supplementary material, which is available to authorized users.

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attributed to mechanisms such as decreased molecular mobility of drug polymer matrix; solubilization of drugs in polymer matrix; molecular interaction between drug and polymers *etc*. (7–9). These mechanisms interfere with the crystallization of drug molecules but are not fully understood. It has been reported that the stabilization effect of a polymer on the crystallization of drugs doesn't correlate with glass transition temperature (Tg), which is an indicator of molecular mobility. Also, it has been found that polymers with low concentration can stabilize drug effectively (10). Based on these observations, it has been proposed that molecular interaction plays a dominant role in preventing crystallization. However, this hypothesis has not been systematically studied.

In this study, the mechanism of polymeric excipients on the precipitation inhibition in solution was studied and correlated to the inhibitory effect of amorphous crystallization prevention in a model compound, IMC. The effects of the commonly used polymeric excipients, including PVP K90, HPMC, Eudragit E100, Eudragit S100, Eudragit L100 and PEG 8000, on the crystallization of IMC in solution and from amorphous solids were studied. Molecular interaction between IMC and polymers were characterized using spectral and computational methods. We hope that understanding and correlating the importance of drug polymer interaction on crystallization inhibition on a model compound can help aid in rationale selection of excipients for stabilizing a metastable formulation delivery.

MATERIALS AND METHODS

Indomethacin (IMC) was purchased from Sigma (St. Louis, Missouri), Polyvinyl pyrrolidine (PVP K90) was purchased from Spectrum (New Brunswick, New Jersey), Hydroxypropyl methyl cellulose (HPMC) was purchased from Dow Company (Midland, Michigan), Eudragit E100, Eudragit S100 and Eudragit L100 were purchased from Degussa (Parsippany, New Jersey) and PEG 8000 was purchased from Sigma (St. Louis, Missouri). Ethanol, methanol, methylene chloride and hydrochloric acid (HCl) were of analytical or HPLC grade. All materials were used as received.

Precipitation Studies

IMC solution of 0.2 mg/mL was prepared using an equal volume mixture of absolute ethanol and 0.01 N hydrochloric acid. PVP K90, HPMC, Eudragit E100, Eudragit S100, Eudragit L100 and PEG 8000 were added in the IMC solution at 5:1 and 1:1 (drug: monomer) molar ratios. For polymers showing precipitation inhibition, other polymer to drug ratios were also investigated as shown in Table I. The precipitation studies were carried out by adding 20 ml (0.2 mg/ml) of the IMC solution to 200 ml of water at a rate of 2 ml/min using a syringe Pump (Harvard 33' Syringe Pump Holliston, MA). The concentration of IMC and the turbidity in the solution phase were determined simultaneously using Cary 50 Probe UV Vis continuous spectrophotometer (Varian analytical instrument, Palo Alto, California) at 254 nm and 500 nm respectively. The initial and final pH and viscosity were measured using Orion 3 star pH meter and Brookfield DV 2 Viscometer respectively. Precipitates from the above experiment were collected by filtering the solution through 0.45 μm membrane filter. The precipitates were then dried overnight under vacuum (Fisher Scientific Isotemp Model 281A Vacuum oven, Pittsburgh, Pennsylvania) and were characterized using X-ray powder diffraction (XRPD), Infrared (IR) spectroscopy and Scanning electron microscopy (SEM).

Table I Experimental Details of the Polymers and their Concentrations Used in Indomethacin Precipitation Studies

S.No	Polymer	Molar ratio (Drug/Monomer)
1	Control	No polymer
2	PVP K90	40:1*,30:1,20:1,10:1,5:1,2:1,1:1
3	HPMC	5:1,3:1, 2:1*,1:1,1:2
4	Eudragit E100	15:1, 10:1*,5:1,1:1
5	Eudragit S100	5:1,1 :1
6	Eudragit L100	5:1,1:1
7	PEG 8000	5:1,1:1

* The highest drug to monomer concentration of each excipient that showed precipitation inhibition

Calculation of the Initiation Time and Rate of Indomethacin Precipitation

The initiation time of IMC precipitation was calculated by determining the slope changes of the absorbance or the turbidity curves (Fig. 1). During precipitation the IMC concentration decreased whereas the turbidity increased. It was found the slope of these two curves change at the same time. Absorbance curves were used to calculate the initiation time and average precipitation rates. The time at which first change in the slope of absorbance curve was observed, minus the 10 min used to add the IMC solution was reported as the precipitation initiation time. During precipitation, the precipitation rate changes as the degree of supersaturation decreases with decreasing concentration of IMC. In the present study, the degree of supersaturation (S) is defined as:

$$S = \left(\frac{C - C^*}{C^*}\right) \quad (1)$$

Where C is the concentration of IMC maintained during the precipitation study and C^* is solubility of IMC with or

without excipients. It was found that the precipitation curves contain several regions where the concentration decreased linearly with time as shown in Fig. 1. For each linear range, the precipitation rate is calculated as the slope of concentration decrease *vs* time and the average concentration of the region is used to calculate the average degree of supersaturation. The average precipitation rate is calculated in terms of moles of IMC precipitating per unit volume with time.

Solubility Determination

An excess amount of IMC was added to the final precipitating medium (10 ml ethanol + 10 ml of 0.01 N HCl mixed with 200 ml of water) in the absence or presence of polymers. The suspension was stirred for 48 h at 25°C and was then filtered using 0.45 μm membrane filters. The IMC concentration was analyzed using an HPLC (Agilient 1200 series with Chemstation software). The mobile phase was water to acetonitrile 1:1 ratio acidified with 0.5% Trifluoroacetic acid. The analysis was conducted with a flow rate of 2.5 ml/min, and a volume of injection of 10 μl, at 254 nm. The column used was C-18, 3.5 μm, 4.6×50 mm Column (Waters, Milford, Massachusetts). Samples were run in triplicates.

Solid Dispersion Preparation and Stability Study

Solid dispersions of IMC with various polymers were prepared by solvent evaporation technique using rotavapor (Buchi rotavapor R 200 series, New Castle, Delaware) or spray-drying (Buchi B-290 with an inert loop attached, New Castle, Delaware). Methanol or methanol/methylene chloride mixtures were used as solvents to prepare solid dispersions. Methylene chloride is used for polymers that are insoluble in methanol. The prepared dispersions were sieved, dried in vacuum oven for 24 h. Physical mixtures of either amorphous or crystalline IMC were prepared by geometrically mixing the required amount of drug with different polymer using a mortar and a pestle. Spray-drying was used to obtain

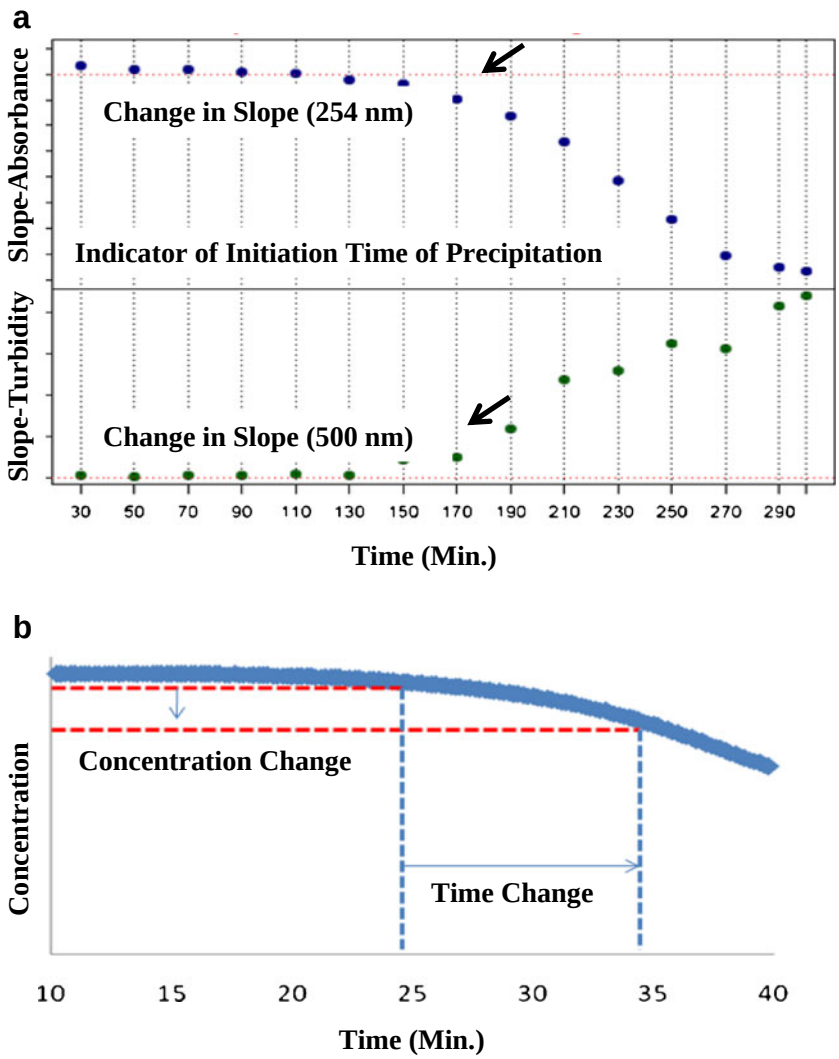

Fig. 1 Determination of (**a**) Precipitation initiation time from slope change of absorbance curves with time, and (**b**) Precipitation rates derived from Concentration *vs*. time. Concentration *vs*. Time curves are derived from indomethacin UV–vis continuous absorbance experiments (Absorbance/Turbidity *vs*. time).

