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Solid State Interaction of Raloxifene HCl with Different Hydrophilic Carriers During Co-grinding and its Effect on Dissolution Rate

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This study investigated the effects of different classes of hydrophilic carriers (poly vinyl pyrrolidones [PVPs] [Plasdone K-25 and Plasdone S-630], cellulosic polymers [hydroxypropyl methyl cellulose and hydroxy propyl cellulose], and Sodium Alginate) on the solid state and dissolution rate of Raloxifene hydrochloride (R-HCl). Solid state characterizations of co-ground mixtures and physical mixtures in 1:1 and 1:2 ratios of drug to polymer were performed by employing laser diffractometer for particle size and differential scanning calorimetry (DSC) for solid state interactions. The results of particle size studies showed that only co-grinding with PVPs was more effective in the reduction of particle size than the milling of drug alone. DSC study indicated that the crystalline nature of the drug was reduced after co-grinding with PVPs when compared with their corresponding physical mixtures. The hydrophilic carriers other than PVPs did not reduce the crystalline nature of the drug significantly. X-ray diffraction and scanning electron microscopy were carried out for selected batches to confirm DSC results. Significant enhancement in dissolution rate and extent was observed with co-ground mixtures of drug and PVPs. Plasdone S-630 was found to be a better carrier for R-HCl in terms of achieving improvement in dissolution. In vitro dissolution data can be described by Hixson–Crowell model, indicating the drug release mechanism predominated by erosion.

Keywords Raloxifene HCl; co-grinding; hydrophilic carriers; BCS II; solid state characterization

INTRODUCTION

The Biopharmaceutical Classification System (BCS) class II active pharmaceutical ingredients (APIs), which have been reported to account for as many as 40% of new chemical entities (Lipinski, 2002), present particular challenges in creating successful drug products. Relative to highly soluble compounds, low drug solubility often manifests itself in a host of in vivo consequences, including decreased bioavailability, increased chance of food effect, more frequent incomplete release from the dosage form, and higher inter-patient variability. Poorly soluble compounds also present many in vitro formulation obstacles, such as severely limited choices of delivery technologies and increasingly complex dissolution testing with limited or poor correlation to the in vivo absorption (Amidon, Lennernas, Shah, & Crison, 1995).

Solubility can be enhanced by several methods. However, techniques such as micronization (Chaumeil, 1998; Otsuka & Kaneniwa, 1984), co-grinding (Friedrich, Nada, & Bodmeier, 2005; Sugimoto, Okagaki, Narisawa, Koide, & Nakajima, 1998; Suzuki, Ogawa, Hironaka, Ito, & Sunada, 2001), solid dispersion (Ahuja, Katare, & Singh, 2007; Yamada, Saito, Anraku, Imai, & Otagiri, 2000), complexation (Veiga, Fernandes, & Maincent, 2001), spray drying (Rogers, Hu, Yu, Johnston, & Williams, 2002), super critical fluid technology (Van Nijlen et al., 2003), and lipid-based drug delivery system (Attama & Mpamaugo, 2006) have commonly been used to improve the dissolution and bioavailability of poorly water soluble drugs.

Grinding is often used as a technique to improve the solubility of drugs, because of the reduction in particle size and affects their dissolution rates (Parrott, 1987). The method is also simple and easy to carry out. However, desired changes sometimes not only occur in physical properties, such as specific area and shape, but also occur in the reduction of drug stability, or polymorphic transformation may also occur (Mura, Cirri, Faucci, Ginès-Dorado, & Bettinetti, 2002). Grinding is not effective if the obtained fine powder forms aggregate.

It is well known that both solid dispersion and co-grinding of poorly soluble drugs with various kinds of polymers are useful for solubilization and enhancement of bioavailability, because the crystalline drug is transformed into an amorphous form in a polymer network (Ahuja et al., 2007; Friedrich et al., 2005). The rationale behind such a strategy is that a highly disordered amorphous material has a lower energetic barrier

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to overcome to enter a solution than a regularly structured crystalline solid. Compared with the other solubilization techniques, the co-grinding method does not need organic solvent processes involving environmental and health concerns. Co-grinding involves cheap and simple instruments whereas other techniques, for example, super critical fluid and spray drying technology involve very expensive sophisticated instruments. The co-grinding method can be carried out under either dry or wet conditions with various milling devices, such as ball, jet, and hammer mills. Milling of a substance that is dispersed in a nonsolvent (wet milling) prevents the formation of dust and particle agglomeration. A common disadvantage of wet milling process is the partial dissolution that can cause an uncontrolled recrystallization (especially during drying) or chemical instability. Most of the investigators used dry milling conditions for co-grinding (Friedrich et al., 2005; Yamamoto, Nakano, Arita, Takayama, & Nakai, 1976). The properties of milled product are dominated by the surface properties of the crystal face. In the case of poorly water soluble substances, the newly created surface is hydrophobic and thus poorly wettable. Because of aerophilicity of such hydrophobic substances, the dissolution rate is not increased as we would expect from the increase in total surface area according to Noyes–Whitney equation. Thus if a poorly soluble drug is micronized in order to increase the dissolution rate and bioavailability, the new surface has to be hydrophilized. The co-milling or co-grinding of a hydrophobic drug with surfactant or hydrophilic polymers increases the wettability, which in turn improves the dissolution rate and dispersibility in a suspension. Many authors have reported on the use of co-grinding method for the enhancement of dissolution rate of various drugs, for example, digoxin, estradiol, sprinolactone (Florence & Salole, 1976), ketoprofen (Mura, Faucci, & Parrini, 2001), indomethacin (Etman & Nada, 1999), and phenytoin (Nada, 1997; Yamamoto et al., 1976). The enhancement of dissolution rate which results in improvement of bioavailability of poorly water soluble drugs has been demonstrated by various investigators (Kubo, Osawa, Takashima, & Mizobe, 1996; Liversidge & Cundy, 1995; Yamamoto et al., 1976).

Various hydrophilic polymers, such as hydroxypropyl methyl cellulose (HPMC), poly vinyl pyrrolidone (PVP), cyclodextrin, poly ethylene glycol (PEG), and so forth, have been used to enhance the dissolution characteristics. Phenytoin and nifedipine when roll mixed with PVP showed improvement in the dissolution rate of the drug (Nozawa, Mizumoto, & Higashide, 1985, 1986). Co-ground mixture of nifedipine prepared with different hydrophilic carriers showed enhancement in the dissolution rate (Friedrich et al., 2005).

Raloxifene hydrochloride (R-HCl), a selective estrogen receptor modulator (SERM) is effective in the prevention of osteoporosis (Delmas et al., 1997), with potential utility as a substitute for long-term female hormone replacement therapy (Mitlak & Cohen, 1997). R-HCl is a poorly water soluble BCS class II drug having an absolute bioavailability of approximately 2% in humans. The absolute bioavailability of R-HCl can be increased by improving its solubility. The inclusion complex of R-HCl with hydroxybutenyl-beta cyclodextrin showed significant improvements in oral bioavailability when compared with R-HCl formulated with microcrystalline cellulose alone (Wempe et al., 2008).

Therefore, this work was proposed to study the effect of co-grinding, which is a relatively simple technique of R-HCl with various hydrophilic carriers (PVPs, HPMC, hydroxy propyl cellulose (HPC), and Sodium Alginate), on particle size, solid state properties, and dissolution performance. To the best of our knowledge, the co-grinding technique using planetary ball mill and the mentioned polymers have not been tried for this drug earlier. In this study, the particle size for physical mixture and co-ground mixture of R-HCl with different hydrophilic carriers has been investigated by laser diffractometry. Solid state characterization of the drug–polymer binary system was carried out using differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), and scanning electron microscopy (SEM) techniques.

The objective was to investigate whether co-grinding could be used as a technique for improving dissolution of R-HCl and to possibly identify a suitable carrier or carriers that could significantly enhance the dissolution of R-HCl.

MATERIALS AND METHODS

Materials

R-HCl was obtained from Golchem Industries, Hyderabad, India. PVP (Plasdone K-25 and Plasdone S-630) and Sodium Alginate (Keltone LV) were generously gifted by ISP, Wayne, NJ, USA. HPMC (Pharmacoat 606) and Hydroxypropyl cellulose (Klucel EF) were purchased from Shinetsu, Tokyo, Japan and Aqualon, Hercules Inc., Wilmington, DE, USA, respectively. All the other chemicals used were of analytical grade.

Preparation of Co-Ground Mixtures

R-HCl (5 g) and polymers (5 and 10 g) having drug to polymer ratios of 1:1 and 1:2 were co-ground at 200 rpm for 120 min using a planetary ball mill (Model-PM 100, Retsch, Haan, Germany). The 120 min consist four cycles each of 30 min. After completion of one cycle, the powder was removed from the wall of the vessel with spatula for proper grinding.

Preparation of Physical Mixture

The corresponding physical mixtures were prepared by triturating in a mortar with a pestle for 30 min.

Particle Size Measurement

The particle size of prepared mixtures was determined by laser diffractometer (Scirocco 2000(A), Malvern Instruments, Worcestershire, UK). The relative frequency of the diameter of the particle was obtained by calculation based on volume

distribution. The particle size at 10% (d_{10}), 50% (d_{50}), and 90% (d_{90}) of total fraction were obtained. The particle size at 90% of total fraction was used. The values were the average of 10 measurements.

