Preparation, Characterization and *in Vitro* Dissolution Studies of Solid Systems of Valdecoxib with Chitosan

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In the present study, the solubilizing and amorphizing properties of Valdecoxib (a poorly water soluble anti inflammatory drug) with low molecular weight chitosan (a polymer), have been investigated. Binary systems of varying drug/polymer ratios were prepared using different techniques (physical mixing, co-grinding, kneading) and were tested for dissolution. Drug carrier interactions were investigated in both the liquid and solid state, by phase solubility analysis, differential scanning calorimetry, powder X-ray diffractometry, FT-IR spectroscopy and scanning electron microscopy. The solubility of the drug increased with increasing polymer concentration showing A_N type phase solubility diagram. Differential scanning calorimetry, powder X-ray diffractometry and scanning electron microscopic studies of binary systems suggested generation of amorphous form of drug (in kneading and co ground mixtures). IR spectroscopy revealed the presence of hydrogen bonding in kneading and co ground mixtures. Drug dissolution was improved with increasing the polymer concentration in the mixture (Kneaded>co ground>physical mixture), which was attributed to the amorphonization and/or decreased drug crystallinity, size and polymer wetting effect. Enhanced dissolution combined with its direct compression feasibility and anti ulcerogenic action results in low molecular weight chitosan for developing fast release oral solid dosage forms of valdecoxib.

Key words   solid dispersion; valdecoxib; solubility; dissolution enhancement; chitosan

Chitosan (CHT) is a linear polycationic copolymer of β (1—4) linked 2-acetamido-2-deoxy-β-D-glucopyranose (Fig. 1) obtained from deacylation of chitin, a naturally occurring polysaccharide abundant in crab and shrimp shells. It has recently emerged as one of the most promising biopolymers used in both the biomedical and pharmaceutical fields as it exhibits several desirable biological properties such as non toxicity, good biocompatibility and biodegrability, accompanied by wide availability in nature and high flexibility in use.[1—3] In addition to its use as an excipient for direct compression[4,5] and it has widely been used in the development of various kinds of drug delivery systems, due to its polymeric cationic character, film forming and gelation abilities, bioadhesiveness and transmucosal penetration enhancer properties.[1—3,6—9] Moreover, its ability of improving dissolution and bioavailability of poorly water soluble drugs has been proved.[10—17] Further, its antacid and antiucler properties can be exploited to prevent or reduce gastric irritation induced by some active compounds.[18—20]

Fig. 1.   Structure of Valdecoxib and Chitosan

Valdecoxib (VLD) is a novel selective cyclooxygenase-2 inhibitor and is one of the recently introduced nonsteroidal anti-inflammatory drugs (NSAIDs) used in the management of oesteoarthritis, pain, and dysmenorhea.[21] VLD has poor solubility in water[21—23] *i.e.*, 10 μg/ml at 25 °C. It is chemically designated as 4-(5-methyl-3-phenyl-4-isoxazolyl)benzenesulfonamide and is a diaryl substituted isoxazole (Fig. 1). For poorly water soluble and highly permeable (Class II) drugs, the rate of oral absorption is often controlled by the dissolution rate in the gastrointestinal tract.[24] Therefore, together with permeability, the solubility and/or dissolution rate of a drug are key determinants of its oral bioavailability[24,25] Thus, increasing the aqueous solubility and dissolution of VLD is of therapeutic importance.

Several attempts have been made to improve the valdecixib dissolution properties *via* rapidly disintegrating tablets[26,27] solid dispersions,[28] or complexation with cyclodextrins.[29]

Taking all this into account, the present study was carried out to investigate the feasibility of CHT enhancing the dissolution of VLD. CHT was selected because of its properties, mainly oral biocompatibility, enhancing dissolution effect of several poorly soluble drugs and direct compression feasibility. The solid systems were investigated using varying drug concentrations and methods. Solubility studies, Fourier Transform Infra Red Spectroscopy (FT-IR), Differential Scanning Calorimetry (DSC), Scanning Electron Microscopy (SEM) and X-Ray Diffraction (XRD) techniques were used for evaluation/characterization of the formulations.