amorphous IMC in the absence of polymer, IMC-PVP K90, IMC-HPMC and IMC-Eudragit S100 dispersion for solid state NMR analysis. A solution of IMC with/without polymer was prepared in methanol and was sprayed using a 1.5 μm nozzle. The inlet temperature was set to 120°C, the aspirator at 100% and the pump at 30%. The condenser was set at −20°C. The dispersions and the mixtures were characterized by XRPD, MDSC, IR and Raman. The different solid dispersions and mixtures prepared in this study are listed in Table II. Solid state stability of IMC dispersions was carried out at 25°C and 60°C for one month.

Characterization of the precipitate obtained from the solution precipitation studies and solid state was carried out by using the following techniques:

Solid State Nuclear Magnetic Resonance (SSNMR)

SSNMR spectra were acquired on Bruker 400 MHz proton frequency wide bore spectrometer. Before obtaining carbon spectra, proton longitudinal relaxation times (^{1}H T_1) were determined by fitting proton saturation recovery data to an exponential function. These values were used to set an optimal recycle delay of carbon cross-polarization magic angle spinning experiment (^{13}C CPMAS), which, typically, was set between $1.2\times{}^1$H T_1 and $1.5\times{}^1$H T_1. The carbon spectra were acquired with 2 ms contact time using linear amplitude ramp on proton channel (from 50% to 100%) and approximately 100 kHz SPINAL-64 decoupling. The typical magic angle spinning (MAS) speed was 12.5 kHz. To limit a frictional heating of sample due to fast spinning, the probe head temperature was maintained at 275 K. Carbon spectra were referenced externally by setting the upfield resonance of solid phase sample of adamantane to 29.5 ppm. Using this procedure, carbon spectra were indirectly referenced to tetramethyl silane at 0 ppm.

Table II Experimental Details of the Polymers and their Concentrations Used in Indomethacin Solid State Studies.

Mixtures	Molar ratio (Drug/Monomer)
All polymers (Crystalline physical mixtures)	5:1, 1:1
All polymers (Amorphous physical mixtures)	1:1
Polymers	**Molar ratio(Drug/Monomer)**
PVP K90	30:1, 20:1, 10:1, 5:1, 3:1, 2:1, 1:1
HPMC	5:1, 3:1, 2:1
Eudragit E100	5:1, 3:1, 2:1
Eudragit S100	5:1, 3:1, 2:1
Eudragit L100	5:1, 3:1, 2:1
PEG 8000	5:1, 3:1, 2:1

Powder X-Ray Diffraction (PXRD)

Samples were analyzed by Powder X-ray diffractometer (PXRD) for crystallinity and form. Bruker AXS-XRD, (Billerica, Massachusetts) with Cu Kα radiation was used. The XRPD patterns were collected in the angular range of 1–40° 2Θ in a step scan mode and analyzed by EVA software (Bruker, Billerica, MA).

Modulated Differential Scanning Calorimetry (MDSC)

Thermal analysis was conducted using MDSC (TA instruments Q 2000 series, New Castle, Delaware) equipped with a liquid nitrogen cooling assembly. Samples of 5–10 mg were prepared in sealed pans. The samples were equilibrated to 10°C; kept isothermal for 5 min, and then heated to 180°C at 2°C per minute ramp with modulation at ± 0.32°C every 60 s. The data was analyzed by TA universal analysis (New Castle, Delaware) software.

Infra Red Spectroscopy

Attenuated total reflectance (ATR) mode was used to obtain IR spectra using Bruker series 80 V IR spectrometer (Billerica, MA). Fifty scans were collected for each sample over a wave number region of 400–4000 cm^{-1}.

Raman Spectroscopy

Raman spectra were obtained using Bruker series 80 V Raman spectrometer; and data processing was performed using OPUS software (Billerica, MA). Data acquisition was done using an accumulation time of 10 s, for 10 accumulations and a laser power setting of 400 mW.

Scanning Electron Microscopy

SEM pictures were obtained using scanning electron microscope Magellon XHR Series (Hillsboro, Oregon) operated between 5 and 24 kV. The specimens were mounted on a metal stub, with double side adhesive tape and coated under vacuum with gold under an argon atmosphere prior to observation.

Molecular Interaction Energy Calculation

The Linux version of the Maestro software suite (Schrödinger, New York, NY) was utilized for molecular modeling. Geometry optimizations using Jaguar were conducted on IMC (B3LYP/6-31G(d)), as well as several excipients: dimers, trimers, tetramers, 5-mers, and 6-mers of PVP, HPMC, Eudragit-S100, Eudragit-L100, and Eudragit-E100. The optimized excipients were subsequently docked onto IMC using

GLIDE with the option of flexible docking and post-docking optimization. The best score from each excipient/API docking result was selected and compared with the best scores from other excipient/API docking results.

RESULTS

Effect of Polymers on the Initiation Time of Indomethacin Precipitation in Solution

The solubility of IMC was found to be 2.11 μg/ml in the final precipitating medium after dilution. Based on Eq. 1, the initial degree of supersaturation was found to be 7.6 after dilution when the starting concentration of IMC is 0.2 mg/ml. To compare the effect of polymer on IMC precipitation, the amount of polymer added is normalized based on drug to monomer molar ratio. At the 1:1 drug to monomer ratio, the solubility of IMC was not changed in the final media. Therefore, the degree of supersaturation in the presence of polymer is comparable to the IMC alone. No change in either the pH or viscosity was detected at these drug/polymers ratios.

The initiation time of precipitation reflects the nucleation rate of precipitation and was determined by reporting the first detectable change in the slope of the precipitation. Without polymers, the precipitation initiation time was 4.7±0.8 min and the precipitated form was found to be the α crystal form of indomethacin. In the presence of polymers at the 1:1 drug to monomer ratio, PVP K90, HPMC and Eudragit E100 increased the precipitation initiation time to 145.6±15.2, 68.6±3.8, 88.4±5.4 min respectively. Furthermore, at a lower polymer concentration ratio of 5:1 (drug: monomer); PVP K 90 and Eudragit E 100 showed an increased initiation time while HPMC didn't showed any effect at this concentration. Eudragit S100, Eudragit L100 and PEG 8000 did not show any significant increase in precipitation initiation time at neither ratios of 1:1 and 5:1 (Figs. 2 and 3).

Among the polymers that showed inhibitory effect on precipitation initiation time, the inhibitory efficiency increased as the concentration of polymers increases except PVP K90. For PVP K90, the inhibitory effects reached a plateau at 10:1 monomer to drug ratio and no further increase in inhibitory efficiency was observed at higher concentrations of PVP K90. HPMC and Eudragit E100 showed concentration dependent increase in IMC precipitation initiation time as shown in Fig. 3. The lowest drug to monomer concentration showing IMC precipitation inhibition was found to be 40:1, 2:1, 10:1 in presence of PVP K90, HPMC and Eudragit E100 respectively (Fig. 3).