Differential Scanning Calorimetry

Thermal curves of each sample were recorded by simultaneous Differential Scanning Calorimeter (TA Instruments Q 1000, Bangalore, India). Each sample (~2.5 mg) was scanned in hermetic pan made of aluminum at a heating rate of 10 C/min over the range of 50–300°C with an empty aluminum pan used as reference. Samples were heated under nitrogen atmosphere (flow rate of N_2—50 mL/min).

FT-IR

FT-IR spectra were obtained using FT-IR spectrometer (Nicolet 5700, Thermo Scientific, Madison, WI, USA) by the conventional KBr pellet method. The samples were ground gently with anhydrous KBr and compressed to form pellet. The scanning range was 400–4,000 cm^{-1} and the resolution was 4 cm^{-1}.

X-Ray Diffraction

Powder XRD patterns were traced employing X-ray diffractometer (Model No. 3000, Seifert, Ahrensburg, Germany) for the samples, using Ni-filtered Cu–K radiation, a voltage of 40 kV, a current of 30 mA radiation scattered in the crystalline regions of the sample, which was measured with a vertical goniometer. Patterns were obtained by using a step width of 0.04° with a detector resolution in 2θ (diffraction angle) between 10° and 80° at ambient temperature.

Scanning Electron Microscopy

The shape and surface characteristics of the mixtures were studied by SEM. The particles were coated with gold–palladium and then observed with an electron microscope (430, Zeiss-Leo, Oberkochen, Germany) at ambient temperature.

In Vitro Dissolution Studies

In vitro dissolution testing employed the United States Pharmacopeia (USP) Apparatus II (VK 7010 Varian, Cary, NC, USA) at 50 rpm with 900 mL of degassed water (DosaprepX[8], DOSA TECH, Middleburg, Netherland) at 37 ± 0.5°C. Six capsules of each batch containing powder sample equivalent to 30 mg R-HCl were tested. The sink condition was maintained in degassed water, as solubility of R-HCl was 627.4 ± 132.0 µg/mL (Scott & Roger, 2002). The sample of the dissolution media was removed using an automated sampling system at a predetermined time interval (0, 5, 10, 15, 30, 45, and 60 min) and was simultaneously analyzed spectrophotometrically at λ_{max} of 285 nm (Cary 50 UV-Spectrophotometer attached with Dissolution Apparatus; Cary, NC, USA). The weight of powder filled in capsule was kept constant by adding lactose in sufficient quantity to make the final weight of 110 mg. In vitro release studies of unmilled R-HCl (UR), milled R-HCl (MR), and batch showing best result were performed in 1,000 mL of 0.1% Tween solution (FDA-recommended media) under same conditions. Data of dissolution study are shown as $M \pm SD$ ($n = 6$).

The percentage dissolution efficiency (%DE) of pharmaceutical dosage form (Khan, 1975) is defined as the area under the dissolution curve up to a certain time, *t*, expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time.

It is calculated by the following equation:

$$DE_T = \frac{\int_0^T y_t \cdot dt}{y_{100} \cdot T},$$

where y_t is the percentage of drug dissolved at any time *t*, y_{100} denotes 100% dissolution, and the integral represents the area under dissolution curve between time 0 and *T*. The time *T* in this study was 60 min.

The time taken to achieve 30% ($t_{30\%}$) and 60% ($t_{60\%}$) drug release in dissolution medium were also calculated from the interpolation of plotted dissolution profiles.

Mathematical Modeling of Release Kinetics

The in vitro drug release data were fitted to various release kinetic models, such as first-order, Higuchi, Hixson–Crowell cube root and zero-order models employing the following set of equations:

First-order model:

$$\ln\left(\frac{M_0}{M_t}\right) = k_1 t$$

Higuchi model:

$$M_t = Kt^{1/2}$$

Zero-order kinetic model:

$$M_0 - M_t = k_0 t$$

Hixson–Crowell cube root model:

$$(M_0)^{1/3} - (M)^{1/3} = k_{1/3} t,$$

where M_t is the amount of drug released at time *t*, M_0; the initial amount of drug, and *M*; the remaining amount.

RESULT AND DISCUSSION

Particle Size Measurement

Table 1 enlists the particle sizes (d_{10}, d_{50}, and d_{90}) of drug and polymers before and after grinding, physical mixtures and co-ground mixtures in 1:1 and 1:2 ratios of all polymers.

Drug and Polymers Before and After Grinding

Milling of drug alone in ball mill for 120 min at 200 rpm results in significant reduction of d_{90} (88 μm), approximately 55% when compared with that of unmilled drug (193 μm). The d_{90} of unmilled Plasdone K-25 and Plasdone S-630 were 120 and 116 μm, respectively. Ball milling of Plasdone K-25 and Plasdone S-630 led to a significant reduction of particle size—d_{90} (50 and 48 μm, respectively). The particle sizes of unmilled HPMC, HPC, and Sodium Alginate were 162, 755, and 166 μm, respectively. The larger particle size of HPC may be because of its fibrous nature. Ball milling of these three polymers did not cause any significant change in d_{90}. The d_{90} of milled HPMC, HPC, and Sodium Alginate were 158, 747, and 171 μm, respectively. The above results indicate that ball milling is ineffective in significant size reduction of these three polymers.

Mixtures of Drug and Polymers Before (Physical Mixtures) and After Grinding (Co-Ground Mixtures)

The d_{90} of physical mixtures of R-HCl and different Plasdones (K-25 and S-630) in 1:1 ratio were 142 and 150 μm,

TABLE 1
Particle Size ($M \pm SD$, $n = 6$) of R-HCl, Hydrophilic Carriers and Prepared Mixtures of R-HCl with Hydrophilic Carriers

Batch Name	Batch Code	d_{10} (μm)	d_{50} (μm)	d_{90} (μm)
Unmilled R-HCl	UR	10.41 ± 0.52	52.54 ± 1.89	193.23 ± 11.26
Milled R-HCl	MR	1.57 ± 0.02	15.14 ± 0.15	88.53 ± 2.29
Unmilled Plasdone K-25	UP1	15.73 ± 0.25	49.04 ± 1.15	119.69 ± 8.01
Milled Plasdone K-25	MP1	3.64 ± 0.05	21.52 ± 0.25	49.89 ± 0.19
PM R-HCl:K-25 1:1	P11	13.78 ± 0.059	51.90 ± 0.27	142.03 ± 0.76
CM R-HCl:K-25 1:1	C11	1.68 ± 0.02	12.64 ± 0.17	54.47 ± 2.24
PM R-HCl:K-25 1:2	P12	14.69 ± 0.04	51.32 ± 0.156	131.99 ± 0.74
CM R-HCl:K-25 1:2	C12	1.24 ± 0.010	9.95 ± 0.113	46.96 ± 0.86
Unmilled Plasdone S-630	UP2	13.73 ± 0.12	42.55 ± 0.54	116.17 ± 2.14
Milled Plasdone S-630	MP2	4.67 ± 0.01	15.75 ± 0.11	48.28 ± 0.95
PM R-HCl:S-630 1:1	P21	13.52 ± 0.14	48.17 ± 0.83	149.81 ± 8.93
CM R-HCl:S-630 1:1	C21	1.76 ± 0.013	12.53 ± 0.09	52.48 ± 1.31
PM R-HCl:S-630 1:2	P22	14.13 ± 0.05	47.33 ± 0.38	145.25 ± 9.06
CM R-HCl:S-630 1:2	C22	2.135 ± 0.014	10.86 ± 0.07	40.54 ± 1.17
Unmilled HPMC	UP3	22.99 ± 0.54	71.41 ± 0.16	161.75 ± 0.33
Milled HPMC	MP3	21.12 ± 0.159	67.86 ± 0.17	157.57 ± 0.19
PM R-HCl:HPMC 1:1	P31	14.33 ± 0.16	61.35 ± 1.00	165.29 ± 1.88
CM R-HCl:HPMC 1:1	C31	3.69 ± 0.32	44.76 ± 1.56	152.81 ± 9.87
PM R-HCl:HPMC 1:2	P32	17.41 ± 0.13	66.45 ± 0.25	166.87 ± 0.06
CM R-HCl:HPMC 1:2	C32	5.99 ± 0.09	57.65 ± 0.77	194.28 ± 10.57
Unmilled HPC	UP4	118.29 ± 9.72	354.20 ± 10.63	755.41 ± 31.62
Milled HPC	MP4	127.96 ± 5.10	361.15 ± 6.77	747.36 ± 16.43
PM R-HCl:HPC 1:1	P41	25.54 ± 0.30	220.45 ± 3.37	625.60 ± 3.40
CM R-HCl:HPC 1:1	C41	12.27 ± 0.52	280.04 ± 5.30	723.28 ± 33.19
PM R-HCl:HPC 1:2	P42	38.57 ± 3.63	275.87 ± 14.21	678.02 ± 33.96
CM R-HCl:HPC 1:2	C42	40.09 ± 1.28	315.02 ± 6.79	771.83 ± 54.36
Unmilled Sodium Alginate	UP5	20.09 ± 0.22	70.92 ± 0.88	165.66 ± 6.07
Milled Sodium Alginate	MP5	20.02 ± 0.287	70.549 ± 1.23	171.39 ± 9.32
PM R-HCl:Sodium Alginate 1:1	P51	13.90 ± 0.20	62.68 ± 1.22	187.96 ± 11.01
CM R-HCl:Sodium Alginate 1:1	C51	3.44 ± 0.05	36.84 ± 0.89	149.07 ± 11.75
PM R-HCl:Sodium Alginate 1:2	P52	15.30 ± 0.09	64.23 ± 0.28	169.14 ± 0.79
CM R-HCl:Sodium Alginate 1:2	C52	4.80 ± 0.10	51.78 ± 1.07	188.10 ± 11.53

PM, physical mixture; CM, co-ground mixture.

respectively, whereas d_{90} of corresponding co-ground mixtures were 54 and 52 μm, respectively (Table 1). The results indicate that a significant reduction in d_{90} can be achieved with co-grinding of R-HCl, Plasdone K-25, and S-630. The d_{90} of physical mixtures of R-HCl and HPMC, HPC and Sodium Alginate in 1:1 ratio were 165, 625, and 188 μm, respectively. The d_{90} of corresponding co-ground mixtures were 153, 723, and 149 μm, respectively. The results indicate that there is no significant difference in the d_{90} of physical mixtures and corresponding co-ground mixtures of these three polymers. Thus, co-grinding of R-HCl and these three polymers was not effective to reduce particle size.