Experimental

Materials   Valdecoxib was obtained as a gift sample from M/s Arthi Drugs Ltd., Tarapur, Maharastra, (India). Chitosan was obtained from India Sea Foods, Cochin, (India). According to supplier specifications, the degree of deacylation of chitosan was 96% and viscosity of 1% w/v solution in 1% acetic acid at 25 °C was 44 cps. All other reagents and solvents were of analytical grade and used as received.
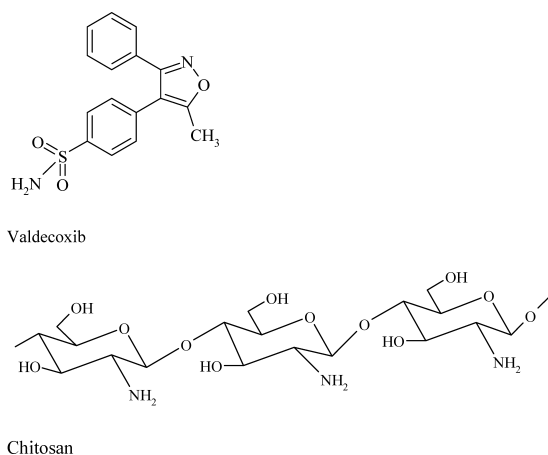
Preparation of Binary Systems Valdecoxib–chitosan binary systems were prepared taking varying drug concentration *i.e.*, 5% w/w (1 : 19), 10% w/w (1 : 9), 20% w/w (1 : 4), 40% w/w (2 : 3) were prepared using different methods. Physical mixtures (PM) of drug and polymer were obtained by simply blending with spatula, co-ground mixtures (CG) by co-grinding of drug and chitosan for 30 min in a ceramic mortar and kneaded mixture (KM) was prepared by kneading the drug-chitosan with ethanol water 6 : 1 (v/v) in a ceramic mortar, followed by drying in oven for 48 h at 40 °C. The powdered products were sieved (75—150 μm) and were used for subsequent studies. The samples were stored in desiccators over silica gel till further use.

Drug Content SDs equivalent to 10 mg of VLD were weighed accurately and suspended in suitable quantity of methanol and it was filtered. The drug content was analyzed using the filtrate at 243 nm using UV spectrophotometer (Perkin Elmer, U.S.A.). Each sample was analyzed in triplicate.

Phase Solubility Studies Solubility measurements[30] of VLD were carried out by adding excess of drug (20 mg) to 20 ml of distilled water in a stoppered conical flask. Similarly, 20 mg of the drug was added to aqueous solutions of chitosan (containing 0.1—2% w/v). The samples were agitated at constant temperature (28±1 °C) until equilibrium was achieved (2 d). The aliquots were filtered through 0.22 μm nylon disc filter, diluted suitably and assayed spectrophotometrically at 243 nm.

Infrared Spectroscopy (IR) IR spectroscopy was performed on Fourier-transformed infrared spectrophotometer (1700, Shimadzu). The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi for 10 min on KBr-press and the spectras were scanned over wave number range of 4500—500 cm^{-1}.

Differential Scanning Calorimetry (DSC) The DSC thermograms were recorded using a differential scanning calorimeter (TA Instruments Q 10, U.S.A.). Approximately 2—5 mg of each sample was heated in an open aluminum pan from 30—300 °C at a scanning rate of 10 °C/min under a stream of nitrogen.

Powder X-Ray Diffraction Analysis (XRD) Powder X-ray diffraction patterns were recorded using a Powder X-ray diffractometer (Philips PW 1729 X-ray generator computer 1710) under the following conditions: target Cu; filter Ni; voltage 35 kV; current 20 mA; reveiving slit 0.2 inches; The data were collected in the continuous scan mode using a step size of 0.01° at 2θ/s. The scanned range was 5—50°.

Scanning Electron Microscopy (SEM) The SEM analysis was carried out using a scanning electron microscope (LEO, 435 VP, U.K.). Prior to examination, samples were mounted on an aluminium stub using a double sided adhesive tape and making it electrically conductive by coating with a thin layer of gold (approximately 20 nm) in vacuum. The scanning electron microscope was operated at an acceleration voltage of 15 kV.