Effect of Polymers on the Precipitation Rate of Indomethacin in Solution

IMC precipitation rate changes during the precipitation process as the degree of supersaturation decreases. After nucleation, the precipitation rates increased initially before reaching a constant precipitation rate. Initial higher precipitation rates can be attributed to a simultaneous occurrence of crystal nucleation and growth followed by a period of steady state precipitation rate. Similar phenomena has been reported in solid state where surface crystallization rate of amorphous IMC below the Tg was two orders of magnitude faster than bulk solid crystallization (11) and absorption of water molecules during the crystallization process increased the molecular mobility (12), overall increasing the initial crystallization rate. The average precipitation rate, which is defined as change in concentration over time during linear precipitation rates ($n=3$), is used to compare the effect of polymers on IMC precipitation. PVP K90, HPMC and Eudragit E100 showed significant decrease in the precipitation rate at 1:1 drug: monomer ratio. At the same concentration Eudragit S100 and Eudragit L100 showed slight decrease whereas PEG 8000 did not show any decrease in IMC precipitation rates (Fig. 4).

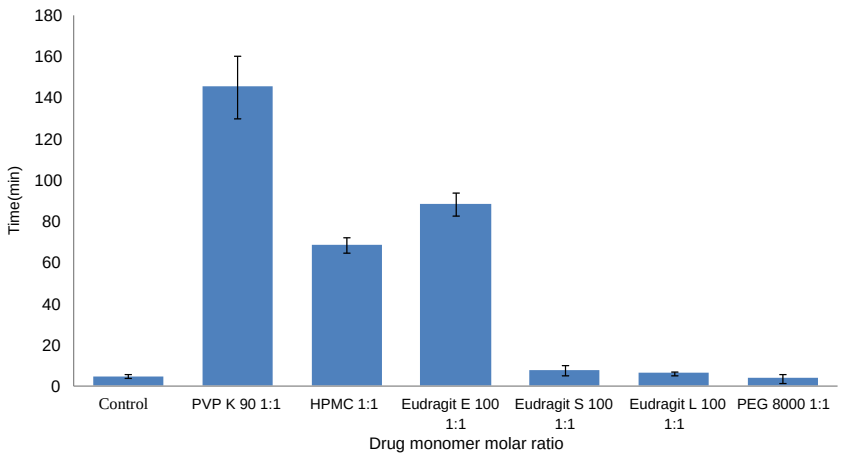

Fig. 2 Comparison of indomethacin precipitation initiation time in the presence of different polymers (Control represents IMC precipitation initiation time in absence of polymer at a supersaturation of 7.6).

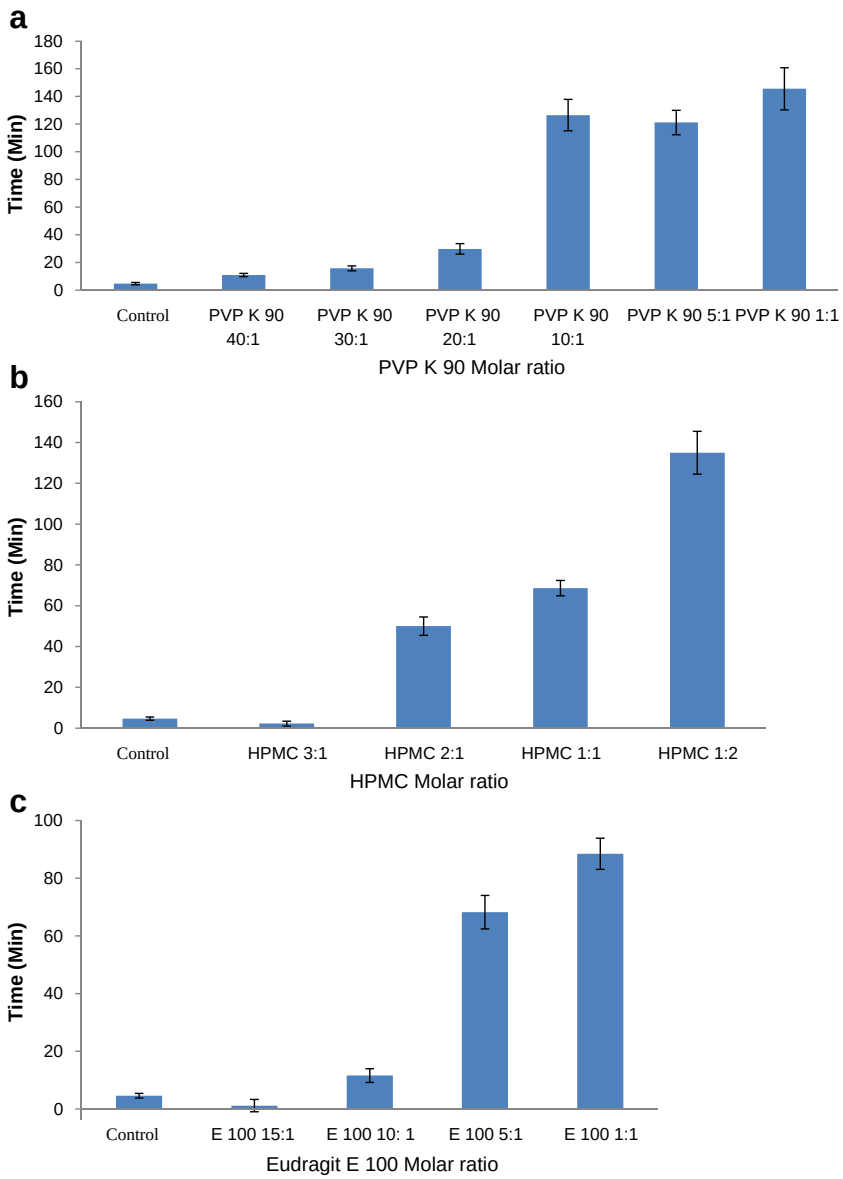

Fig. 3 Precipitation initiation time of indomethacin at different drug to monomer ratios (**a**) PVP K90; (**b**) HPMC and (**c**) Eudragit E100. (Control represents IMC precipitation initiation time in absence of polymer at a supersaturation of 7.6).

Figure 5 shows decrease in indomethacin precipitation rates in presence of PVP K90, HPMC and Eudragit E100 at various drug to monomer concentrations. Similarly, significant decrease in precipitation rate of IMC was observed with PVP K 90 (50:1); HPMC (3:1) and Eudragit E100 (15:1). These detailed study on the effect of PVP K90, HPMC and Eudragit E100 on precipitation rates showed that these polymers started to show effect on the precipitation rate at a lower concentration as compared to the precipitation initiation time, which suggests that polymers can influence crystal growth rate at a lower concentration than nucleation rate.

In presence of PVP K90, HPMC and Eudragit E100, formation of irregular aggregates of α IMC was observed unlike in the presence of Eudragit S100, Eudragit L100 and PEG 8000 where well defined needle shaped crystals of α and γ mixtures were formed (Fig. 6).

Effect of Polymer on the Solid State Stability of Indomethacin Dispersion

Solid dispersions of IMC with or without polymers were characterized by PXRD for crystallinity. At low polymer concentrations, it was observed that polymers that showed precipitation inhibition effects in solution were able to stabilize the dispersion to keep the IMC in the amorphous form while IMC alone or IMC with excipients without precipitation inhibition readily crystallized during preparation or characterization. The rank order of polymer effect on stabilizing amorphous IMC from crystallization based on the amount of polymer needed is: PVP > Eudragit E100 > HPMC > Eudragit S100 & Eudragit L100 > PEG 8000. As shown in Table III, the lowest drug to PVP K 90 monomer ratio that formed solid dispersion was 10:1; unlike HPMC and Eudragit