The effect of two different drug polymer ratios (1:1 and 1:2) was studied on the particle size of physical and co-ground mixtures of drug and polymers. The d_{90} of physical mixtures of R-HCl and Plasdone (K-25, S-630) in ratios 1:1 and 1:2 were 142, 150 and 132 μm, 145 μm, respectively (Table 1). Also the d_{90} of co-ground mixtures of R-HCl and Plasdones (K-25, S-630) in ratios 1:1 and 1:2 were 54, 52 and 47 μm, 40 μm, respectively.An increase in the amount of polymer in physical and co-ground mixtures (drug and polymer ratio changed from 1:1 to 1:2) did not result in significant reduction of $d_{90.}$

The d_{90} of physical mixtures of R-HCl and HPMC, HPC, and Sodium Alginate in ratios 1:1 and 1:2 were 165, 625, 188 μm and 167, 678, 169 μm, respectively (Table 1). The d_{90} of co-ground mixtures of R-HCl and HPMC, HPC and Sodium Alginate in ratios 1:1 and 1:2 were 153, 723, 149 μm and 194, 771, 188 μm, respectively (Table 1). These results indicate that increase in the ratio of polymer did not affect the particle size in physical mixtures of these polymers, whereas increase in the amount of polymer in co-ground mixtures of these polymers resulted in increase in particle size. The increase in the particle size of co-ground mixtures of 1:2 ratio can be explained based on previous observations, where the particle size of polymers alone was found to be not affected by milling; on the contrary, milling reduced the particle size of drug. The net effect during co-grinding in the case of higher amount of above polymers was the increase in particle size.

A significant reduction was observed between d_{90} of milled drug (88 μm) and co-ground mixtures of R-HCl and Plasdones (K-25 and S-630) in both the ratios 1:1 and 1:2. The smallest particles were obtained by co-grinding of R-HCl with Plasdone S-630 (batch C22); the d_{90} being 40 μm. The particle size by co-grinding with Plasdone S-630 was reduced by approximately 54% when compared with milled drug. The reduction in particle size of drug was approximately 78% when compared with the particle size of unmilled drug. It is also evident that ball milling is not effective in reducing the particle size of cellulosic polymers and Sodium Alginate (Table 1). An increase in the ratio of polymer (Plasdone K-25, Plasdone S-630) also does not result in significant reduction in the particle size of drug.

When the drug alone is ball-milled, mechanically micronized substances are electrostatically charged and, in most cases, they are agglomerated because of their cohesive behavior (De Villiers & Tiedt, 1996). The agglomeration of particles during ball-milling can be prevented by using homopolymer of PVP (PlasdoneK-25) and co-polymer of PVP (Plasdone S-630) and cellulose ethers (HPMC and HPC). These polymers are adsorbed onto the newly created particle surfaces (Liversidge, 1997; Liversidge et al., 2000). In this study, among all the polymers the co-polymer of PVP (Plasdone S-630) is the best one to stabilize the system and results in least particle size.

Differential Scanning Calorimetry

DSC studies were performed on the individual components and on the freshly prepared co-ground and physical mixtures in order to study the interaction between R-HCl and the carriers in the solid state (Figure 1).

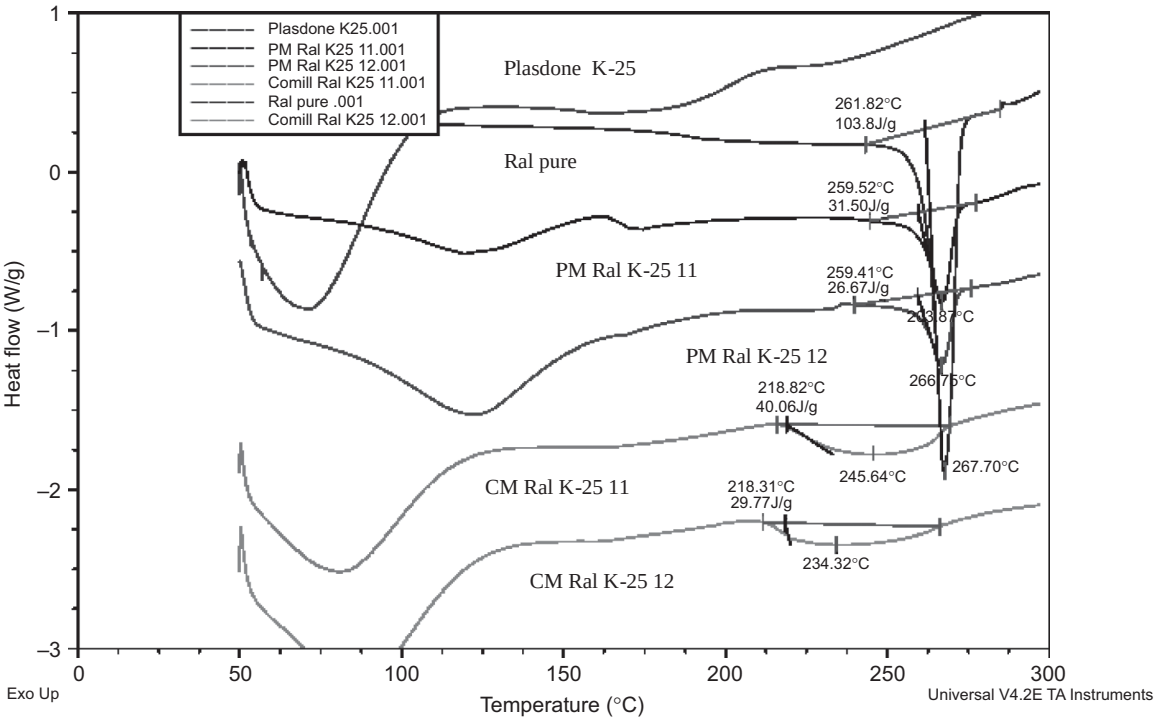
R-HCl exhibited a single sharp melting endothermic peak at 267°C. The DSC thermograms of hydrophilic polymers (Plasdone K-25, Plasdone S-630, HPMC, HPC, and Sodium Alginate [Keltone]) showed a broad endothermic peak in the range from 50 to 130°C, which may be because of endothermic relaxation (Figure 1). The DSC thermograms indicated that polymers (except Sodium Alginate) are amorphous and hydrated compounds. The DSC thermogram of Sodium Alginate (Figure 1E) showed an additional degradation exothermic peak at 252°C (Sankalia, Mashru, Sankalia, & Sutariya, 2005).

The DSC thermograms of all the physical and co-ground mixtures showed two endothermic peaks. The first broad endothermic peak in the range of 50–130°C corresponds to endothermic relaxation of the polymers and the second endothermic peak represents the melting temperature of drug in mixtures.

