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Enhancement of dissolution rate and bioavailability of aceclofenac: A chitosan-based solvent change approach

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Abstract

In this study the significant effect of chitosan on improving the dissolution rate and bioavailability of aceclofenac has been demonstrated by simple solvent change method. Chitosan was precipitated on aceclofenac crystals using sodium citrate as the salting out agent. The pure drug and the prepared co-crystals with different concentrations of chitosan (0.05–0.6%) were characterized in terms of solubility, drug content, particle size, thermal behaviour (differential scanning calorimetry, DSC), X-ray diffraction (XRD), morphology (scanning electron microscopy, SEM), *in vitro* drug release and stability studies. The *in vivo* performance was assessed by preclinical pharmacodynamic (analgesic and anti-inflammatory activity) and pharmacokinetic studies. The particle size of the prepared co-crystals was drastically reduced during the formulation process. The DSC showed a decrease in the melting enthalpy indicating disorder in the crystalline content. The XRD also revealed a characteristic decrease in crystallinity. The dissolution studies demonstrated a marked increase in the dissolution rate in comparison with pure drug. The considerable improvement in the dissolution rate of aceclofenac from optimized crystal formulation was attributed to the wetting effect of chitosan, decreased drug crystallinity, altered surface morphology and micronization. The optimized co-crystals exhibited excellent stability on storage at accelerated conditions. The *in vivo* studies revealed that the optimized crystal formulation provided a rapid pharmacological response in mice and rats besides exhibiting improved pharmacokinetic parameters in rats.
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Keywords: Aceclofenac; Chitosan; Crystals; Sodium citrate; Dissolution; Pharmacokinetics; Pharmacodynamics

1. Introduction

The rate of absorption and bioavailability of poorly water-soluble drugs is often controlled by the rate of dissolution of the drug in the gastrointestinal tract. Many technological methods of enhancing the dissolution characteristics of slightly water-soluble drugs have been reported in various literatures (Moneghini et al., 2001). These include reducing particle size to increase surface area (Nijlen et al., 2003), solubilization in surfactant systems, formation of water-soluble complexes, use of pro-drug, drug derivatization and manipulation of solid state of drug substance to improve drug dissolution, i.e. by decreasing crystallinity of drug substance (Nokhodchi, 2005). Recently, natural polymers such as polysaccharides and proteins have received much attention in the pharmaceutical field owing to their good biocompatibility and biodegradability (Imai et al., 1991; Zhang et al., 2002; Cai et al., 2005). Among polysaccharides, chitosan has been considered to be one of the most promising biopolymer for drug delivery purposes. Chitosan (β-(1-4)-2-amino-2-deoxy-D-glucose) is a linear hydrophilic polysaccharide polymer of D-glucosamine. It is a non-toxic natural poly cationic polymer that is degraded by the microflora in the colon. It is abundant in nature and is present in the exoskeleton of crustaceans such as crabs and shrimp (Fukuda et al., 2006). Chitosan, being a cationic polysaccharide in neutral or basic pH conditions, contains free amino groups and hence, it is insoluble in water. In acidic pH, amino groups can undergo protonation thus, making it soluble in water. It breaks down slowly to harmless products (amino sugars), which are completely absorbed by the human body (Agnihotri et al., 2004).

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Chitosan has been demonstrated to be a good vehicle for enhancing the dissolution properties and bioavailability of a number of poorly water-soluble drugs (Portero et al., 1998). Low molecular weight chitosan can function as drug release enhancers for poorly water-soluble drugs due to an improvement in wettability resulting from the solubility of low molecular weight chitosan in water (Fukuda et al., 2006).

Aceclofenac (2-[(2,6-dichlorophenyl) amine] phenylacetoxyacetic acid) is an orally effective non-steroidal anti-inflammatory drug (NSAID) of the phenyl acetic acid group, which possesses remarkable anti-inflammatory, analgesic and antipyretic properties (British Pharmacopoeia, 2005; Parfitt, 1999). The analgesic efficacy of aceclofenac 100 mg is more prolonged than that of acetaminophen 650 mg. Aceclofenac appears to be particularly well-tolerated among the NSAIDs, with a lower incidence of gastrointestinal adverse effects (Gowda et al., 2006).

Aceclofenac exhibits very slight solubility in water and as a consequence it exhibits low bioavailability after oral administration (Lee and Jung, 1999; Kim et al., 2001). Therefore, the improvement of aceclofenac dissolution from its oral solid dosage forms is an important issue for enhancing its bioavailability and therapeutic efficacy. In an earlier study, we have reported the enhancement in the dissolution rate and *in vivo* effectiveness of aceclofenac when prepared in the form of spherical agglomerates (Mutalik et al., 2007a). The solid systems for several drugs using chitosan have been reported with solid dispersions, co-ground mixture and solid complexes, physical mixture and co-ground products and spray dried products at different ratios (Portero et al., 1998; Hel et al., 1999; Mura et al., 2003; Maestrelli et al., 2004; Kumar and Mishra, 2006). There are hardly any reports on the improvement of dissolution rate and bioavailability of poorly water-soluble drugs by precipitation of chitosan using a simple solvent change method. Hence the objectives of present study were (i) to assess the feasibility of chitosan in enhancing the solubility and dissolution rate of acelofenac by preparing its co-crystals using solvent change method and (ii) to evaluate the *in vivo* performance of optimized co-crystals with respect to pharmacodynamics and pharmacokinetics in animal models.