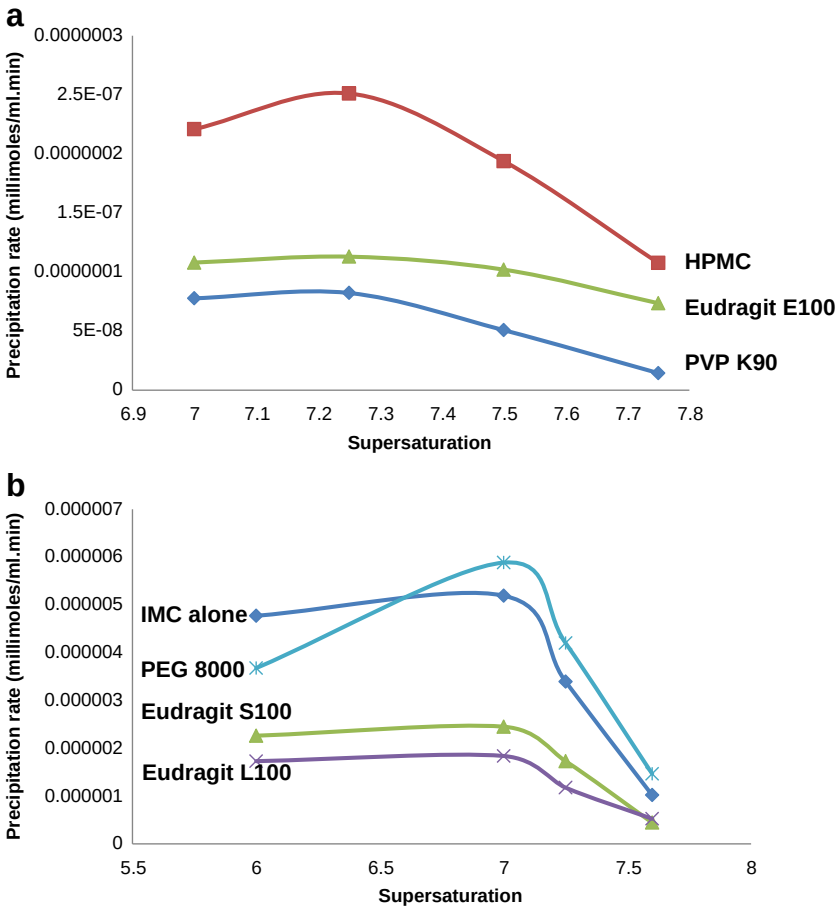

Fig. 4 Indomethacin precipitation rate at 1: 1 drug to monomer concentrations (**a**) HPMC; Eudragit E100; PVP K90 and (**b**) IMC alone; PEG 8000; Eudragit S100; Eudragit L100. (Note: The precipitation rate is calculated as the slope of concentration decrease *vs* time and the average concentration of the region is used to calculate the average degree of supersaturation. The linear ranges of precipitation curve are selected for calculation of precipitation rate.).

E100, which required higher polymer concentration of 5:1 ratio. Eudragit S100, Eudragit L100 formed solid dispersions at 2:1 whereas PEG 8000 was not able to form any dispersion at all the studied concentrations. The solid dispersion of IMC with PVP K90 and Eudragit E100 5:1 ratio showed no crystallization after drying under vacuum for 1 day, whereas HPMC required a minimum of 3:1 IMC: monomer ratio to stabilize the drug in an amorphous form for 1 day. After one month of storage at 25^0 C and 60^0 C, no further crystallization was observed in any of the dispersions that were amorphous initially.

Characterization of Molecular Interaction between Indomethacin and Polymers

XRPD, IR and Raman of IMC physical mixtures showed no change in crystalline form and no detectable interactions were observed. For the dispersion of IMC, peak shifts were observed when comparing these dispersions with physical mixtures of amorphous IMC and polymers.

The stable γ form of IMC at carbonyl stretching region shows strong IR peaks at 1717 cm^{-1} and 1692 cm^{-1} corresponding to asymmetric acid C=O of a cyclic dimer and benzoyl C=O peak respectively. A shift in the above peaks to 1710 cm^{-1} and 1684 cm^{-1} respectively was observed in the amorphous form. In addition the non-hydrogen bonded acid C=O appears as a shoulder at1735 cm^{-1}. Crystalline γ is known to exist as a cyclic dimer, whereas the amorphous form consists mainly of cyclic dimers with a small portion of molecular hydrogen bonded to form a chain. Raman active peaks of benzoyl C=O were observed at 1698 cm^{-1} and 1681 cm^{-1} in the crystalline and amorphous form respectively (13).

For the dispersion of IMC-PVP, the IR spectrum indicates the gradual decrease in the intensity of peak at 1735 cm^{-1} and 1710 cm^{-1}. At higher concentrations both peak disappears along with the appearance of new peak at 1725 cm^{-1}. Slight shifting of benzoyl C=O toward lower wave number was also observed in the spectra. Also, a shoulder at 1638 cm^{-1}started to appear with higher PVP content. This shoulder is assigned to PVP carbonyl group hydrogen bonded to acid hydrogen of IMC. Raman spectra does not show any change in this region (Figs. 7 and 8). For the solid dispersions of IMC-HPMC, IR spectra showed a disappearance of 1710 cm^{-1} peak and an increased intensity of peak at 1735 cm^{-1} (Fig. 9). No change in the shift of benzoyl C=O was observed in the IR spectra but a slight shift was observed in Raman spectra (Fig. 10). For the solid dispersions of IMC-Eudragit E100, significant changes in the carbonyl region could be observed compared to

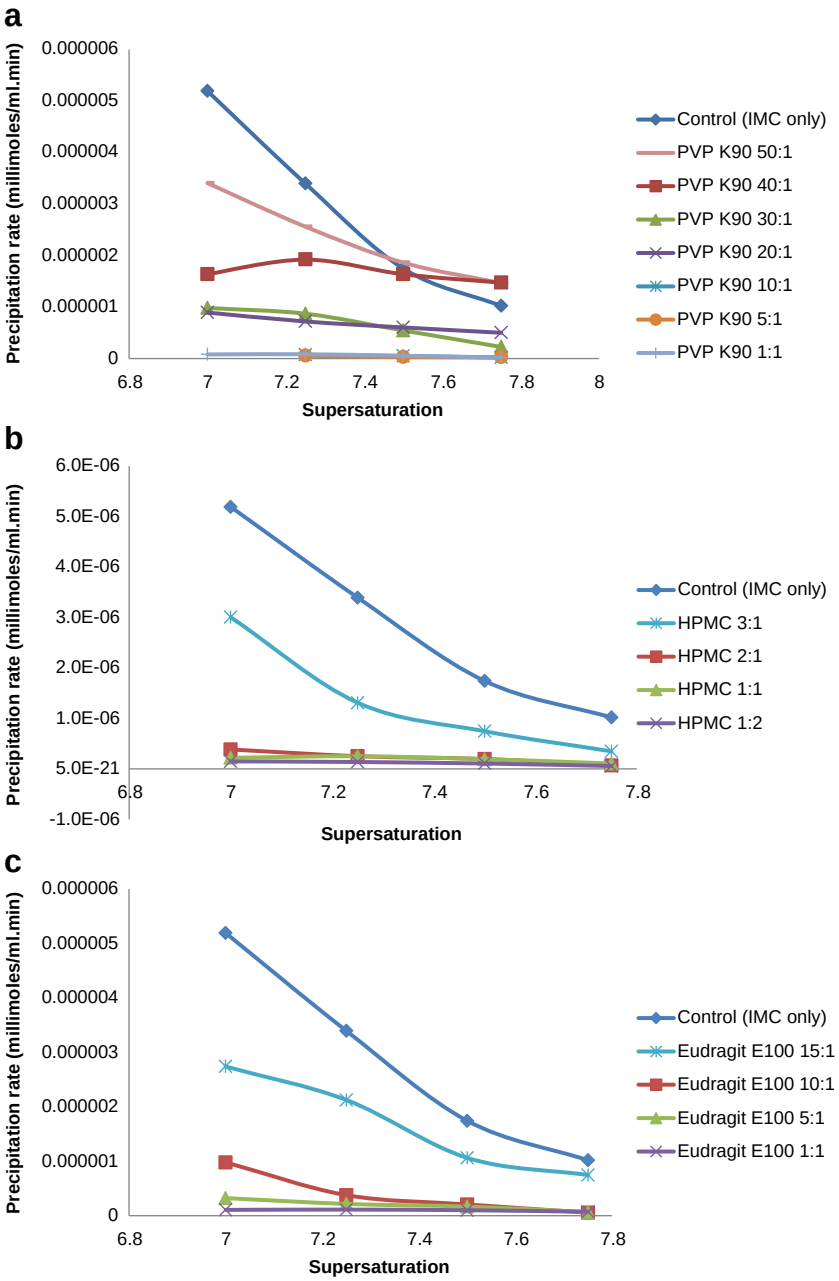

Fig. 5 Indomethacin precipitation rates with various drug to monomer concentrations (**a**) PVP K90 (50:1,40:1, 30:1, 20:1, 10:1, 5:1, 1:1) (**b**) HPMC (3:1,2;1, 1:1, 1:2) and (**c**) Eudragit E100 (15:1,10:1, 5:1, 1:1). (Control represents IMC precipitation rate in absence of polymer).