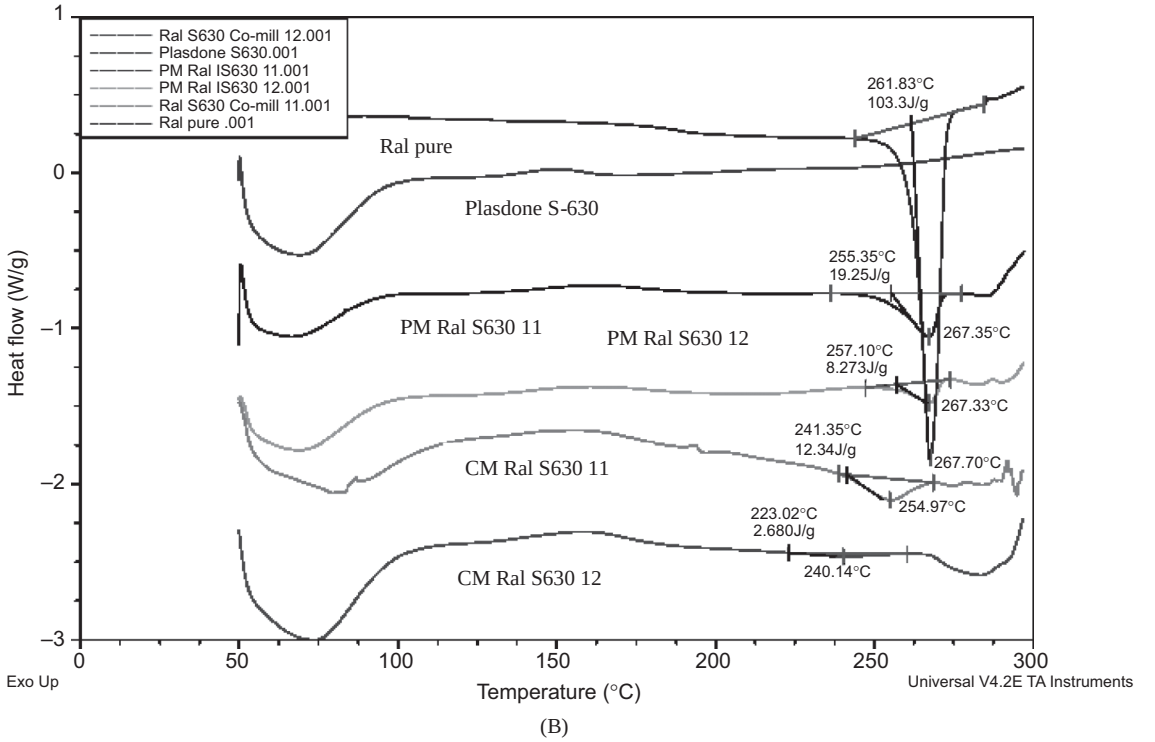
Physical Mixtures

Physical mixtures of R-HCl and hydrophilic carriers result in decrease in the intensity of melting endothermic peak as evidenced by ΔH_m values in J/g than pure drug (Figure 1). Physical mixtures of R-HCl and PVP (Plasdone K-25 and Plasdone S-630) exhibited less melting endothermic peak than physical mixtures of cellulosic polymers (HPMC and HPC) with R-HCl. Physical mixture of R-HCl and Sodium Alginate exhibited an exothermic peak similar to the thermogram of Sodium Alginate.

The DSC thermogram of physical mixture of R-HCl and Plasdone K-25 in ratios 1:1and 1:2 revealed an endothermic drug peak with no shifting of melting temperature of the drug. However, fusion enthalpy was found to decrease from 103.8 J/g (drug) to 31.50 J/g (1:1) and 26.67 J/g (1:2), respectively (Figure 1A). The above results indicate that the presence of higher amount of Plasdone K-25 in physical mixture could not cause a significant change in fusion enthalpy. The DSC thermograms of physical mixture of R-HCl and Plasdone S-630 in 1:1 and 1:2 ratios revealed

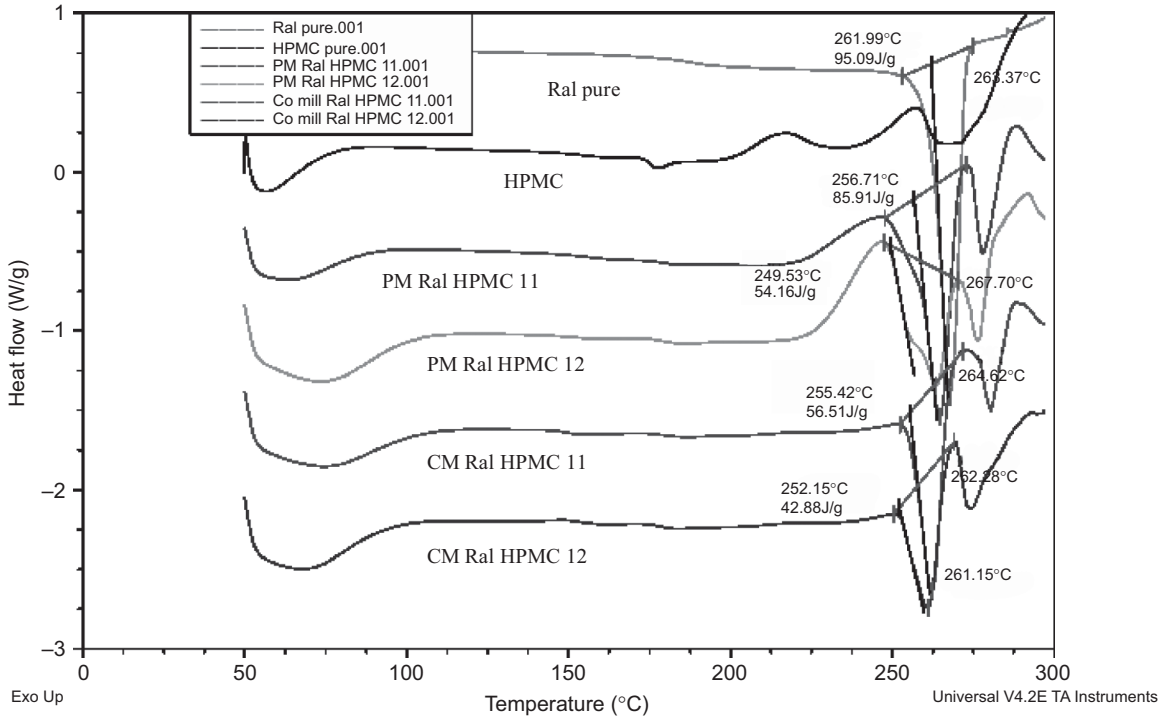


(A)

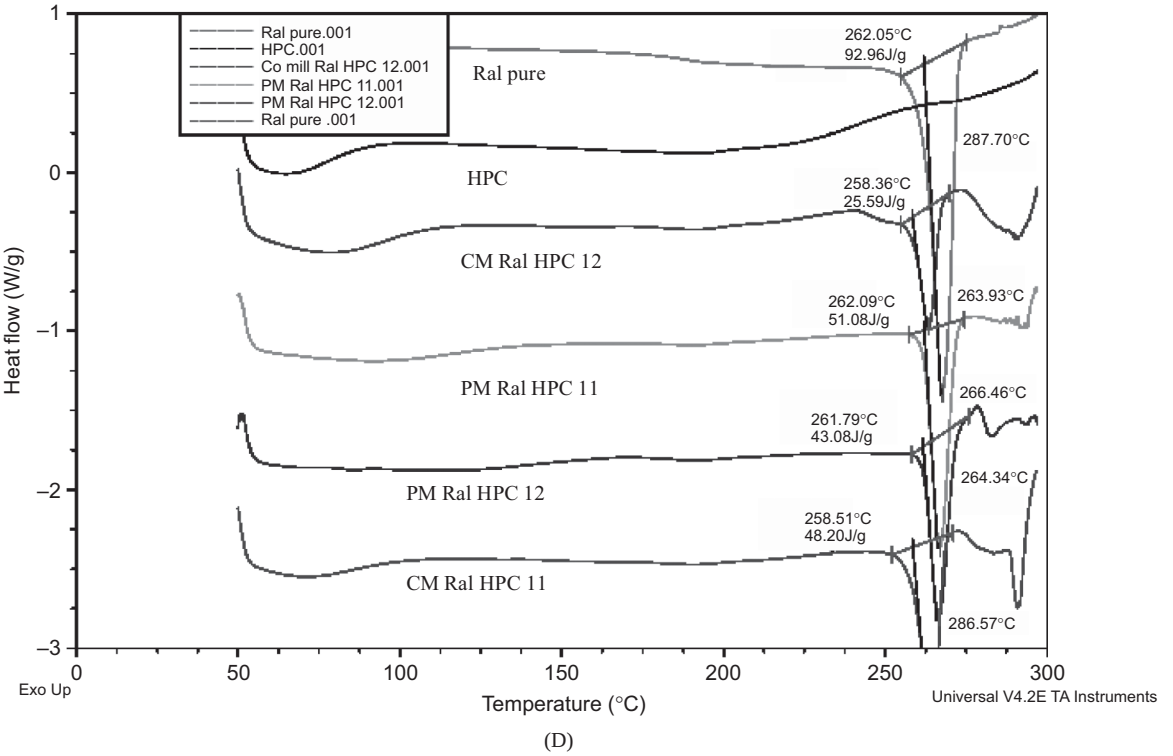


(B)

FIGURE 1. DSC thermogram of (A) R-HCl and Plasdone K-25 mixtures; (B) R-HCl and Plasdone S-630 mixtures; (C) R-HCl and HPMC mixtures; (D) R-HCl and HPC mixtures; and (E) R-HCl and Sodium Alginate mixtures.