2. Materials and methods

2.1. Materials

Aceclofenac was obtained as gift sample from Lupin Research Park, Pune, India. Chitosan (from crab shells; minimum 85% deacetylated) and Tween-80 (Specific gravity: 1.07 at 25 °C; HLB value: 15.0; critical micellar concentration: 13–15 mg/L; viscosity: 400–620 cps at 25 °C) were purchased from Sigma–Aldrich, MO, USA. Hydrochloric acid (35–38%) and glacial acetic acid (99.5%) were purchased from Labort Fine Chem Pvt. Ltd., Surat, India and Qualigens Fine Chemicals, Mumbai, India, respectively. Sodium citrate (99%) was purchased from SRL Pvt. Ltd., Mumbai, India. The solvents for analysis used were of HPLC grade. All other chemicals used were of analytical grade.

2.2. Preparation of co-crystals

The composition of different crystal formulations is given in Table 1. Chitosan solution was prepared by soaking chitosan in 1% glacial acetic acid for 3 h. A weighed amount of the drug was dispersed in chitosan solution by using high dispersion homogenizer (Polytron, PT-MR 3100, Kinematica AG, Switzerland) at 15,000 rpm for 5 min. This dispersion was then added to distilled water (D-1 to D-8) or sodium citrate solution (C-1 to C-10) to precipitate chitosan on drug crystals. The precipitate obtained was filtered through Whatmann No. 1 filter paper using vacuum filtration unit and dried at 45 °C for 24 h. The dried product was then passed through sieve No. 60 to obtain a uniform size distribution. A control crystal formulation (C-11) without chitosan was also prepared. The practical yield of the prepared crystals was calculated.

2.3. Solubility, drug content and particle size determination

An excess quantity of aceclofenac or prepared co-crystals was placed in the bottles containing 10 ml of different solutions. The bottles were agitated in a shaking water bath (100 agitations/min) for 24 h at room temperature. The solution was then passed through a membrane (0.45 μm) and the amount of the drug dissolved was analyzed spectrophotometrically (UV-1601PC, Shimadzu, Japan) at 275 nm. This study was also carried out to select a suitable dissolution medium for the *in vitro* drug release studies.

For the determination of drug content, prepared crystals (10 mg) were triturated with phosphate buffer (pH 6.8) and finally the volume was made up to 100 ml with the same. The solution was filtered through a membrane (0.45 μm) and analyzed spectrophotometrically for drug content after sufficient dilution with phosphate buffer (pH 6.8).

The mean particle size of pure drug and prepared crystals was determined by laser light scattering technique using Ankersmid CIS-50 particle size analyzer (Ankersmid, USA).

2.4. Infrared (IR) spectroscopy

IR spectroscopy was conducted using a Shimadzu FT-IR 8300 Spectrophotometer (Shimadzu, Tokyo, Japan) and the spectrum was recorded in the wavelength region of 4000–400 cm^{-1}. The procedure consisted of dispersing a sample (drug alone, mixture of drug and polymer or prepared co-crystals) in KBr and compressing into discs by applying a pressure of 5 t for 5 min in a hydraulic press. The pellet was placed in the light path and the spectrum was recorded. All spectra were collected as an average of three scans at a resolution of 2 cm^{-1}.

2.5. Differential scanning calorimetry (DSC)

DSC was performed using DSC-60 (Shimadzu, Tokyo, Japan) calorimeter to study the thermal behaviour of drug alone, mixture of drug and polymer or prepared co-crystals. The instrument comprised of calorimeter (DSC 60), flow controller (FCL

Table 1
Composition of aceclofenac-chitosan crystal formulations

Ingredients	D-1	D-2	D-3	D-4	D-5	D-6	D-7	D-8	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11
Aceclofenac (mg)	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400
Chitosan	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.75	0.05	0.1	0.2	0.3	0.4	0.5	0.15	0.25	0.2	0.2	–
Glacial Acetic acid (ml)	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Distilled water (ml)	100	100	100	100	100	100	100	100	–	–	–	–	–	–	–	–	–	–	–
Sodium citrate 1% (ml)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	100	–	–
Sodium citrate 2% (ml)									100	100	100	100	100	100	100	100	–	–	100
Sodium citrate 3% (ml)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	100	–

60), thermal analyzer (TA 60) and operating software (TA 60). The samples were heated in hermetically sealed aluminum pans under nitrogen flow (30 ml/min) at a scanning rate of 5 °C/min from 24 ± 1 °C to 250 °C. Empty aluminum pan was used as a reference. The physical mixture of drug with excipients for compatibility studies was prepared by triturating the drug with excipient in a dried mortar for 5 min.

2.6. X-ray diffraction (XRD)

The X-ray diffraction patterns of pure drug and the optimized crystals formulation were recorded using Philips X-ray diffractometer (Model: PW 1710) with a copper target. The conditions were: voltage −30 kV; current −30 mA; scanning speed −1°/min; temperature of acquisition: room temperature; detector: scintillation counter detector; sample holder: non-rotating holder.

2.7. Scanning electron microscopy (SEM)

The surface characteristics of the pure drug and prepared crystals were studied by SEM (JEOL, JSM 50A, Tokyo, Japan) at 1600×. The samples were mounted on double-sided adhesive tape that has previously been secured on copper stubs and then analyzed. The accelerating voltage was 20 kV.