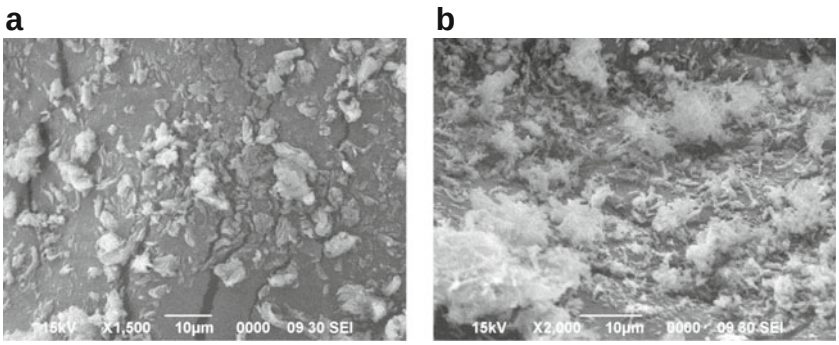

Fig. 6 Indomethacin morphology in presence of (**a**) PVP K90 and (**b**) PEG 8000.

Table III Indomethacin Solid Dispersions*

Polymer	Molar ratio (Drug/monomer)	Drug :polymer formulation (before drying)	Drug :polymer formulation (Vacuum drying 1 day)
IMC:PVP K90	30:1, 20:1	Crystalline	Crystalline
IMC:PVP K90	10:1	Amorphous	Crystalline
IMC:PVP K90	5:1, 3:1, 2:1, 1:1	Amorphous	Amorphous
IMC:HPMC	5:1	Amorphous	Crystalline
IMC:HPMC	3:1, 2:1	Amorphous	Amorphous
IMC:Eudragit E100	5:1, 3:1, 2:1	Amorphous	Amorphous
IMC:Eudragit S100	5:1, 3:1	Crystalline	Crystalline
IMC:Eudragit S100	2:1	Amorphous	Amorphous
IMC:Eudragit L100	5:1, 3:1	Crystalline	Crystalline
IMC:Eudragit L100	2:1	Amorphous	Amorphous
IMC:PEG 8000	3:1, 2:1, 1:1	Crystalline	Crystalline

*No crystallization in any of the amorphous dispersions was observed after 1 day at 25°C and 60°C for 1 month

amorphous IMC and amorphous physical mixture of IMC and Eudragit E 100 (Fig. 11). Similar to IMC PVP spectra, disappearance of peak at 1735 cm^{-1} and 1710 cm^{-1} was observed with the appearance of 1721 cm^{-1} assigned to hydrogen bonding between hydrogen of IMC carboxylic group and Eudragit E100 dimethylamino group. Disappearance of 2770 and 2822 assigned to dimethylamino groups of Eudragit E100 in all solid dispersions indicate the interaction of this group with the indomethacin. Raman spectra also shows a shift of benzoyl C=O toward lower wave number (Fig. 12). In general, the shift is more pronouncing in the IR spectra than Raman spectra. Other polymers showed no changes in IR and Raman spectra.

Solid state NMR chemical shift differences between neat amorphous IMC and IMC-polymer dispersion can be used to evaluate the IMC-polymer interaction. In general, no chemical shift differences are expected to be observed for phase separated drug-polymer systems with domains on the order of several nanometers or larger. For molecular level dispersions, depending on the specifics of the local molecular level interactions, various extends of chemical shift differences are expected. The chemical shift differences in the polymer (neat polymer *vs*. drug-polymer dispersion) can be used to characterize the interaction as well. However, it appears to be less obvious how to compare and contrast these between different polymers. Also note that the broad peak nature of the amorphous phase resonances limits the observation of the chemical shift differences. In Fig. 13, some signal intensity differences between neat IMC and IMC-polymer can be observed. These are less useful as a measure of the interaction as the carbon cross-polarization magic angle spinning (^{13}C CPMAS) signal depends on the sample relaxation parameters as well as the cross-polarization kinetics, both of which can differ depending on the structure and thermal history of the sample. The aromatics region of the spectrum (160–110 ppm) is free from polymer overlap for all three dispersions tested and can be used to compare the IMC chemical shift differences. Subjectively, the aromatic chemical shift differences follow the order of PVP K 90>HPMC>Eudragit S100 and corroborate the data from the complementary techniques above. The interaction between the carbonyl and carboxylic moieties of IMC with the polymers are expected to be significant. Indeed, for IMC-HPMC, which does not show any polymer resonances in this 182–165 ppm region, IMC chemical shift (as well as

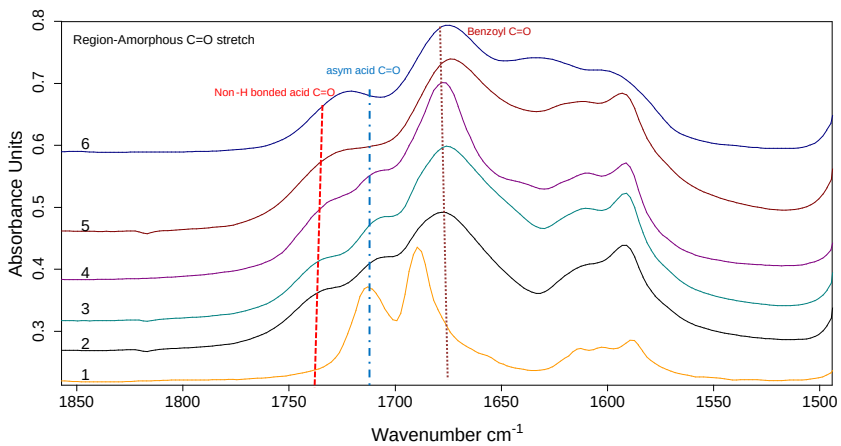

Fig. 7 IR spectra of amorphous carbonyl region 1) Crystalline γ IMC 2)Amorphous IMC 3) Amorphous physical mixture 4)Solid dispersion, IMC-PVP K 90(3:1) 5) Solid dispersion, IMC-PVP K 90(2:1), 6) Solid dispersion, IMC-PVP K 90(1:1).

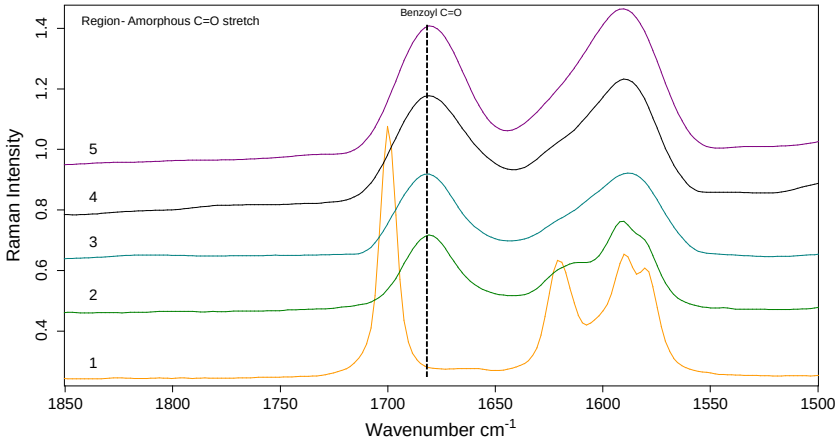

Fig. 8 Raman spectra of amorphous carbonyl region 1) Crystalline γ IMC 2) Amorphous physical mixture 3) Solid dispersion, IMC-PVP K 90(3:1) 4) Solid dispersion, IMC-PVP K 90(2:1), 5) Solid dispersion, IMC-PVP K 90(1:1).

signal intensity) differences are observed. It should be cautious, however, that the observed change may be a result of formation of molecular dispersion which is different to the strictly defined molecular interaction. Unfortunately, the peak overlap for the remaining two polymers (PVP and Eudragit S100) precludes more detailed analysis.

The change in melting event based on MDSC has been used to characterize the miscibility between drug and polymers (14,15). A change in melting doesn't necessary mean the existence of interaction as a melted polymer, *e.g.* PEG 8000, can dissolve drug and change the melting event. However, a lack of changes in melting in the presence of polymer might indicate the absence of drug-polymer interaction. In our studies, we observed that IMC didn't show any change in the melting endotherm during MDSC in the presence of Eudragit S100, Eudragit L100 and PEG 8000 at low concentration, indicating lack of interaction with these polymers (Data not shown). The observed decrease of melting point in the presence of polymers suggests that IMC and polymer is miscible in the melted state, which suggests some degree of interaction.