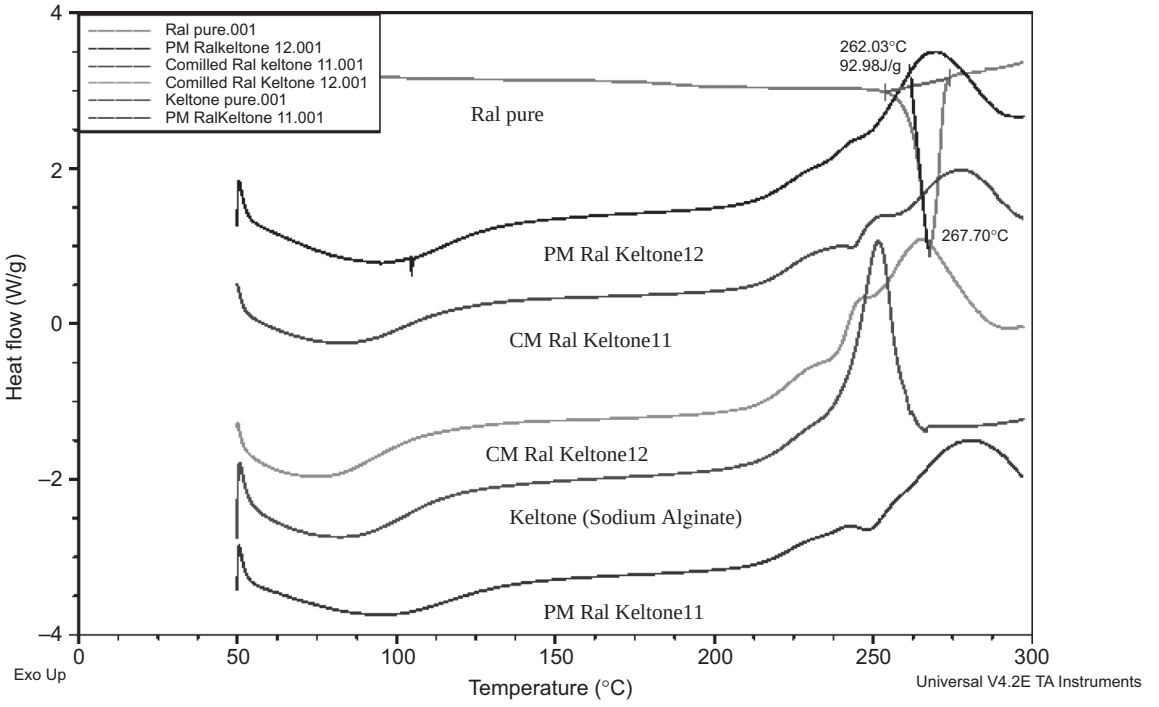


(C)



(D)

FIGURE 1. (*Continued*)



(E)

FIGURE 1. (*Continued*)

the drastic decrease in fusion enthalpy from 103.3 J/g (drug) to 19.25 and 8.273 J/g in ratios 1:1 and 1:2, respectively, with no shifting of melting temperature (Figure 1B). Thus, it can be inferred that the crystallinity of drug was not affected by increase in the amount of Plasdone K-25 in physical mixture. However, Plasdone S-630 in higher ratio results in reduction of crystallinity.

Co-Ground Mixtures

Co-ground mixtures of R-HCl and PVPs lead to further decrease or broadening of melting endothermic peak of drug than their corresponding physical mixtures. Co-ground mixture of R-HCl and Plasdone K-25 showed a broad endothermic peak at 245.64 and 234.32°C in 1:1 and 1:2 ratios, respectively (Figure 1A). This indicates a decrease in the crystallinity of the drug, which can be attributed to the presence of Plasdone K-25 during co-grinding. As the amount of Plasdone K-25 in the mixture was increased the apparent crystallinity of the drug decreased.

Co-ground mixtures of R-HCl and Plasdone S-630 showed a broad endothermic peak with shifting of melting temperature from 267.7 (drug) to 254.97 and 240.14°C in 1:1 and 1:2 ratios, respectively, and decrease in fusion enthalpy from 103.3 J/g (drug) to 12.34 and 2.68 J/g in ratios 1:1 and 1:2, respectively. In higher ratio of Plasdone S-630, a very small endothermic peak was observed in the DSC thermogram (Figure 1B). It clearly indicates the loss of the crystalline nature of the drug during co-grinding of R-HCl with Plasdone S-630 in ratio 1:2.

An increase in the width of melting endothermal peak in co-ground mixtures of drug and both types of PVP may be because of the decreased interparticular spaces resulting from the co-grinding and more surface area available for interactions between drug and PVP than their corresponding physical mixtures (Mura, Zerrouk, Meennini, Maestrelli, & Chemtob, 2003). Co-ground mixtures of R-HCl and Plasdone S-630 showed lower crystallinity than co-ground mixtures of R-HCl and Plasdone K-25 (Figure 1A and B). Plasdone S-630 was adsorbed readily on the newly created particle surface than Plasdone K-25 during grinding because of its partial hydrophobic nature (Chee, Johansen, Gu, Karlsen, & Heng, 2005). The drug is hydrophobic in nature, therefore, newly created particle surface was also hydrophobic.

However, a significant reduction or broadening of melting endothermal peak was not observed in the case of co-ground mixtures of R-HCl with cellulosic polymers than their corresponding physical mixtures. It can be explained because co-grinding of drug and cellulosic polymers was ineffective in the reduction of particle size and therefore, unable to provide more surface area than their corresponding physical mixtures.

In Vitro Release Studies in Water

Table 2 enlists the various dissolution parameters, such as $DP_{60,}$ $\%DE_{60}$, $t_{30\%}$, and $t_{60\%}$ of R-HCl, physical mixtures,

TABLE 2
Dissolution Parameters of R-HCl and Different Prepared Mixtures

S. no	Batch	DP_{15}[a]	DP_{60}[b]	$\%DE_{60}$[c]	$t_{30\%}$[d](min)	$t_{60\%}$[e](min.)
1	UR	8.13 ± 2.14	17.11 ± 2.63	9.04	NA	NA
2	MR	11.58 ± 1.46	30.16 ± 3.38	14.66	60.0	NA
3	P11	4.59 ± 2.60	21.65 ± 2.31	8.77	NA	NA
4	C11	10.10 ± 2.51	47.58 ± 0.41	20.77	27.52	NA
5	P12	8.24 ± 2.7	26.92 ± 1.25	12.55	NA	NA
6	C12	8.06 ± 2.25	58.06 ± 1.47	23.48	25.73	NA
7	P21	1.24 ± 0.06	20.54 ± 1.04	5.55	NA	NA
8	C21	19.30 ± 2.65	63.55 ± 3.63	29.61	20.77	49.35
9	P22	3.14 ± 0.06	24.69 ± 0.65	8.11	NA	NA
10	C22	11.29 ± 2.19	63.45 ± 2.88	22.77	27.74	55.08
11	C31	2.78 ± 0.91	26.34 ± 2.57	5.55	NA	NA
12	C32	2.39 ± 0.30	20.22 ± 2.95	4.72	NA	NA
13	C41	16.59 ± 1.88	32.45 ± 1.75	11.40	51.38	NA
14	C42	2.92 ± 0.8	10.30 ± 1.52	3.83	NA	NA
15	P51	3.52 ± 0.87	23.3 ± 2.26	6.55	NA	NA
16	C51	3.05 ± 0.13	11.47 ± 2.88	4.0	NA	NA
17	P52	3.27 ± 0.36	20.54 ± 3.38	5.5	NA	NA
18	C52	2.85 ± 0.3	13.68 ± 1.41	4.166	NA	NA

NA, not achieved.
[a]Drug percentage release at 15 min ($M \pm SD$, n= 6).
[b]Drug percentage release at 60 min ($M \pm SD$, n = 6).
[c]Percentage dissolution efficiency at 60 min.
[d]Time in minutes to achieve 30% drug release.
[e]Time in minutes to achieve 60% drug release.

and co-ground mixtures of R-HCl with hydrophilic carriers used in the study.

The percentage dissolution efficiency at 60 min ($\%DE_{60}$) was computed to compare the relative performance of various carriers in co-ground and physical mixtures. It seems to be a better parameter than drug percentage released for comparison as it includes both rate and extent of release.

As is evident from Table 2 the dissolution rate of pure unmilled drug (UR) is very low. Only 17% of the drug is released in the dissolution medium after 1 h. Milling of the drug without any carrier showed enhancement in dissolution rate as $\%DE_{60}$ increases from 9 to 14.5%. This result can be correlated with the results of particle size.

Mixtures of R-HCl and PVP

Physical Mixtures. Physical mixtures of R-HCl and Plasdone K-25 in ratio 1:1 (P11) did not show any significant difference in $\%DE_{60}$ (8.77%) when compared with UR (9%). An increase in the amount of Plasdone K-25 (drug and K-25 in 1:2 ratio) in physical mixture (P12) results in slight improvement in $\%DE_{60}$ (12.55%) when compared with UR (Table 2). Physical mixture of drug and Plasdone S-630 in 1:1 (P21) showed a decrease in $\%DE_{60}$ from 9.04 to 5.55 which may be only because of the presence of polymer. Physical mixture of drug with Plasdone S-630 in 1:2 ratio enhanced $\%DE_{60}$ when compared with that in 1:1 ratio ($\%DE_{60}$ of P21 and P22 are 5.55 and 8.11, respectively). Higher proportion of S-630 in the mixture results in more loss of crystallinity than lower proportion as shown in the DSC thermograms (Figure 1B).

Co-Ground Mixtures. In general, co-grinding of drug with Plasdone K-25 (homopolymer) and Plasdone S-630 (co-polymer) showed significant enhancement in the dissolution rate and dissolution extent when compared with their corresponding physical mixtures. Co-ground mixture of R-HCl and Plasdone S-630 in 1:1 ratio (Batch C21) showed highest value of $\%DE_{60}$ (29.61%) which indicates highest dissolution rate. Co-grinding of R-HCl with Plasdone K-25 also results in significant improvement of $\%DE_{60}$ (20.77%). Amorphous forms of pharmaceuticals are markedly more soluble than their crystalline counterparts (Hancock & Parks, 2000) and improve the dissolution rate (Ahuja et al., 2007; Friedrich et al., 2005). This higher

$\%DE_{60}$ value of batch C21 was because of lower crystallinity than batch C11 as already discussed in the DSC results.