2.8. Dissolution studies

The *in vitro* dissolution studies were carried out using eight-station USP type 1 dissolution apparatus (Electrolab, Mumbai, India). The study was carried out in 900 ml of 0.1N HCl containing 2% Tween 80. Dissolution medium was kept in a thermostatically controlled water bath, maintained at 37 ± 0.5 °C. The basket was rotated at 75 rpm. At predetermined time intervals, 5 ml of samples were withdrawn and assessed for drug release spectrophotometrically. At each withdrawal, 5 ml of fresh dissolution medium was added to dissolution jar.

2.9. Stability studies

After determining the drug content, the optimized crystals were charged for the accelerated stability studies according to ICH guidelines (40 ± 2 °C and 75 ± 5% RH) for a period of 6 months in stability chamber (Thermolab, Mumbai, India). The samples were placed in USP type-1 flint vials and hermetically sealed with bromobutyl rubber plugs and aluminium caps. Five milligrams of the stored crystals ($n = 3$) were taken out at 15, 30, 60, 90 and 180 days, and evaluated for the drug content and physical changes.

2.10. In vivo studies

The preclinical studies (anti-inflammatory, analgesic and pharmacokinetic studies) were carried out in Wistar rats and Swiss albino mice. Male Wistar rats (200–250 g) and Swiss albino mice (25–30 g) were obtained from the Central Animal House, Manipal University, Manipal. They were housed

in elevated wire cages, four animals per cage, with free access to food (Lipton, Mumbai, India) and water. The preclinical study protocol was approved by the Institutional Animal Ethical Committee, Kasturba Medical College, Manipal (Approval No. IAEC/KMC/06/2006-2007).

2.10.1. Anti-inflammatory activity

Carrageenan-induced rat paw edema model was used to assess the anti-inflammatory effect of the pure drug and optimized crystals (Kulkarni, 1997). The overnight fasted rats were divided into three groups ($n = 6$) and treated as following:

Group I: aceclofenac (10 mg/kg) in 0.5% sodium carboxymethylcellulose (CMC); p.o.
Group II: C-3 crystals containing equivalent amount of aceclofenac (10 mg/kg) in 0.5% CMC; p.o.
Group III: 2.5 ml of 0.5% CMC; p.o.

After 30 min of drug administration, all the rats were challenged by a subcutaneous injection of 0.05 ml of 1% solution of carrageenan in saline into the plantar site of the left hind paw. The paw volumes were measured with a plethysmometer, prior to administration of carrageenan and after 1–5 h of administration. The percent inhibition of edema for all time intervals was calculated.

2.10.2. Analgesic activity

Writhing test was used to evaluate the analgesic activity of the drug when administered as pure drug and prepared crystal formulation (C-3) (Kulkarni, 1997). The overnight fasted mice were divided into three groups ($n = 6$) and treated orally as given in the above procedure. After 1 h of post-dose, they were injected with 1% acetic acid (0.1 ml/10 g) intraperitonially (i.p.). Then the number of writhings was recorded for 30 min. The analgesic activity was evaluated in terms of the percentage of writhe inhibitions.

2.10.3. Pharmacokinetic study

The pharmacokinetic studies were carried out in Wistar rats. The overnight fasted animals were divided into three groups ($n = 6$) and treated orally as shown below.

Group I: pure aceclofenac (10 mg/kg) in 0.5% CMC.
Group II: C-3 crystals containing equivalent amount of aceclofenac (10 mg/kg) in 0.5% CMC.

Then blood samples were collected at predetermined intervals (0, 0.5, 1, 2, 4, 6 and 8 h) of post-dose into heparinized tubes from the orbital sinus. The plasma was separated immediately by cold centrifugation (Remi Equipments Ltd., Mumbai, India) at 3000 rpm for 15 min and the plasma was stored at −72 °C until analysis.

2.11. HPLC analysis of drug

A sensitive high performance liquid chromatographic (HPLC) method was used to analyze aceclofenac (Mutalik et al., 2007b). The HPLC system (Shimadzu Class *VP* series having Class *VP* 6.12 version software) with two pumps (LC-10AT *VP*), a variable wavelength programmable UV–vis detector (SPD-10A *VP*), a system controller (SCL-10A *VP*) and an RP C-18 column (Hypersil BDS C_{18}) was used.

Preparation of stock and working standard solutions: Stock solutions of 1 mg/ml of aceclofenac and venlafaxine (internal standard) were prepared separately using methanol. From the stock solutions, working standard solutions were prepared to contain 1, 2, 5, 10, 20, 30, 50 and 70 μg/ml of aceclofenac and 500 μg/ml of venlafaxine using methanol and water (Milli-Q, Millipore, USA) in the mixture of 80:20 (v/v) as diluent.

Preparation of calibration standards in plasma curve: the studies were carried out using aliquots of plasma (95 μl) pipetted into micro centrifuge tubes, spiked with 5 μl of the working standard solutions of drug (to give final concentration of 50, 100, 250, 500, 1000, 1500, 2500 and 3500 ng/ml, respectively). To this, 25 μl of 500 μg/ml internal standard and 200 μl of acetonitrile was added and mixed for a minute. Six hundred and seventy-five microliters diluent was added to make up the volume up to 1.0 ml and vortexed for 60 s. The plasma sample was centrifuged at 10,000 rpm for 10 min in cooling centrifuge at 4 °C. After centrifugation, the supernatant layer was separated and injected to the HPLC system. Standard curves were obtained from the linear square regression analysis of drug/internal standard peak area ratio as a function of theoretical concentration. Slopes, intercept and correlation coefficients were determined.