Dissolution Studies *In vitro* dissolution studies of VLD, PM and SD were carried out using USP paddle method by dispersed powder technique.[31] Samples equivalent to 10 mg of VLD was added to 900 ml of distilled water (pH <6.2) containing 0.25% w/v sodium lauryl sulphate at 37±0.5 °C and stirred at 50 rpm. An aliquot of 5 ml was withdrawn at different time intervals, filtered through 0.45 μm nylon filter disc. The withdrawn volume was replenished immediately with the same volume of the prewarmed (37±0.5 °C) dissolution medium in order to keep the total volume constant. The filtered samples were suitably diluted, if necessary, and assayed spectrophotometrically at 243 nm. The mean of at least three determinations was used to calculate the drug release.

Results and Discussion

Drug Content The drug content of the prepared binary systems were found to be in the range of 99.6—101.2% indicating the applications of the present method for the preparation of solid system with high content uniformity.

Phase Solubility Studies In order to gain insight into the nature of a possible interaction between Valdecoxib and Chitosan in solution, phase solubility experiments were performed. The drug solubility was increased with increase in polymer concentration (Fig. 2), and type A_N phase solubility diagram obtained.[30] These results are consistent with the formation of weak soluble complexes between VLD and CHT.[16,31] The presence of electrostatic interactions favouring and stabilizing complexation may be assumed, due to the anionic nature of the of the drug and the strong positive charge
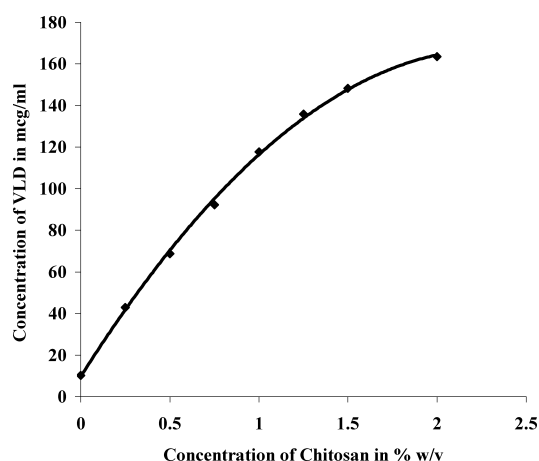

Fig. 2. Effects of Increasing Weight Fractions of Chitosan on Solubility of VLD

of the polymer at pHs <6.5.[3] The negative curvature of A_N diagrams, was probably due to self association phenomena of chitosan molecules at higher concentrations. The slope of the initial straight line part of the curve, demonstrated the relative affinity of the drug for polymer.[32] More than 16 times increase in drug solubility was observed in the presence of 2% w/v polymer. The results were found in accordance with the established formation of soluble complex between chitosan and poorly soluble drug.[16]

Infrared Spectroscopy (IR) The interaction between the drug and the carrier often leads to identifiable changes in the FT-IR profile of solid systems. FT-IR spectra for VLD, CHT, PM, CG and KM of 1 : 9 drug/polymer ratio have been depicted in Fig. 3. The spectrum of VLD exhibited characteristic signals at 3375.2 and 3249.8 cm^{-1} (N–H stretching vibrations), 1620.1 cm^{-1}, (C=N stretching vibrations), 1332.7, 1149.5 and 1091.6 cm^{-1} (S=O stretching vibrations), respectively. CHT showed a broad band at 3413.8 cm^{-1} due to a stretching vibration in the hydroxyl group and a band at 1620.1 cm^{-1} (N–H bending vibrations). The spectra of PM were equivalent to the addition spectrum of polymer and the drug indicating no interaction occurring in the simple physical mixture of drug and the polymer. The spectrum of KM exhibited significant decrease in the intensities of N–H and S=O stretching vibration peaks of VLD, a slight shift alongwith decrease in intensity of C=N stretching vibrations and hydroxyl stretching vibration peak (3377.1 cm^{-1}). Moreover, the surface of KM exhibited a C–H stretching vibration peak (2881.5 cm^{-1}) of CHT. These observations indicated the possibility of intermolecular hydrogen bonding *via* N–H, S=O and C=N groups of VLD and hydroxyl groups of CHT. The results were consistent with the reported results.[8] However, in the low frequency region (1000—400 cm^{-1}) of the spectra of binary systems, the peaks characteristic of VLD were almost unchanged. This indicated that although the drug molecule is hydrogen bonded with the polymer, the overall symmetry of the molecule is not significantly affected.[28]