Co-grinding of drug with Plasdone S-630 (Co-povidone) in 1:1 and 1:2 ratios enhanced $\%DE_{60}$ 3.27 and 2.52 times of unmilled drug, respectively ($\%DE_{60}$ of batches C21 and C22 are 29.61 and 22.77, respectively, whereas $\%DE_{60}$ of UR is 9.04). A decrease in dissolution rate, with an increase in PVP S-630 content can be explained by leaching out the polymer from co-ground mixtures containing higher proportion of polymer during dissolution, which could form a concentrated layer of the polymer around the drug particles; therefore, the migration of drug particles into the bulk of dissolution medium would be slowed (Akbuga, Gursoy, & Kendi, 1988; Ford, 1986; Zingone & Rubessa, 1994).

Mixtures of R-HCl and Cellulosic Polymers

No significant difference was observed in solid state characteristics (particle size and DSC thermograms) of physical mixture and co-ground mixture of drug and cellulose polymers (HPMC and HPC). Therefore, dissolution studies have been performed for only co-ground mixtures of these polymers.

Co-Ground Mixture of R-HCl and HPMC. Co-ground mixtures of drug and HPMC in 1:1 and 1:2 ratios lead to decrease in $\%DE_{60}$ (5.55 and 4.72, respectively) because of the formation of lump when they come in contact with water. Thus, co-ground mixtures of R-HCl and HPMC provide a slow release of drug.

Co-Ground Mixture of R-HCl and HPC. Co-ground mixture of drug with HPC in 1:1 ratio showed an increase in $\%DE_{60}$ (11.40) when compared with $\%DE_{60}$ of unmilled drug (9.04). However, this increase in $\%DE_{60}$ is not comparable to co-ground mixture of drug and Plasdone in the same ratio. However, co-ground mixture of drug with HPC in 1:2 ratio dramatically decreases the $\%DE_{60}$ (3.83). During the dissolution study, the formation of lump was observed in C42 which may be because of higher proportion of HPC in formulation and this provides a very slow release of drug.

Mixtures of R-HCl and Sodium Alginate

$\%DE_{60}$ of P51, P52 and C51, C52 are 6.55, 5.5, and 4, 4.16, respectively. Physical mixture and co-ground mixture of drug with Sodium Alginate form a lump because of swelling of Sodium Alginate during dissolution studies. Therefore, a decrease in $\%DE_{60}$ of physical mixture and co-ground mixture were observed. However, co-ground mixture showed lower $\%DE_{60}$ than physical mixture of drug and Sodium Alginate. This may be because of the higher physical interaction between Sodium Alginate and drug particles in co-ground mixture than physical mixture (Figure 1E).

Thus, the result of dissolution studies showed that co-grinding of R-HCl with Plasdone (PVP) could increase the rate and extent of dissolution of drug, whereas co-grinding with HPMC, HPC, and Sodium Alginate is not effective.

In Vitro Release Studies in 0.1% Tween Solution

Tween solution of 0.1% is US FDA-approved dissolution media for R-HCl. Therefore, dissolution studies of UR, MR, and batch C21 were carried out in this dissolution medium for comparison. Batch C21 showed approximately 80% drug release within 60 min, whereas UR and MR showed 52 and 62% drug release within 60 min, respectively (Figure 2).

Mathematical Modeling

Table 3 enlists the regression parameter obtained after fitting various release kinetic models to the in vitro dissolution data. Hixson–Crowell cube root model and zero order described drug release kinetics in the most befitting manner. Highest rate constants for both models were observed for batch C21. It indicates fast release of drug from C21 among all the batches. According to Hixson–Crowell cube root model, cube root constant is inversely proportional to the diameter of the particle of powder (Hixson & Crowell, 1931). The drug particle surface can be approximated to receding sphere as it dissolves. Thus, our mathematical modeling results are in accordance with the particle size data and in vitro release study. Hixson–Crowell equation also suggests the release mechanism of drug through erosion.

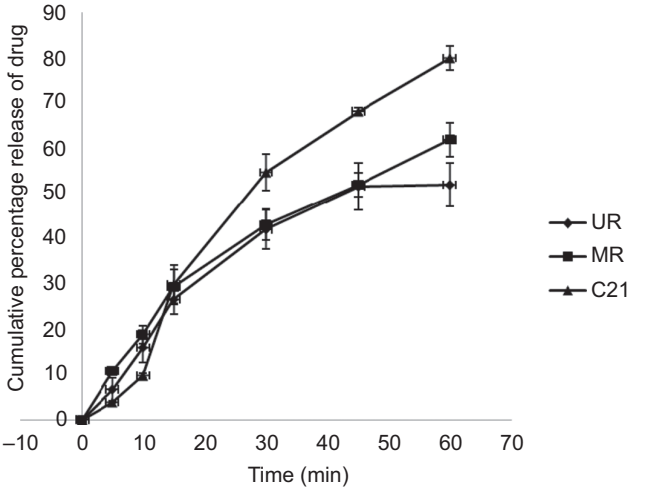


FIGURE 2. Dissolution profiles of unmilled R-HCl (UR), milled R-HCl (MR) and co-ground mixture of R-HCl and Plasdone S-630 in 1:1 ratio (C21) in 0.1% Tween solution (each data represented as $M \pm SD$, $n = 6$).

TABLE 3
Statistical Parameters of Various Prepared Mixtures Obtained After Fitting the Drug Release Data to Various Release Kinetic Models

	Hixson–Crowell		Higuchi		First Order		Zero Order	
Batch	Slope	R^2	Slope	R^2	Slope	R^2	Slope ($\times 10^{-4}$)	R^2
UR	0.0004	.9173	0.0006	.9501	–0.0339	.7163	–0.9	.9386
MR	0.0006	.9683	0.0011	.9073	–0.0433	.7144	–2	.9618
P11	0.0004	.9803	0.0007	.8157	–0.0558	.8373	–1	.9833
C11	0.0011	.9432	0.0016	.7807	–0.0556	.7271	–3	.943
P12	0.0006	.9633	0.0006	.9633	–0.0542	.7498	–1	.9575
C12	0.0014	.9413	0.0019	.7559	0.061	.7832	–3	.9488
P21	0.0004	.9311	0.0006	.6949	–0.0701	.8938	–1	.968
C21	0.0016	.9706	0.0022	.8403	–0.0591	.712	–4	.955
P22	0.0005	.9553	0.0007	.7372	–0.0633	.832	–1	.981
C22	0.0014	.968	0.002	.7847	–0.0656	.8074	–3	.9869
C31	0.0004	.8822	0.0006	.6498	–0.0676	.8973	–1	.9321
C32	0.0003	.9281	0.0005	.6946	–0.0689	.8621	–1	.9647
C41	0.0007	.9493	0.0012	.9466	–0.034	.7666	–2	.9533
C42	0.0002	.9894	0.0003	.873	–0.0359	.9345	–0.5	.9916
P51	0.0004	.9615	0.0007	.7537	–0.052	.9473	–1	.9818
C51	0.0002	.9977	0.0004	.8684	–0.0388	.9204	–0.6	.9983
P52	0.0003	.9515	0.0006	.7449	–0.0486	.9643	–1	.9708
C52	0.0002	.9784	0.0004	.8007	–0.0431	.9466	–0.7	.9847

FT-IR

FT-IR studies showed that there was no significant change in the spectrum of co-ground mixture when compared with drug alone, as incorporation of R-HCl into the polymers did not change the position of its functional groups. The absence of shifts in the wave numbers of the FT-IR peaks (Figure 3) of the co-ground mixture vis-à-vis the physical mixture indicates the lack of significant interaction between the drug and the carrier in the mixture (Valizadeh et al., 2004; Vippangunta, Maul, Tallavajhala, & Grant, 2002). Thus these results ratify the absence of any well-defined interaction between R-HCl and the polymers used, more particularly with Plasdone S-630.

X-Ray Diffraction

XRD studies were undertaken to consolidate the DSC data indicating the reduction of the crystallinity of R-HCl with PVP. Therefore, the XRD patterns of R-HCl (unmilled and milled), Plasdone S-630, physical mixture, and co-ground mixture in 1:1 ratio were observed. The diffraction spectrum of UR showed that the drug was crystalline in nature, as demonstrated by numerous distinct peaks observed at 2θ of 13.4, 14.4, 15.7,19.0, 20.9, 21.1, 22.6, and 25.9 (Figure 4A). The milled drug also showed similar superimposable diffraction pattern indicating that the crystallinity of the drug was unaffected by milling of drug alone (Figure 4B). XRD pattern of Plasdone S-630 showed no sharp peaks, indicating its amorphous nature (Figure 4C).