Preparation of sample solutions: To 100 μl of rat plasma, 25 μl of internal standard solution (500 μg/ml) and 200 μl of acetonitrile was added and mixed for a minute. To this, diluent was added (675 μl) up to 1 ml. The resulting solution was vortexed for 60 s and centrifuged at 10,000 rpm for 10 min. The supernatant layer was separated and analyzed using HPLC system.

Chromatographic conditions: Mobile phase—methanol: 0.3% TEA pH 7.0 (60:40, v/v); column: Hypersil BDS C_{18} (250 cm × 4.6 mm), 5 μm; flow rate: 1.0 ml/min; injection volume: 20 μl; temperature: 25 °C; run time: 25 min; detection wavelength: 275 nm; internal standard: venlafaxine.

Method: The standard and sample solutions were injected with the above chromatographic conditions and the chromatograms were recorded. The response factor (peak area ratio of drug peak area to the internal standard peak area) of the standard solution and the sample were calculated and the concentration of the aceclofenac present in the plasma samples was calculated from the calibration curve. The blank plasma samples were analyzed prior to the analysis of aceclofenac standard preparations. No interference from the blank plasma was observed for the analysis of drugs. The peaks were well resolved and the retention time of aceclofenac and venlafaxine were 10.285 and 18.303 min, respectively in rat plasma.

2.12. Data analysis

Student's *t*-test was employed to analyze the results (Graph Pad Prism Software). Difference below the probability level of 0.05 was considered statistically significant. The pharmacoki-

Table 2
Practical yield, drug content and solubility data for the pure drug and prepared crystals

Formulation	Yield (%)	Drug content (%)	Solubility	
			Water (mg/ml)	0.1N HCl (mg/ml)
Aceclofenac	–	–	0.053 ± 0.004	0.013 ± 0.001
C-1	87.54	99.76 ± 1.24	0.069 ± 0.006	0.013 ± 0.004
C-2	85.32	99.23 ± 1.67	0.074 ± 0.004	0.019 ± 0.003
C-3	82.54	98.87 ± 1.34	0.078 ± 0.009	0.027 ± 0.002
C-4	70.19	98.35 ± 1.53	0.063 ± 0.005	0.013 ± 0.003
C-5	62.12	98.19 ± 2.73	0.069 ± 0.006	0.013 ± 0.003
C-6	60.59	98.07 ± 1.56	0.075 ± 0.006	0.017 ± 0.002
C-7	84.32	99.02 ± 2.51	0.075 ± 0.006	0.021 ± 0.003
C-8	75.62	98.45 ± 1.58	0.068 ± 0.004	0.025 ± 0.002
C-11	95.92	99.25 ± 0.55	0.054 ± 0.005	0.013 ± 0.001
D-1	93.54	99.49 ± 2.17	0.062 ± 0.003	0.015 ± 0.001
D-2	90.75	99.26 ± 1.34	0.063 ± 0.004	0.015 ± 0.003
D-3	88.28	99.15 ± 1.41	0.060 ± 0.006	0.014 ± 0.003
D-4	85.61	99.06 ± 2.69	0.057 ± 0.009	0.016 ± 0.004
D-5	80.41	98.88 ± 1.37	0.061 ± 0.008	0.015 ± 0.003
D-6	76.34	98.76 ± 1.29	0.061 ± 0.008	0.017 ± 0.001
D-7	70.29	98.54 ± 2.62	0.063 ± 0.008	0.018 ± 0.002
D-8	60.17	98.49 ± 1.88	0.062 ± 0.004	0.018 ± 0.002

netic parameters were calculated by using PK Solutions 2.0™ Noncompartmental pharmacokinetic data analysis software.

3. Results and discussion

The solubility, dissolution behaviour and permeability of a drug are the key determinants of its oral bioavailability. The solubility data of aceclofenac reveals that it is poorly soluble in water. Therefore, the improvement of aceclofenac dissolution from its oral solid dosage forms is of great concern.

3.1. *Practical yield, drug content, particle size and solubility study*

The practical yield was ranged from 60.17 to 95.92% (Table 2). The practical yield was found to be decreased with the increase in polymer concentration due to the formation of a thick viscous chitosan solution from which separation of the drug crystals was difficult. The drug content was found to be good and uniform among the different batches of crystals prepared and ranged from 98.07 to 99.76% (Table 2).

The particle size of all the crystal formulation prepared was considerably reduced during the preparation process. This might be due to high attrition during the homogenization process. Pure aceclofenac showed maximum particle size (D_{10} = 34.72 μm; D_{50} = 62.62 μm; D_{90} = 95.65 μm; D_{97} = 96.25 μm). The highest size reduction was achieved with C-3 crystals (D_{10} = 0.11 μm; D_{50} = 0.53 μm; D_{90} = 0.95 μm; D_{97} = 1.20 μm) which exhibited highest solubility and dissolution rate for aceclofenac; whereas the size reduction was less in the absence of chitosan (C-11, without chitosan; D_{10} = 0.39 μm; D_{50} = 2.27 μm; D_{90} = 5.22 μm; D_{97} = 6.55 μm).

Based on the solubility studies of drug in 0.1N HCl containing different concentrations of Tween 80, suitable dissolution medium was selected for *in vitro* drug release studies (Table 3). The solubility of aceclofenac in HCl was very less when compared to that of distilled water. Of the various methods investigated to select suitable dissolution medium, the use of media containing surfactants was proposed as a suitable method for solubilizing such drugs, because various surfactants are present in the gastrointestinal fluid, e.g., bile salts, lecithin, cholesterol and its esters (Park and Choi, 2006). Hence Tween 80 was used as surfactant in this study. Tween 80 has been successively used to develop dissolution media for poorly water-soluble drugs (Banakar, 1992; Balakrishnan et al., 2004; Pennings et al., 2006). Aceclofenac showed sufficient solubility in 0.1N HCl + 2% Tween 80 which was adequate to maintain sink condition and was selected as the dissolution medium.