Differential Scanning Calorimetry (DSC) The DSC thermogram of VLD exhibited a typical of a crystalline anhydrous substance, showing a sharp endothermal peak (T_{onset}=159.21 °C, T_{peak}=171.83 °C, fusion enthalpy 109.2 J/g) corresponding to the melting point of VLD. The DSC
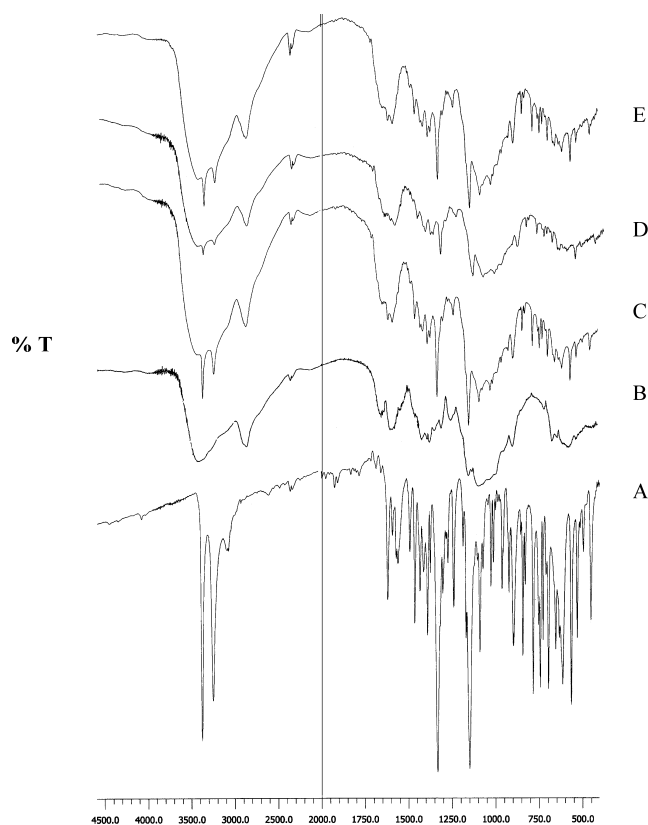
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Fig. 3. FT-IR Spectra of VLD (A), CHT (B), CCPM 10% (C), VCCG 10% (D), VCKM 10% (E)

Fig. 4. DSC Thermographs of Solid Systems VLD (A), CHT (B), VCPM 10% (C), VCCG 10% (D), VCKM 10% (E)

trace of CHT was typical of an amorphous hydrated compound, showing a broad endothermal effect, ranging between 40 and 130 °C (Fig. 4). Physical mixture revealed the addition of both polymeric curve and drug peaks, with slight shift in the melting temperature of the drug along with significant decrease in fusion enthalpy. Co-ground mixture exhibited a short and broader drug peak, while kneaded mixture show only a mild drug peak indicating the change of nature of drug form crystalline to amorphous one.

Powder X-Ray Diffraction Analysis (XRD) Powder X-ray diffraction patterns in the 5—50° at 2θ range showed that the typical diffraction peaks of VLD were still detectable and emerged on the diffuse background of the amorphous additive in the respective physical mixtures (Fig. 5). An evident loss of crystallinity was observed in co ground and kneaded mixtures. Crystallinity was determined by comparing a representative peak height in the diffraction patterns of the binary systems with those of a reference. The crystallinity of the drug was calculated in terms of relative degree of crystallinity (RDC)$=I_{sam}/I_{ref}$, where I_{sam} is the peak height of the sample under investigation and I_{ref} is the peak height of the reference at the same angle showing the highest intensity.[33,34] Pure drug peak at 22° (2θ) was used for calculating RDC of PM, CG and KM. The RDC values of VLD in physical mixture, coground mixture and kneaded mixture at 10% w/w drug concentration were 0.2083, 0.1654 and 0.1417 respectively, suggesting, the presence of VLD in the solid dispersion mostly in amorphous state with a few partially crystallized drug molecules.[35]