All the principal peaks of R-HCl were present in their physical mixtures and co-ground mixtures, although with lower intensity. No new peaks could be observed, suggesting the absence of interaction between the drug and the carrier (Ahuja et al., 2007; Hancock & Zografi, 1997; Williams, Timmins, Lu, & Forbes, 2005). The prominent peaks from R-HCl at 2θ of 14.4, 15.7, 19.0, 21.1, and 22.6 were clearly seen at the same position in the physical mixture (Figure 4D). However, in co-ground mixture, similar diffraction peaks were obtained at 2θ of 14.4, 15.7, 21.1, and 22.6. These peaks were broadened and reduced in intensity when compared with the diffraction peaks of corresponding physical mixture (Figure 4E). A relative reduction of diffraction intensity of R-HCl in co-ground mixture than physical mixture at these angles suggest that either the crystal quality is reduced or change is induced in the crystal orientation or only some of the drug is still present in the crystalline form (Betageri & Makarla, 1995; Valizadeh et al., 2004; Vippangunta et al., 2002). R-HCl therefore existed in a very less crystalline state in co-ground mixture with Plasdone S-630. The corresponding physical mixture showed a higher degree of crystallinity than the co-ground mixture. These results are similar to DSC results.

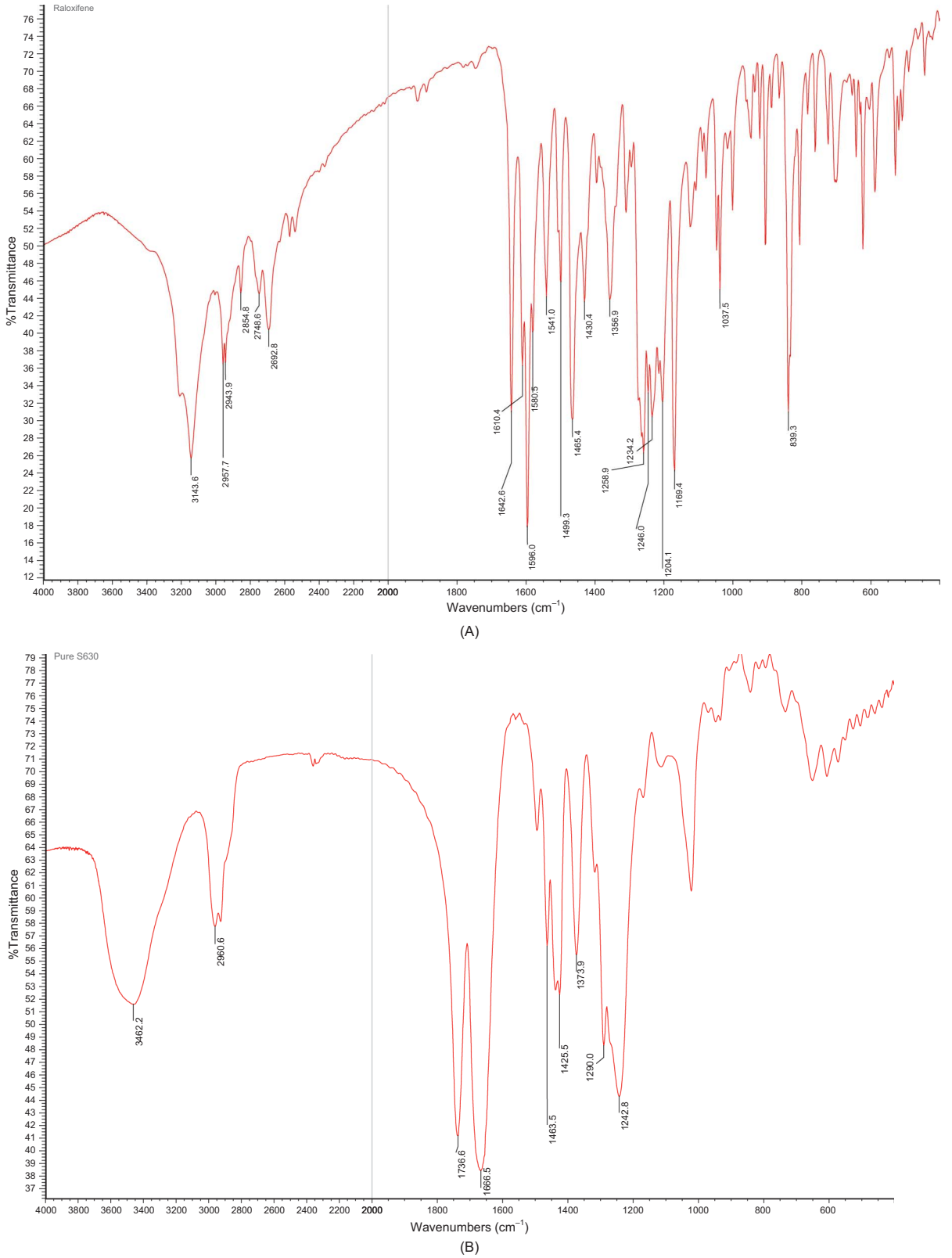


FIGURE 3. FTIR spectra of (A) R-HCl; (B) Plasdone S-630; (C) physical mixture of R-HCl and Plasdone S-630 in 1:1 ratio (P21); and (D) co-ground mixture of R-HCl and Plasdone S-630 in 1:1 ratio (C21).

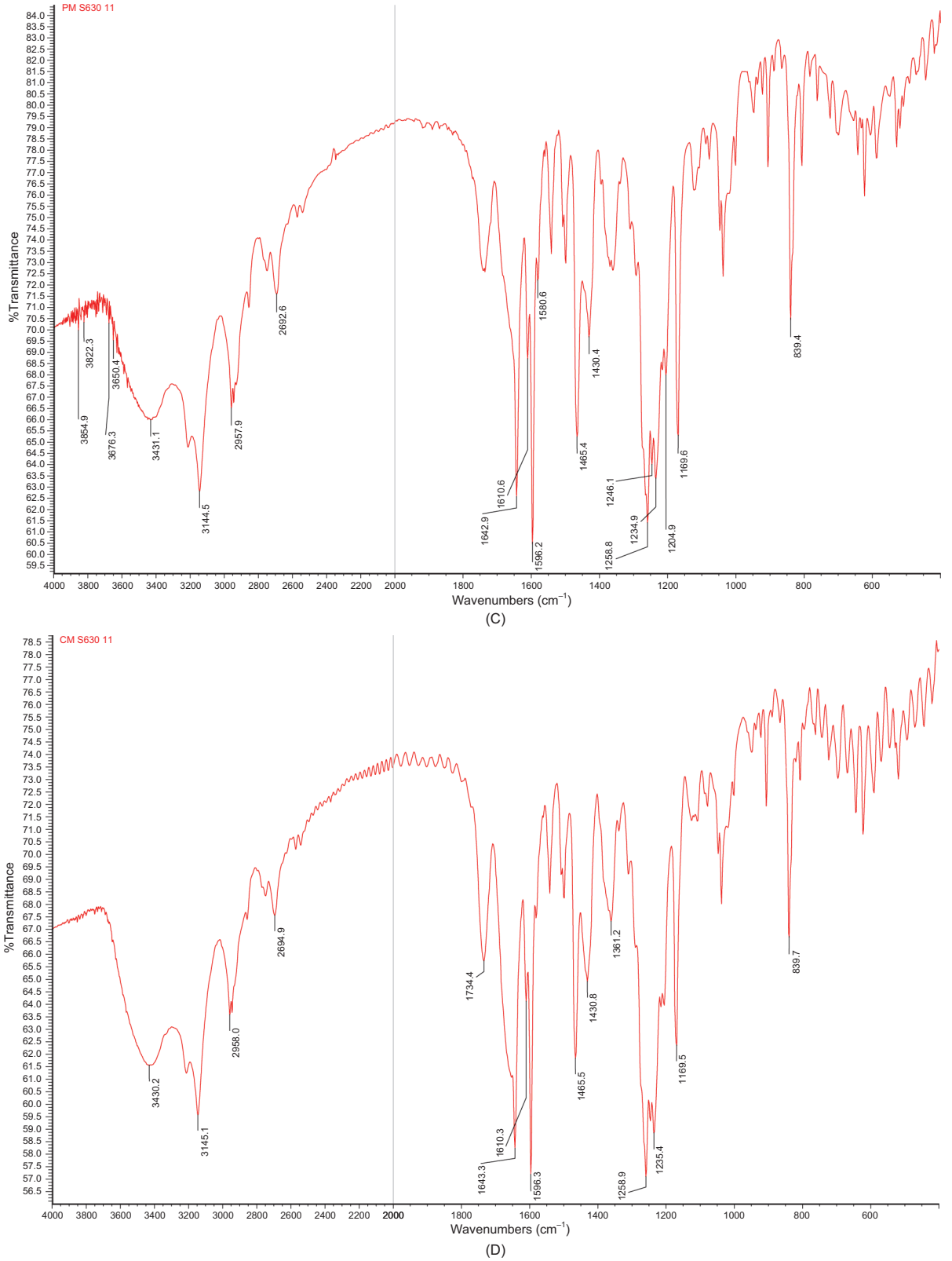


FIGURE 3. (*Continued*)