In the solubility studies of the prepared crystals, C-3 crystals showed highest solubility of drug in both water (0.078 mg/ml) and 0.1N HCl (0.027 mg/ml) in comparison with pure drug (water: 0.053 mg/ml; 0.1N HCl: 0.013 mg/ml). In addition, as the concentration of chitosan increased in the formulation, the solubility gradually increased up to a certain concentration followed by decrease in the solubility, however the variability in the solubility of drug in the presence of chitosan was not so substantial.

Table 3
Solubility data of aceclofenac in different media

Media	Solubility (mg/ml)
Water	0.053 ± 0.004
0.1N HCl	0.013 ± 0.003
0.1N HCl + 0.5% Tween 80	0.183 ± 0.009
0.1N HCl + 1% Tween 80	0.287 ± 0.009
0.1N HCl + 2% Tween 80	0.521 ± 0.013
0.1N HCl + 3% Tween 80	0.693 ± 0.009
0.1N HCl + 4% Tween 80	0.736 ± 0.019
0.1N HCl + 5% Tween 80	0.826 ± 0.015
0.1N HCl + 6% Tween 80	0.970 ± 0.049

3.2. IR, DSC, XRD and SEM studies

The possible interaction between the drug and the excipients were studied by IR spectroscopy and DSC. IR spectra of pure aceclofenac, its physical mixture with chitosan, and the prepared crystal formulations are shown in Fig. 1. Pure aceclofenac showed major peaks at 3319.3, 2970.2, 2935.5, 1716.5, 1589.2, 1506.3, 1479.3, 1344.3, 1280.6, 1255.6, and 665.4 cm^{-1} (Mutalik et al., 2007a, 2007b). The results revealed no considerable changes in the IR peaks of aceclofenac in the physical mixture or in the prepared crystals when compared to pure drug thereby indicating the absence of any interaction.

The results of DSC studies are given in Fig. 2. Pure aceclofenac showed a sharp endotherm at 154.49 °C corresponding to its melting point (Mutalik et al., 2007a, 2007b). There was no appreciable change in the melting endotherms of the physical mixture (aceclofenac + chitosan) as compared to pure drug. This observation further supports the IR spectroscopy results, which indicated the absence of any interactions between drug and excipient used in the preparation. However there was slight decrease in the melting point of drug when prepared in the form of crystals. It was also observed that there was a noticeable reduction in the enthalpy of the crystals in comparison with pure aceclofenac. C-3 crystals showed a lowest enthalpy of −97.07 J/g along with a lowest melting point (152.66 °C). This reduction in melting point and enthalpy accounts for increased solubility and reduced crystallinity of aceclofenac with C-3 crystals (Kapoor et al., 1998; Paradkar et al., 2002; Rawlinson et al., 2007).

The XRD patterns of the pure drug and C-3 crystals are shown in Fig. 3. The XRD scan of plain aceclofenac showed intense peaks of crystallinity; whereas the XRD pattern of the prepared