Scanning Electron Microscopy (SEM) The morphology of the Valdecoxib chitosan systems prepared by the dif-
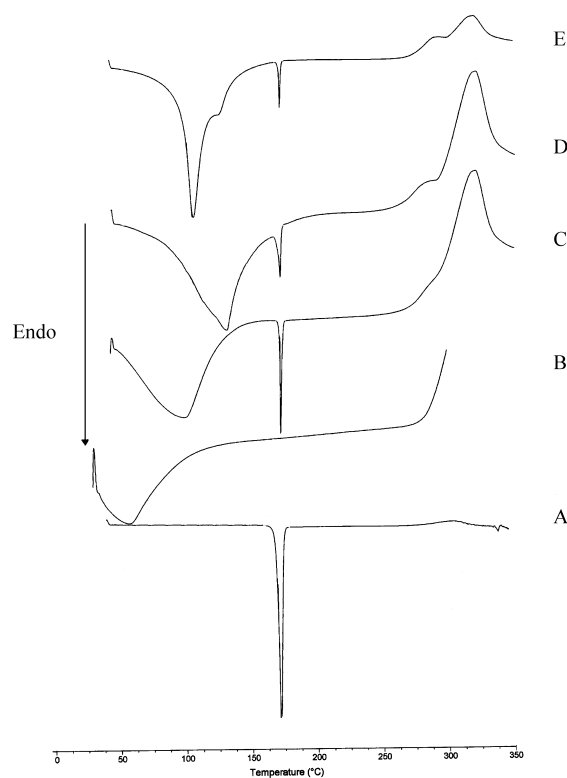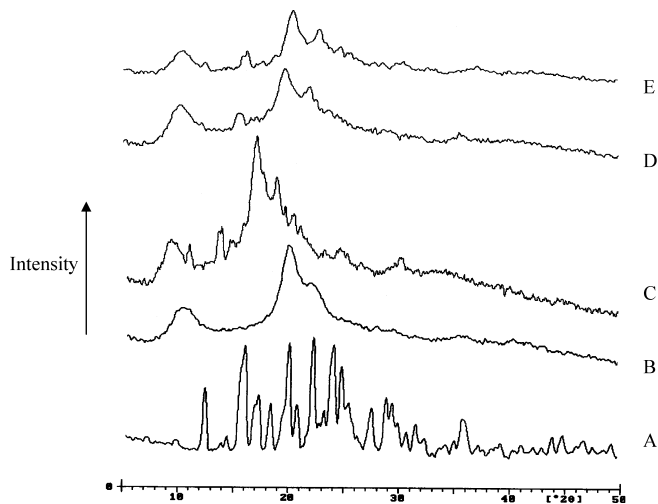

Fig. 5. XRD Patterns of Solid Systems VLD (A), CHT (B), VCPM 10% (C), VCCG 10% (D), VCKM 10% (E)

ferent methods was investigated by SEM analysis (Fig. 6). VLD particles appear as small plate like crystals with smooth surfaces of homogenous morphology, whereas chitosan consisted of amorphous particles of rather irregular size and shape. Crystals of VLD mixed with carrier particles were clearly evident in physical mixture whereas in corresponding kneaded system, it appeared as a substantially amorphous product. These observations indicate the evidence of interaction between drug and polymer and are in accordance to the results obtained from FT-IR and DSC studies.