In an attempt to rank order the strength of molecular interaction, docking score between IMC and the polymer was calculated. The docking score is a combination of intermolecular lipophilic interactions, hydrogen-bond interactions, van der Waals interactions, and Coulomb interactions. Due to the limitation calculation power, it is not possible to dock the polymer with full chain length. Dimer through sixmer was docked to minimized structure of IMC. The rank order of docking studies for strength of molecular interaction using computational method was found to be PVP K90 (−2.918) > Eudragit E100 (−2.791) > HPMC (−2.175) > Eudragit L100 (−1.023) > Eudragit S100 (−0.130). Docking attempt with ethylene glycol dimers through sixmers didn't return any score suggesting that docking is not favored. The rank order of docking score is in line with the rank order of polymer effects on precipitation inhibition and amorphous stabilization. The drug polymer interaction in a solution is different to the conditions assumed under the current calculation. Nevertheless the calculation attempt provides insights to the role of molecular interaction on the crystallization inhibition. The application of Glide to calculate the interaction score of drug and polymers is a novel usage of the software. As the calculation of drug-polymer interaction is still a field in its infancy, more investigation is needed to identify or develop the software package that will be most suitable for the calculation of drug polymer interaction.

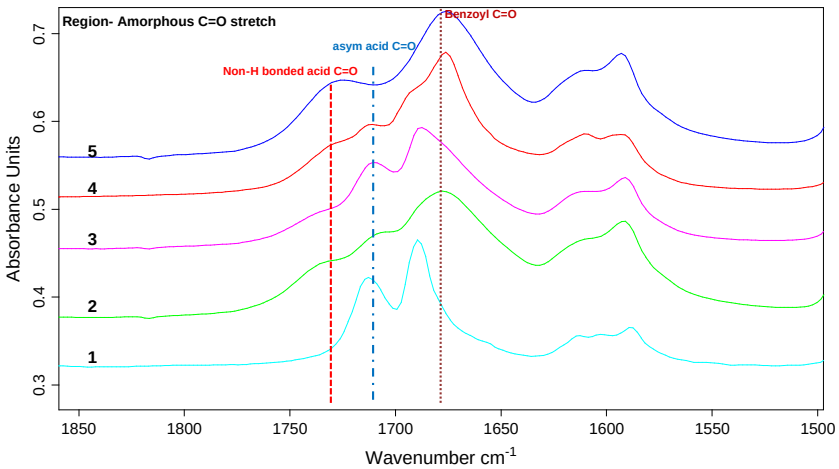

Fig. 9 IR spectra of amorphous carbonyl region 1) Crystalline γ IMC 2) Amorphous IMC 3) Amorphous physical mixture 4) Solid dispersion, IMC-HPMC (3:1) 5) Solid dispersion, IMC-HPMC(2:1).

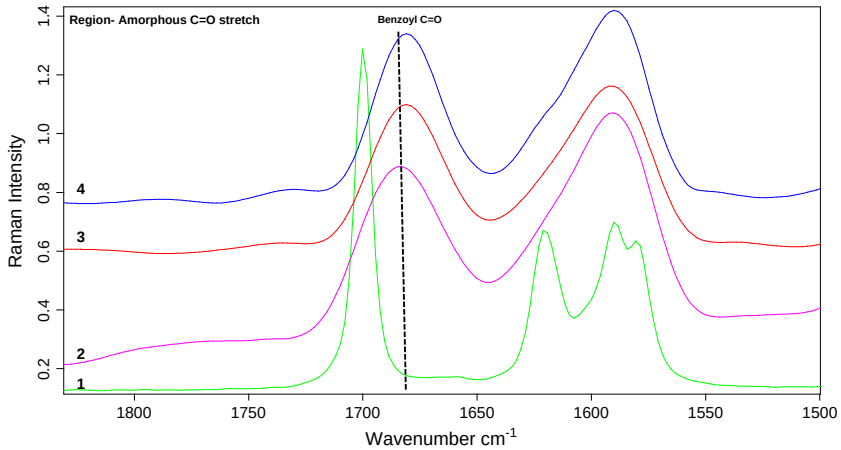

Fig. 10 Raman spectra of amorphous carbonyl region 1) Crystalline γ IMC 2) Amorphous physical mixture 4) Solid dispersion, IMC-HPMC (3:1) 5) Solid dispersion, IMC-HPMC (2:1).

DISCUSSION

Effect of Polymers on Indomethacin Precipitation in Solution

Based on our results PVP K90, HPMC and Eudragit E100 were shown to delay IMC precipitation during initial screening while other polymers showed no effect. These polymers significantly delay IMC precipitation initiation time indicating inhibition of crystal nucleation event and rank ordering of these polymers was – PVP K90>Eudragit E100>HPMC> Eudragit L100, Eudragit S100 and PEG 8000. Further, rank ordering can be done based on minimum polymer concentration needed to delay IMC precipitation *i.e.* PVP K90 (40:1), Eudragit E100 (10:1) and HPMC (2:1) which is in agreement with the above rank order. Similar rank order was obtained on polymers efficiency on retarding crystal growth rate. It was found that polymers, such as Eudragit S100 and Eudragit L100 (high molecular weight), which have no effect on nucleation rate can decrease the crystal growth rate during precipitation. It is clear that the three most hydrophobic polymers Eudragit L100, Eudragit S100 (anionic polymers in acidic media) and PEG 8000, have the least ability to inhibit nucleation, which can be attributed to the limited ability of the polymers to interact with the IMC polar groups, involved in the crystal formation(−COOH, -C=O) and interfere with the crystallization process. This is similar to the recently reported phenomena in which hydrophobicity of the polymer was found to influence the ability of the polymer to form polymer-solute interactions relative to polymer-solvent and polymer-polymer interactions (16). These polymer-solute interactions can hinder the reorganization of a cluster of solute molecules into an ordered crystal structure. Further, the crystal growth rate decrease may be attributed to the steric hindrance caused by size of these polymers as PEG 8000 (relatively low molecular weight) showed no effect on either nucleation or crystal growth (17). Lynne *et al*. recently reported that interactive forces like hydrophobicity and specific drug polymer interactions can promote adsorption of drugs into polymer surface and thus can significantly influence growth rate (18).

Since low concentrations of polymers were used in the experiments and no change in pH and viscosity was observed, this inhibition of nucleation and crystal growth reflects the

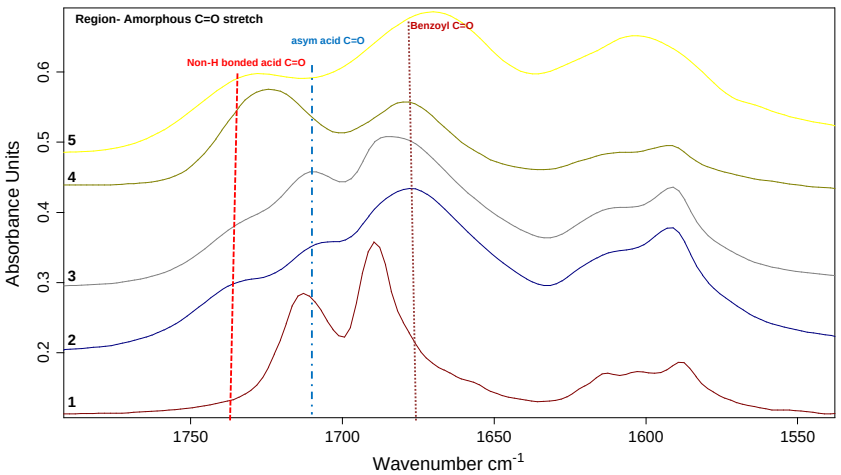

Fig. 11 IR spectra of amorphous carbonyl region 1) Crystalline γ IMC 2) Amorphous IMC 3) Amorphous physical mixture 4) Solid dispersion, IMC-Eudragit E 100 (3:1) 5) Solid dispersion, IMC-Eudragit E 100 (2:1).