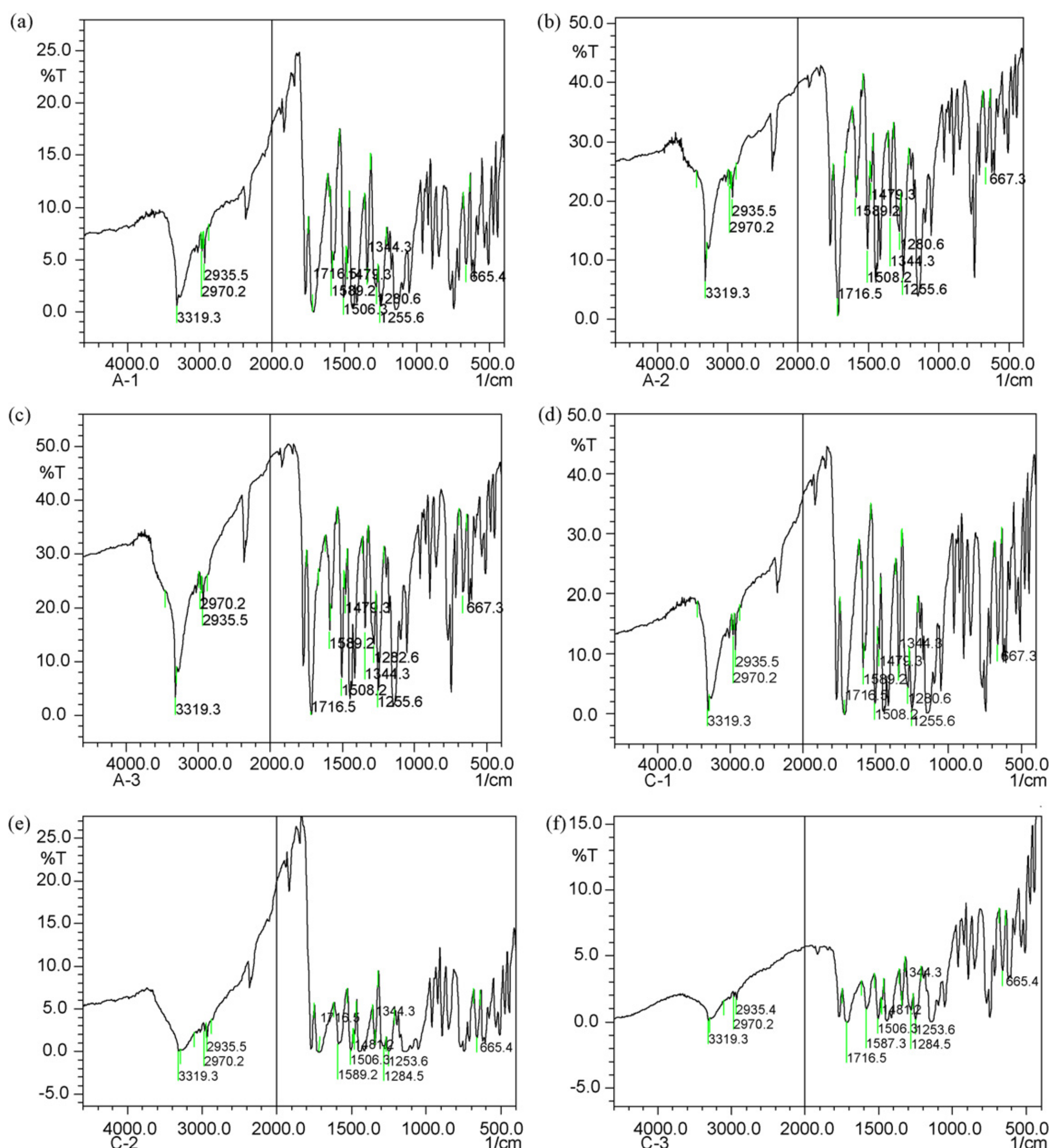

Fig. 1. IR spectra: (a) aceclofenac; (b) physical mixture; (c) C-11; (d) C-1; (e) C-2; (f) C-3; (g) C-4; (h) C-5; (i) C-6; (j) D-7.

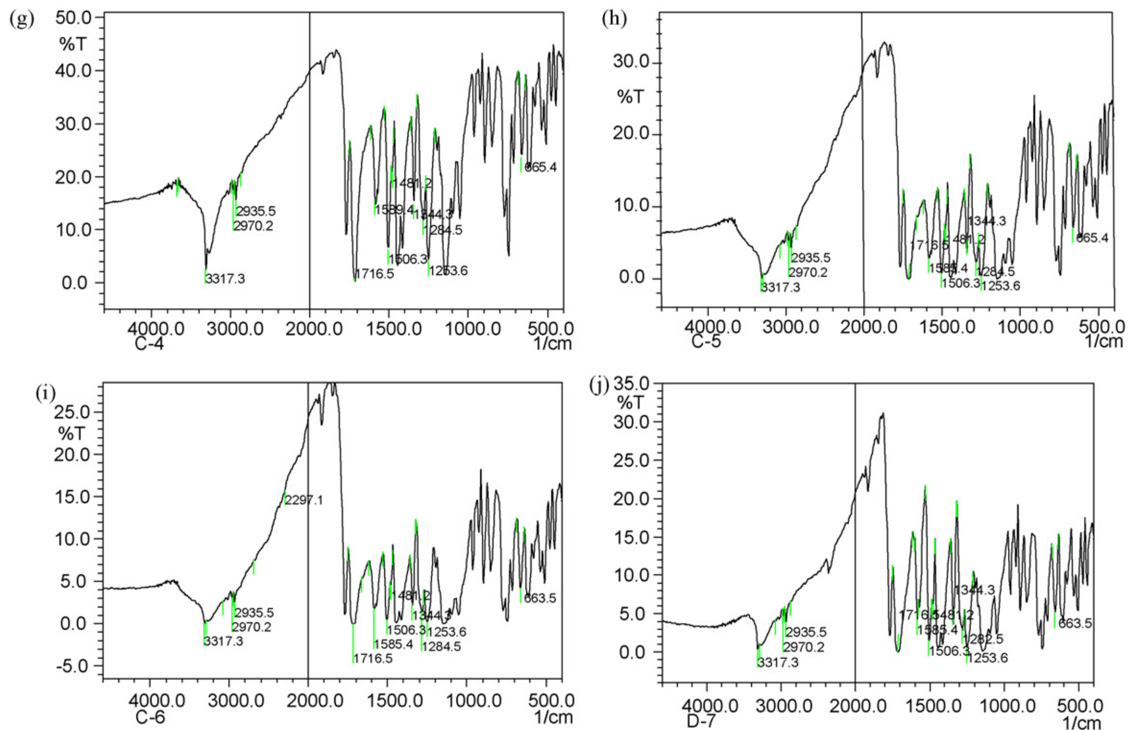

Fig. 1. (*Continued*).