Dissolution Studies The dissolution of poorly water-soluble drugs requires a dissolution medium entirely different
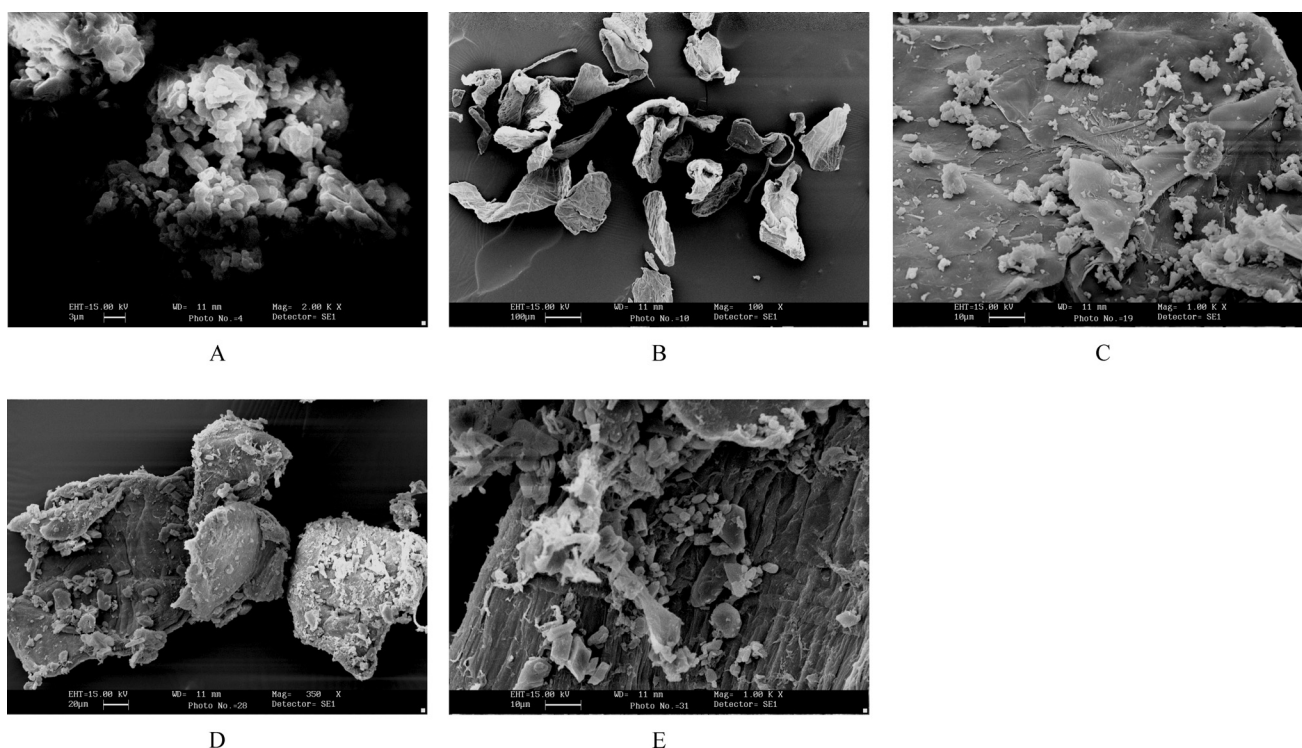
Fig. 6.   SEM Images of Valdecoxib (A), Chitosan (B), and Its Physical Mixture (C), Co-ground Mixture (D) and Kneaded Mixture (E) at 10% w/w Drug Concentration

from those used for water-soluble drugs. One of the techniques that have been useful in dissolution of insoluble drugs is the incorporation of a small amount of surfactant in the dissolution medium.[36] The use of surfactant in the dissolution medium is physiologically meaningful, due to the presence of natural surfactants (like bile salts) in the gastrointestinal tract. The ability of surfactants to accelerate the *in vitro* dissolution of poorly water-soluble drugs has been attributed to wetting, micellar solubilization, and/or defocculation. It is easy to understand that a biorelavent medium needs similar surface activity as bio fluids. Studies on sodium lauryl sulphate have shown to satisfy this needs.[37] Based upon these facts, dissolution of pure VLD, PM, CG and KM were carried out in distilled water (pH <6.2) containing 0.25% w/v sodium lauryl sulphate. The dissolution profiles of PM, CG and KM of VLD with CHT are shown in Fig. 7. Dissolution had progressively been improved with increasing polymer concentration in the mixture and were found in the order of Kneaded>co-ground>physical mixture>pure drug.