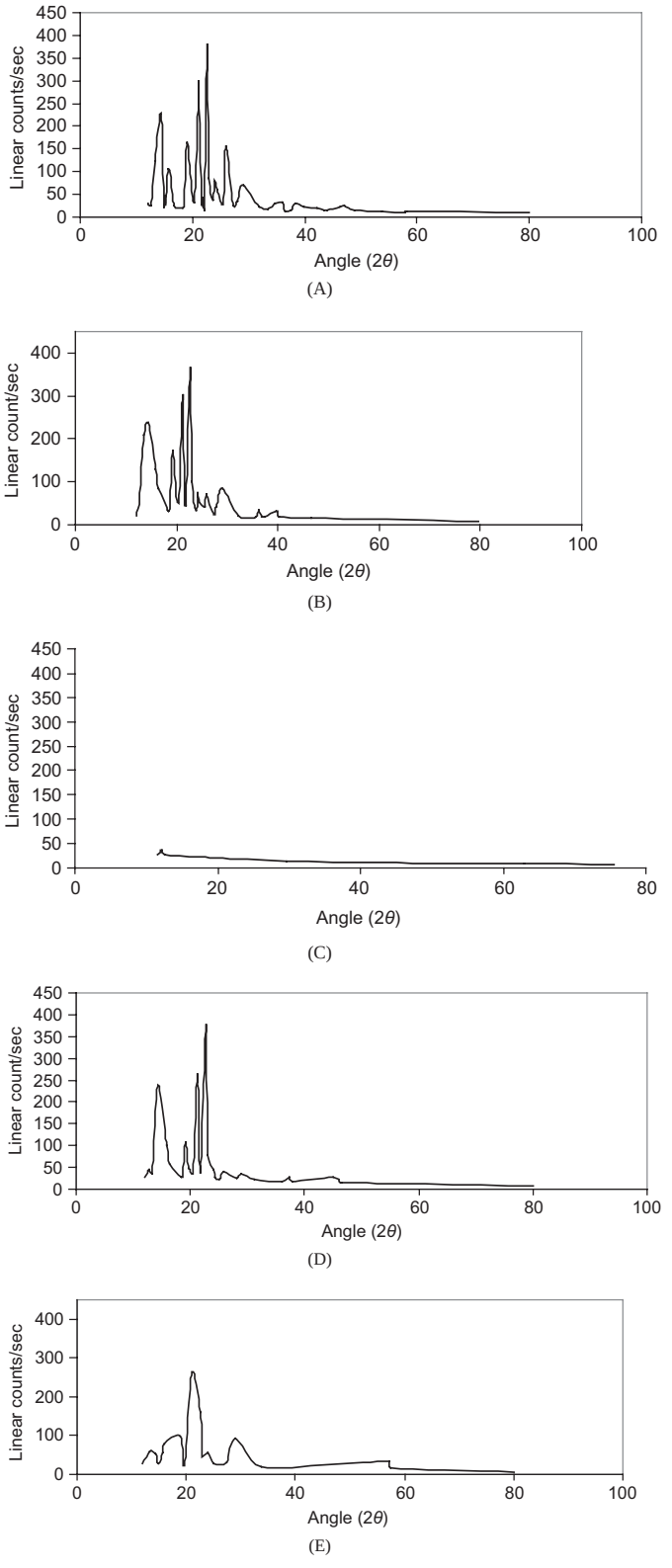


FIGURE 4. XRD spectra of (A) Un-milled R-HCl (UR); (B) Milled R-HCl (MR); (C) Plasdone S-630 (UP2); (D) physical mixture of R-HCl and Plasdone S-630 in 1:1 ratio (P21); and (E) co-ground mixture of R-HCl and Plasdone S-630 in 1:1 ratio (C21).

Scanning Electron Microscopy

In physical mixture (P21) two types of particles can be clearly observed (Figure 5A). Rectangular-type particles with sharp edges represent R-HCl particles and round-shaped particles represent Plasdone S-630 particles. Figure 5B showed the destruction of particles of drug after milling but still structured particles with sharp edge can be seen, indicating that the crystalline nature of the drug was not reduced by milling the drug alone. Figure 5C showed the shape of co-ground particles of R-HCl and Plasdone S-630 in 1:1 ratio (C21). The original morphology of both the drug and Plasdone S-630 disappeared in the co-ground mixtures. All particles are similar and spherical in shape. Particles with sharp edges are not observed in Figure 5C, which indicated that crystalline structure was absent in C21 (Mura et al., 2003). This loss of crystallinity has been also shown by DSC and XRD data. Thus, SEM confirmed the loss of the crystalline nature of R-HCl in co-ground mixture with Plasdone S-630. However, SEM of co-ground mixture of R-HCl with HPMC and Sodium Alginate (C31 and C51, respectively) showed particles with sharp edges similar to milled drug (Figure 5D and E). This observation supported the DSC data where no reduction in crystallinity was observed for batches C31 and C51.

CONCLUSION

Co-grinding of R-HCl and PVP improved the dissolution rate to variable extent. It depends upon the ratio and the grade of PVP used. In vitro dissolution data was well described by Hixson–Crowell model, indicating the possible drug release mechanism predominated by erosion. Batch C21 showed highest drug percentage release and $\%DE_{60}$. This effect was not only because of particle size reduction but also because of loss of the crystalline nature of the drug during co-grinding. Solid state characterization studies revealed that co-grinding with PVP co-polymer (Plasdone S-630) produced remarkable decline in the drug crystallinity and played a pivotal role in governing the dissolution characteristics of the drug. PVP co-polymer was found to be a better carrier for raloxifene in terms of achieving improvement in dissolution. However, it needs to be established whether the improvement in dissolution imparted by the PVP co-polymer translates into improved bioavailability in vivo in a suitable animal model. Further, the amorphous stability of these compositions needs to be ascertained on long-term storage, which is currently under progress.

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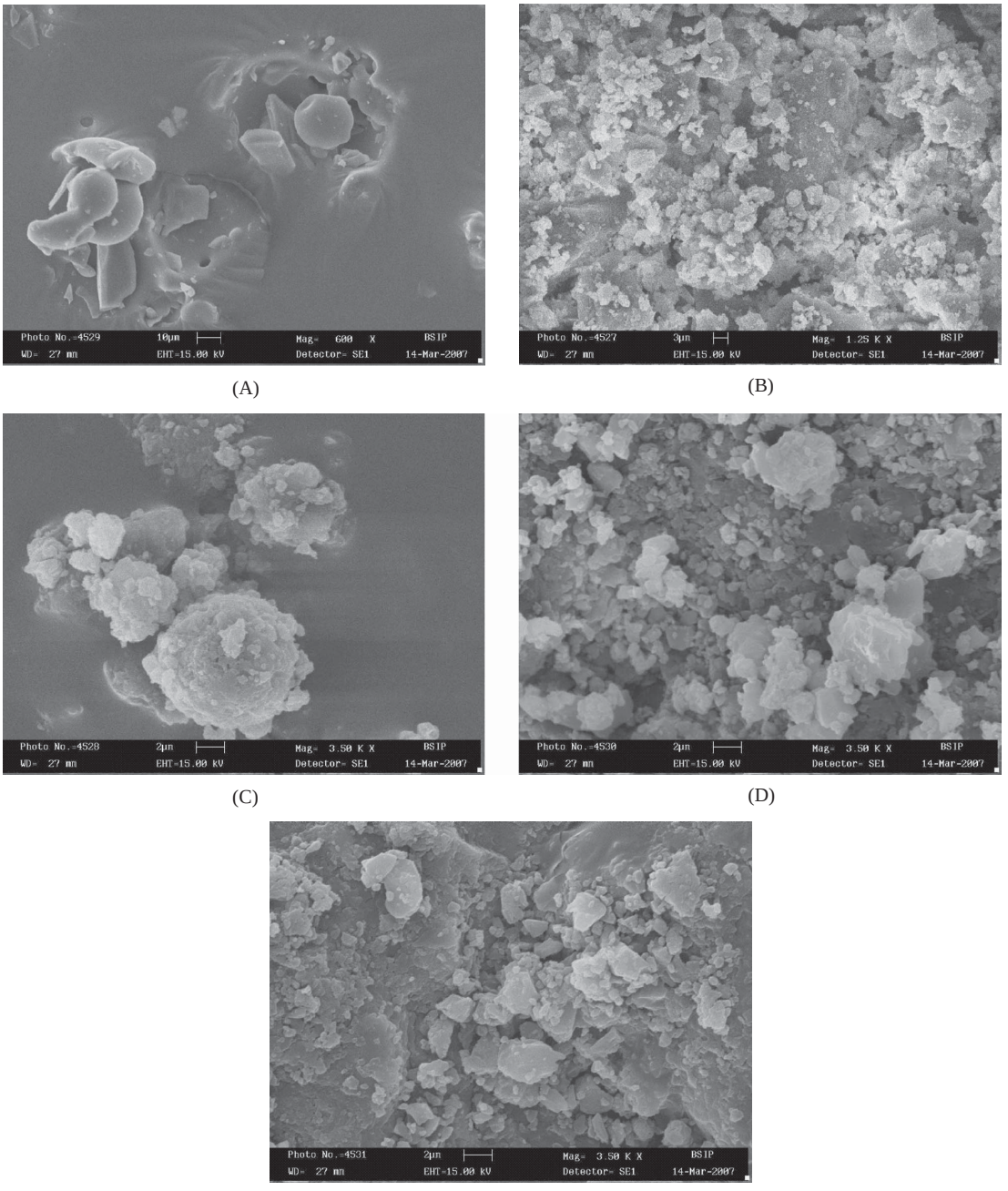

FIGURE 5. Scanning electron microphotographs of (A) physical mixture of R-HCl and Plasdone S-630 (P41); (B) Milled R-HCl (MR); (C) co-ground mixture of R-HCl and Plasdone S-630 (C21); (D) co-ground mixture of R-HCl and HPMC (C31); and (E) co-ground mixture of R-HCl and Sodium Alginate (C51).

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