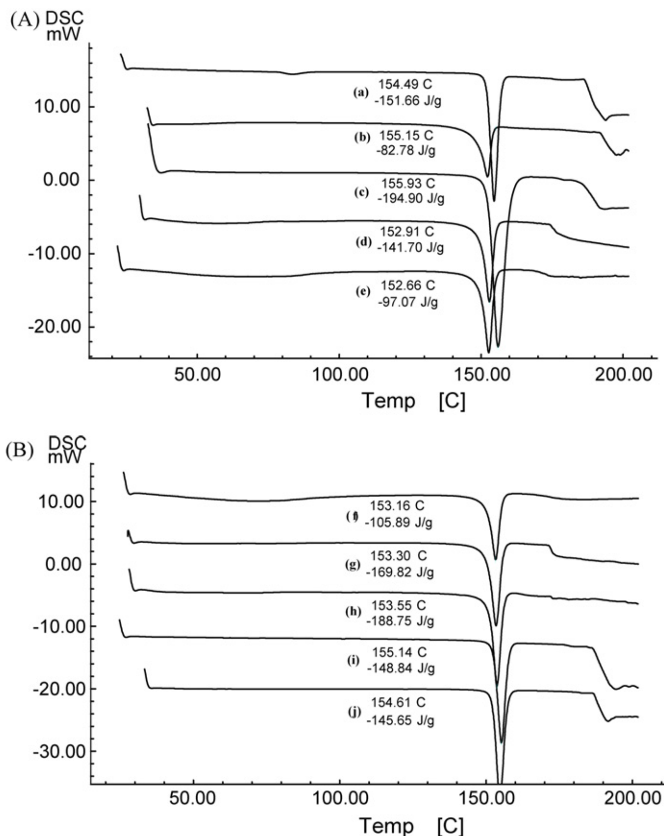

Fig. 2. DSC thermograms: (a) aceclofenac; (b) physical mixture; (c) C-1; (d) C-2; (e) C-3; (f) C-4; (g) C-5; (h) C-6; (i) C-11; (j) D-7.

crystals exhibited reduction in both number and intensity of peaks compared to plain aceclofenac indicating the decrease in crystallinity or partial amorphization of the drug in C-3 crystal forms (Babu et al., 2002; Moneghini et al., 2001; Portero et al., 1998). Thus XRD data supports the DSC studies which indicated the decreased crystallinity of drug in the prepared crystals by exhibiting lower values of enthalpy and melting points.

The SEM photomicrographs of pure aceclofenac and the crystal formulations (C-3 and D-7) are given in Fig. 4. The pure aceclofenac was characterized by crystals of bigger size and regular shape with an apparently smooth surface. In contrast, C-3 crystals were present in was in the form of fine powder. Additionally, the C-3 crystals were fluffy and possess porous and rough surface which might have resulted in the enhanced dissolution rate as compared to pure drug. The size of C-3 crystals was comparatively less than that of plain aceclofenac, which further supports the results of particle size determination. Although D-7 crystals were present in the form of fine powder with porous and hard surface, the magnitude was less than that of C-3 crystals.

3.3. Dissolution studies

The results of *in vitro* drug release studies in 0.1N HCl containing 2% Tween 80 for 3 h are depicted in Fig. 5. The pure drug showed a release of 54.13% at the end of 3 h. The control crystals (without chitosan; C-11) showed 60.49% drug release in 3 h. Various crystals (D-1 to D-8) were prepared using increasing concentrations of chitosan (Table 1) using distilled water as dispersion medium. All the prepared crystals showed a better drug release when compared to that of pure drug. The D-1