CG and KM exhibited a higher relative dissolution rate at 10 min ($RDR_{10\,min}$) than the PM, when compared to the pure drug. Incorporation of VLD with CHT especially in CG and KM form significantly improved the dissolution of the drug as compared to the PM and the pure drug. Dissolution efficiency (DE)[38] which is defined as the area under dissolution curve up to the time (t) is expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time.

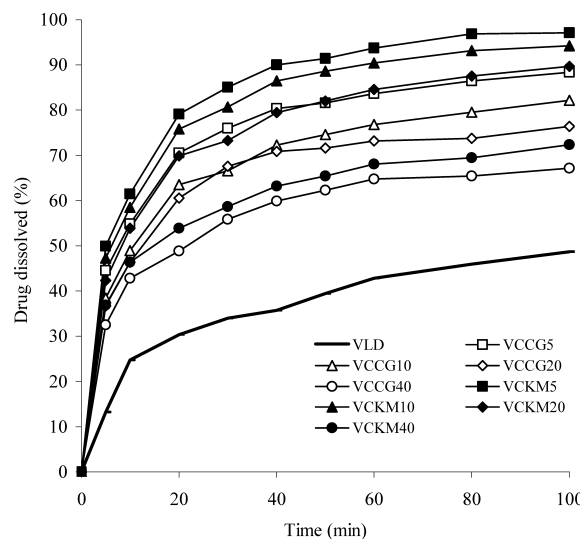$$\text{Dissolution efficiency (DE)} = \left(\frac{\int_0^t y \times dt}{y100 \times t} \right) \times 100$$

Fig. 7.   Dissolution Profile of Pure Valdecoxib, and Valdecoxib in Kneaded Mixtures and Co-ground Mixtures

Each point represents as mean±S.D., $n=3$.

The dissolution efficiency can have a range depending upon the time interval selected. In any case, a constant time interval be chosen for better comparison. In the present investigation, $DE_{10\,min}$ values were calculated from the dissolution data of each product and used for comparison. CG and KM revealed significantly higher dissolution efficiency ($DE_{10\,min}$) compared to PM and the pure drug. Further, PM showed a higher $DE_{10\,min}$ values than the pure drug.

The slight positive effect on drug dissolution shown by simple physical mixtures could be the result of reduction of the interfacial tension between the hydrophobic drug parti-

Table 1.　Percent Drug Dissolved at 30 min (DP_{30}), Dissolution Efficiency at 10 min (DE_{10}) and Relative Dissolution Rate at 10 min (RDR_{10}) of Pure VLD, Physical Mixtures (PM), Co-ground Mixtures (CG) and Kneaded Mixtures (KM)

Sample	DP_{30}	DE_{10}	RDR_{10}
VLD	33.98±0.84	12.79	1.00
VCPM 5	47.96±0.95	22.56	1.38
VCPM 10	44.41±1.67	19.55	1.32
VCPM 20	42.39±0.65	17.42	1.16
VCPM 40	36.04±0.27	15.79	1.04
VCCG 5	74.74±1.12	35.71	2.20
VCCG 10	66.02±0.56	30.88	1.93
VCCG 20	67.04±0.56	30.06	1.88
VCCG 40	55.06±0.76	26.81	1.71
VCKM 5	84.38±0.68	39.39	2.45
VCKM 10	80.19±0.51	37.94	2.35
VCKM 20	73.24±0.33	34.8	2.17
VCKM 40	58.28±0.48	29.61	1.87

Each value represents as mean±S.D., $n=3$.

cles and the dissolution medium, owing to the presence of the hydrophilic polymer and the local solubilizing effect acting at the early stage of the dissolution process in the micro environment surrounding the drug particles. The best performance shown by kneaded mixture over co-ground mixture could be the intimate contact between VLD and CHT leading to a better dispersion of drug into the hydrophilic carrier in KM. The solubilizing effect of CHT in drug–carrier complexes also hypothesized, beyond the particle size reduction brought about by the mechanical treatment, and the decrease in drug crytallinity during grinding with amorphous carrier.[11—13]

In conclusion, this study showed that chitosan can favorably enhance dissolution. Moreover, the possibility of obtaining tablets by direct compression using this polymer is advisable for the development of a fast release solid dosage form of Valdecoxib for oral administration.

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