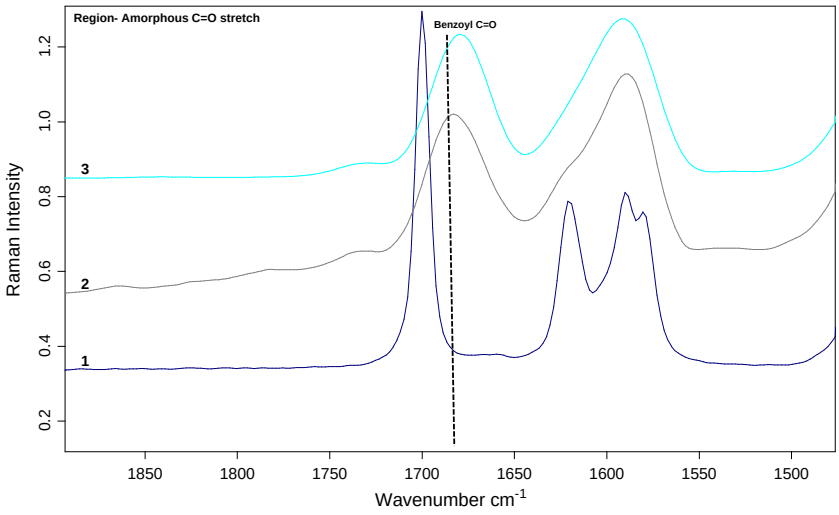

Fig. 12 Raman spectra of amorphous carbonyl region 1) Crystalline γ IMC 2) Amorphous physical mixture 3) Solid dispersion, IMC-Eudragit E 100 (2:1).

polymers ability to inhibit crystallization in solution, which may be an indicator of the interaction strength of IMC with these polymers. Similar observations were made for Celecoxib where it was suggested that different drug/polymer interactions can give rise to the variation in dissolution with different polymers (19). The change in the crystal form and morphology are the additional evidence that drug polymer interaction played a role in modifying the crystallization kinetics.

It's known that impurities can inhibit crystallization based on surface adsorption (20–25). We attempted to fit the inhibition data presented here to different surface adsorption models, namely Cabrera and Vermileya's model; Davey's model and Leeden's model. The fitting of nucleation rate, which is the inverse of precipitation time, and the average precipitation rate, to each model is presented in Figs. 14 and 15. A single best model which describes the effect of polymers on precipitation

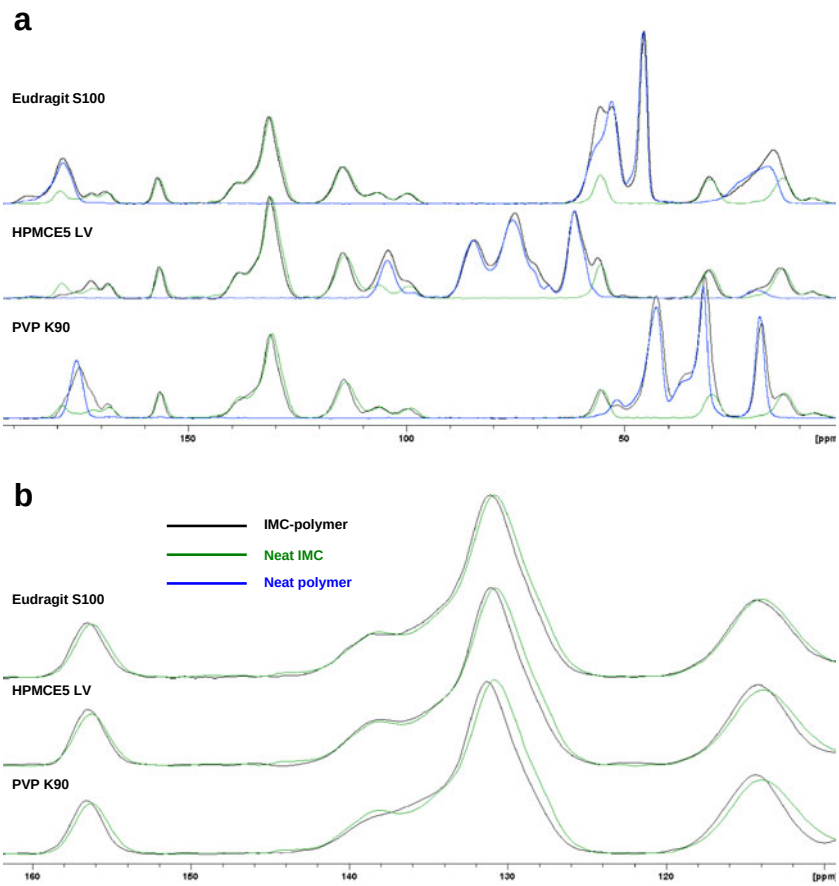

Fig. 13 SSNMR Overlays of carbon solid state NMR spectra. Green traces: neat amorphous IMC (the same spectrum in all cases); black traces: IMC-polymer dispersions; blue traces: polymer only. (**a**) Full chemical shift range. (**b**) Only aromatic chemical shift region shown. Note that since none of the polymers shows any resonances in this region, the IMC chemical shift differences between neat IMC and IMC-polymer can be directly compared.

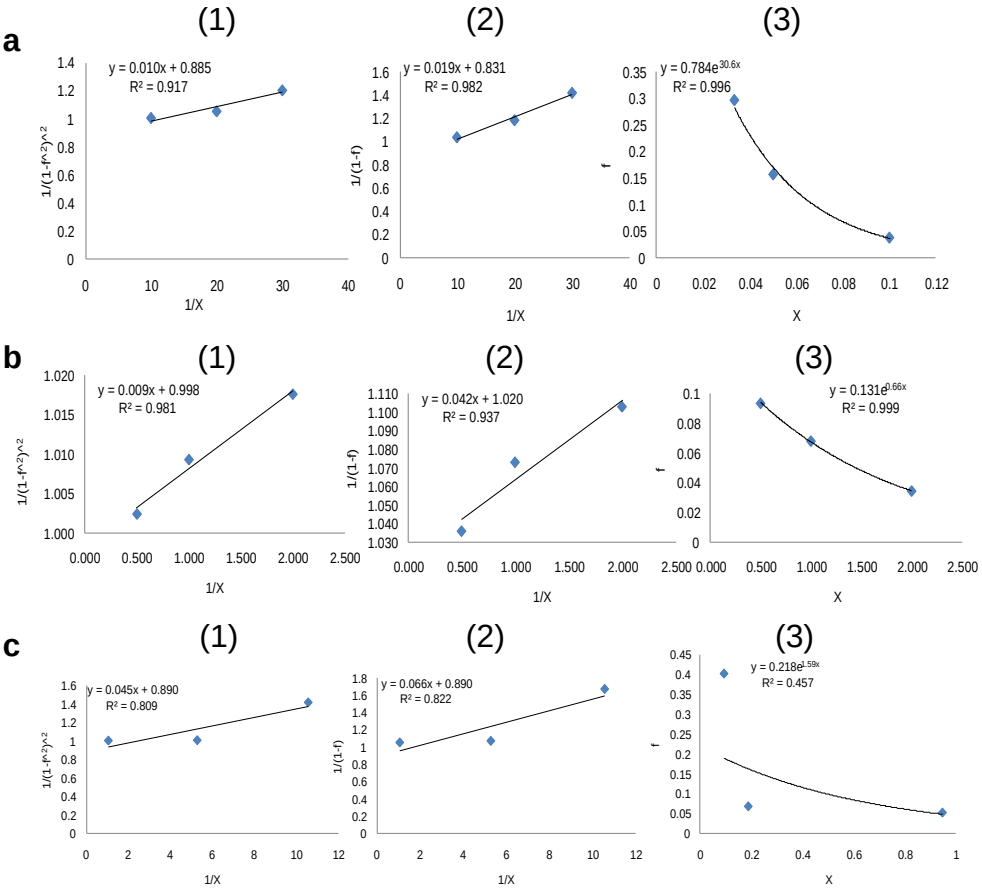

Fig. 14 Fitting to surface adsorption models based on initiation time (**a**) IMC-PVP K 90 (1) Cabrera and Vermileya's model (linear) (2) Davey's model (linear) (3) Leeden's model (exponential); (**b**) IMC-HPMC (1) Cabrera and Vermileya's model (linear) (2) Davey's model (linear) (3) Leeden's model (exponential); (**c**) IMC-Eudragit E 100 (1) Cabrera and Vermileya's model (linear) (2) Davey's model (linear) (3) Leeden's model (exponential).

inhibition of IMC cannot be determined because of the good fit to multiple models and the limited number of data points. However, it can be concluded that the effect of polymers on the precipitation inhibition follows surface adsorption mechanism in general. On the crystal growth rate, the effect of polymers can be fitted well with Davey's surface adsorption models.

Amorphous Stabilization of Indomethacin by Polymers at Solid State

Overall ranking of amorphous stabilization of IMC by different polymers based on polymer concentration and stability studies was found to be PVP K90 > Eudragit E100 > HPMC > Eudragit S100, Eudragit L100 > PEG 8000. We observed that very low amount of effective polymers can stabilize indomethacin. This is necessary so that solubilized drug form is present in polymeric matrix. Andrews *et al*. showed that mefenamic acid can be present both in solubilized and dispersed form in solid state which can affects the dissolution advantages of the prepared solid dispersion's (26). Further, it appears that there is some degree of miscibility between IMC and these effective polymers as observed by melting point depression of IMC in the physical mixtures of PVP K90, HPMC and Eudragit E100. The rank order of amorphous stabilization at such low level of polymer is an indication of the ability of polymers to disrupt crystallization of IMC, which may be an indicator of strength of molecular interaction. Recently, molecular interactions have been found to play an important role in solubility and stability enhancement of Bicalutamide-PVP and Celecoxib-PVP solid dispersions (27,28). Bicalutamide molecular dispersions were found to have a significant increase in release rate and were found to be stable for 12 weeks at 20°C, 40% RH (27,29). In our case, no change in T_g was observed at low polymer levels, suggesting changing in molecular mobility is unlikely the reason for stabilization. Although, stability and solubility enhancement of many drugs like Ellagic acid, quercetin and resveratrol has been observed, but in these cases, high concentration of polymers were used which significantly changes the Tg of the solid dispersions (30–32). The observation that Eudragit E100 has stabilization effect on amorphous IMC despite its very low Tg further support that the stabilization is more through molecular interaction than mobility reduction.

IR and Raman data confirm the presence of different types of interaction between IMC and PVP K90, HPMC and Eudragit E100. PVP K90 appears to interact through the formation of hydrogen bond between IMC hydroxyl group and the polymer carbonyl group, resulting in the disruption of

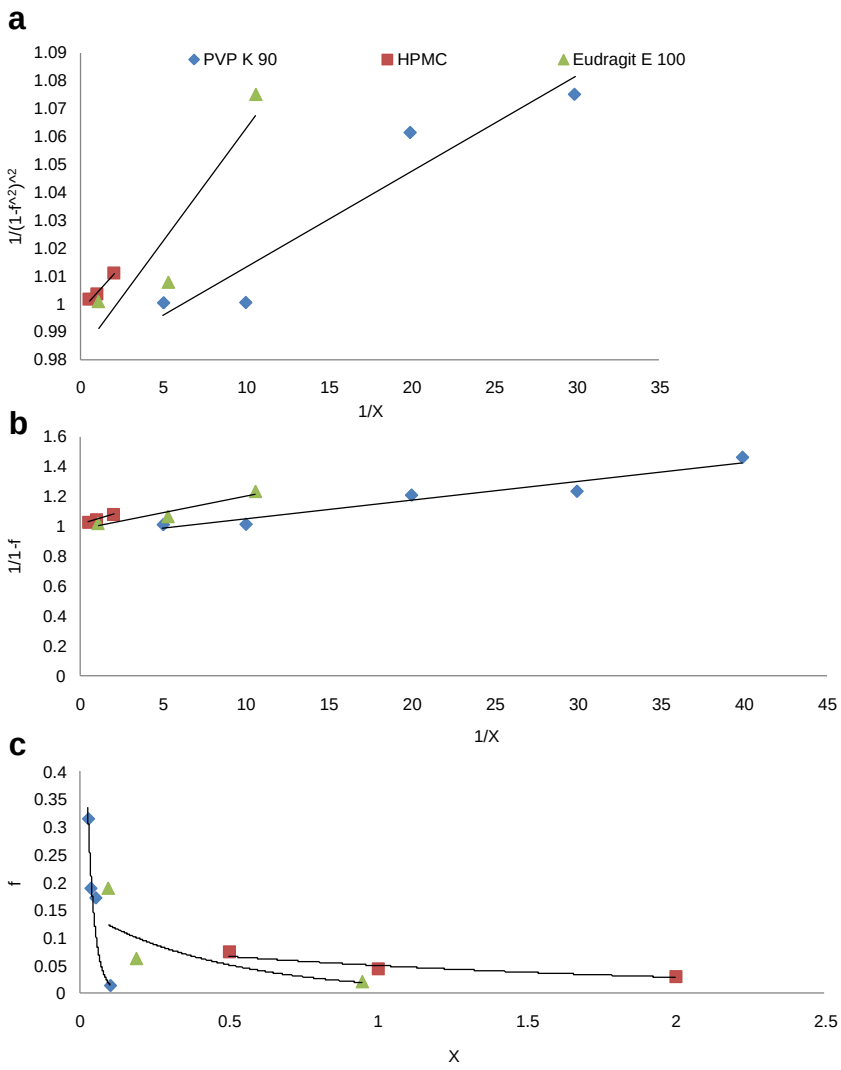

Fig. 15 Fitting of various surface adsorption models based on precipitation rate of IMC in presence PVP K 90, HPMC and Eudragit E 100 (**a**) Cabrera and Vermileya's model (linear) (**b**) Davey's model (linear) c) Leeden's model (exponential).

IMC dimer formation and their self-association as reported previously (13). Eudragit E100, a cationic polymer, has a potential for ionic interaction with the weak acidic drug IMC (4) whereas HPMC was found to form hydrogen bonds between hydroxyl group of HPMC and carboxylic group of IMC (33). Significant changes in IR spectra confirm interaction between IMC to these polymers, but more studies are needed to confirm the interaction mechanisms. Using solid-state NMR, we found clear interaction between IMC and PVP but less obvious interaction for Eudragit and HPMC, which also supports the strongest inhibitory effect of PVP. The rank order of stabilization was also found to agree with interaction strength based on Glide score. Polymers effective at inhibiting crystallization and their effectiveness usually depend upon the type and strength of their intermolecular interactions. Similar observations are recently reported for resveratrol where strength of molecular interaction was found to play an important role in crystallization inhibition from its amorphous form and improving its overall stability (34).

Correlation of Polymer Precipitation Inhibitory Effects on IMC in Solution to Amorphous Stabilization in Solid State

Correlation was observed as PVP K90>Eudragit E100> HPMC were found to be most effective in solution as well as in solid state. Eudragit S100, Eudragit L100 and PEG 8000 were the least effective polymers in both the states. We have chosen PVP K90 to represent PVP in the detail studies. We have observed that other grade of PVPs, such as PVP K30; also provide stronger precipitation inhibition, comparable to PVPK90. The fact that the degree of stabilization is independent of polymer molecular weight may supportsthe argument that molecular interaction plays the most important role for stabilization. The correlation of crystallization inhibition in both the states indicates that strength of molecular interaction may be the underlying mechanism in inhibiting crystallization. Both spectra and in silico calculation suggest that the rank order of crystallization inhibition is related to the strength

of molecular interaction attributed to the drug-polymer interactions. Although hydrogen bonding interaction is often suggested as the primary interaction responsible for stabilization, it is possible that other types of interactions, such as hydrophobic interaction, also play important roles. The main finding in the current study is that molecular interaction is important for crystallization inhibition, which links the rank order of polymer effect in the solution and solid states. The exact type of interaction is however, difficult to determine." Upon studying the effective polymer concentration, we found that polymers at lower drug to monomer ratios are needed to be effective crystallization inhibitor in solution whereas in solid state higher concentrations of polymer are needed for the amorphous stabilization. This may be a general phenomenon as molecular movement is much faster in the solution than in the solid state. Therefore, low amount of polymer is needed in solution to be at the nucleation or crystal growth site to prevent crystallization.

CONCLUSIONS

Correlation is observed in polymers ability to inhibit precipitation in solution and amorphous stabilization in solid state of IMC with a rank order of PVP K90 > Eudragit E100 and HPMC. The correlation may indicate that ability of polymer to prevent crystallization based on strength of molecular interaction play a significant role in crystallization inhibition, which applies to both precipitation inhibition in solution and amorphous stabilization in solid state. It may be possible to select excipients to stabilize amorphous in the solid state based on rank order of precipitation inhibition in solution, which is a more straight forward technique.

ACKNOWLEDGMENTS AND DISCLOSURES

Vertex Pharmaceuticals Incorporated (Cambridge, MA) and Massachusetts College of Pharmacy and Health Sciences (Boston, MA) are acknowledged for the use of instruments and funding in completing this project.

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