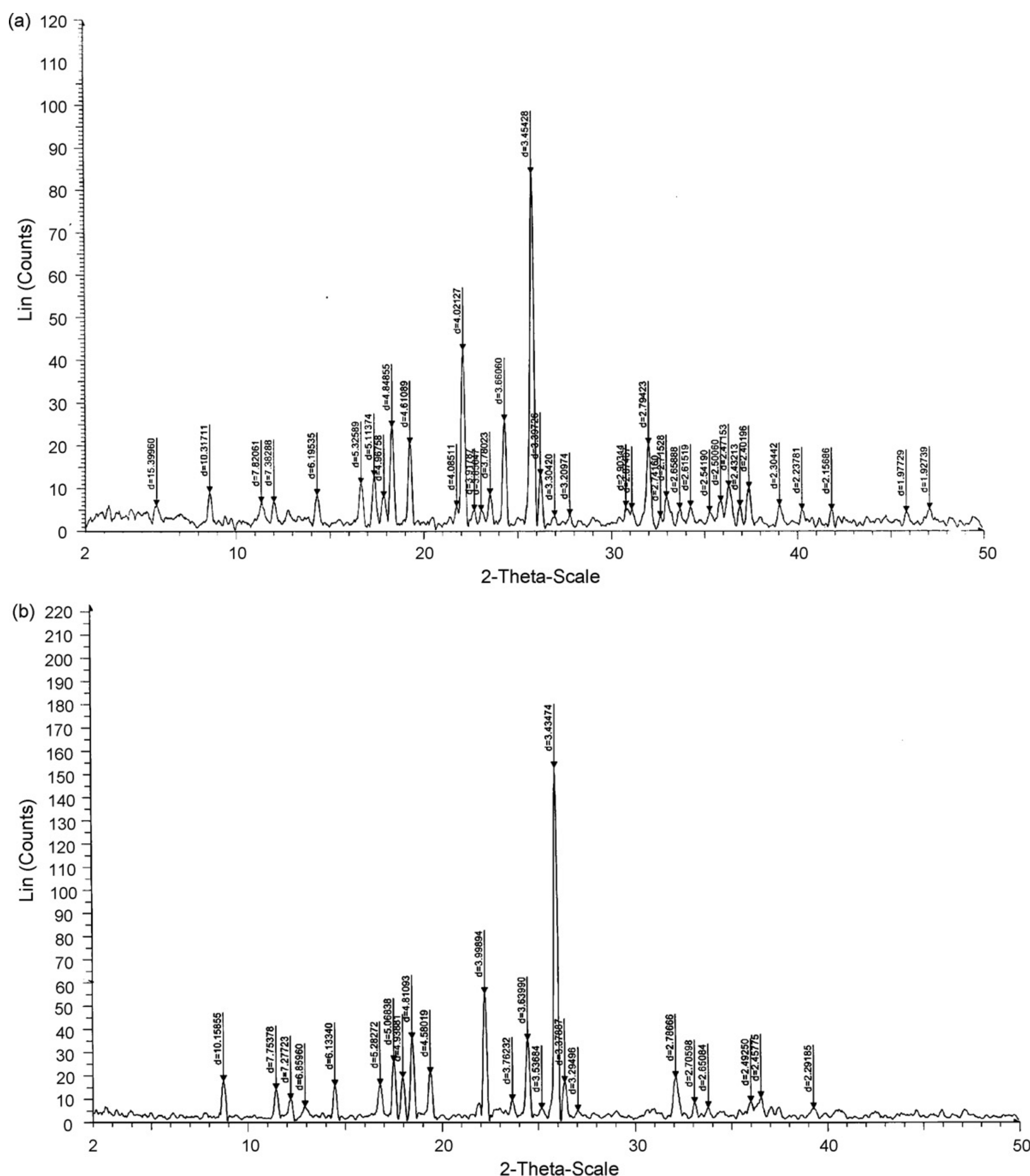

Fig. 3. X-ray diffraction patterns of pure aceclofenac (a) and C-3 crystals (b).

crystals containing 0.05% chitosan showed a release of 55.22% at the end of 3 h. In order to achieve a better release, the formulations were prepared again by increasing the concentration (0.1–0.6%) of chitosan. D-7 crystals containing 0.6% chitosan showed a release of 95.27%. Chitosan concentration was further increased to 0.75% (D-8) but the release rate decreased to 91.79%. This decrease in dissolution rate can be explained based on the viscous gel formation by chitosan at higher concentration; where as at lower concentration, easy solubilization of chitosan may aid increased dissolution rate in acidic condition. Further trials to increase the chitosan concentration were found to be difficult due to the formation of a very thick dispersion as a result of increased viscosity.

Further, the crystals were developed by replacing distilled water with sodium citrate solution (2%), which acts as an efficient salting-out agent for chitosan. The chitosan salted-out with the sodium salts of citric, tartaric, malic and malonic acids was in general more soluble in dilute aqueous HCl or dilute aqueous acetic acid (Shu and Zhu, 2002; Dupuis and LeHoux, 2007). The C-1 crystals containing 0.05% chitosan, showed a drug release of 65.50% in 3 h. Crystals C-2 to C-6 were again prepared by increasing the chitosan concentrations in small increments of 0.1%. C-2 crystals containing 0.1% chitosan showed a release of 83.13% at the end of 3 h. However highest drug release was observed with C-3 crystals with 0.2% chitosan (96.32% in 45 min and 99.16% in 1 h). Further increase in concentration of

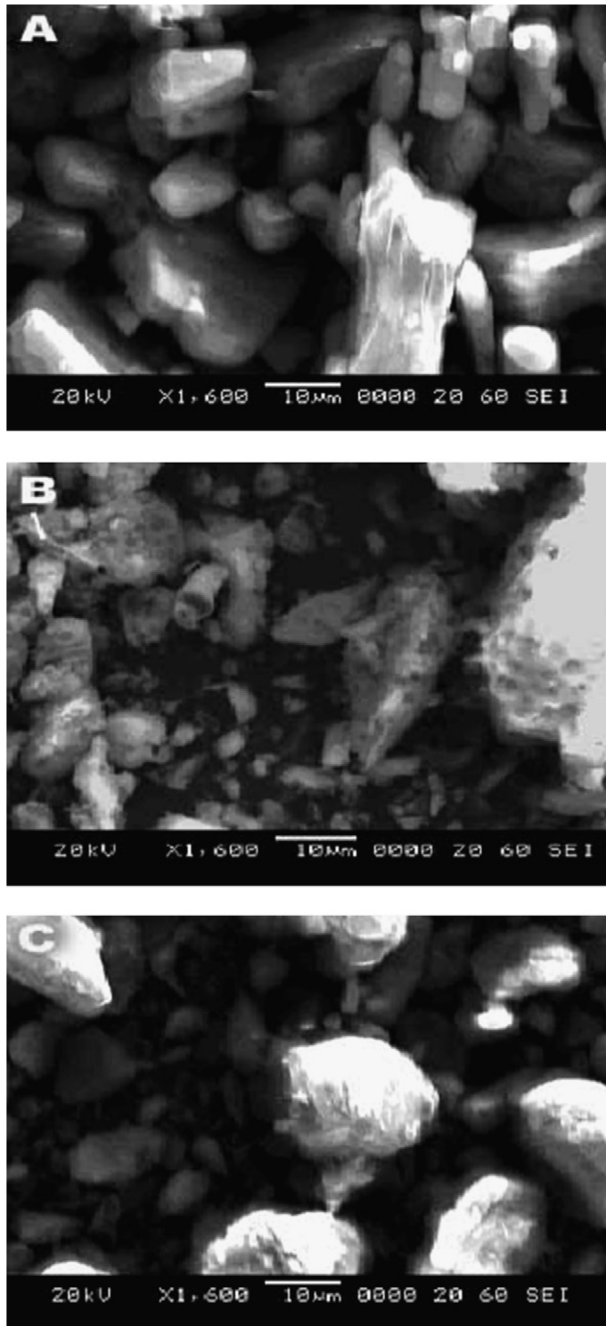

Fig. 4. SEM photomicrographs of aceclofenac (A) and its chitosan crystals (B: C-3 crystals; C: D-7 crystals).

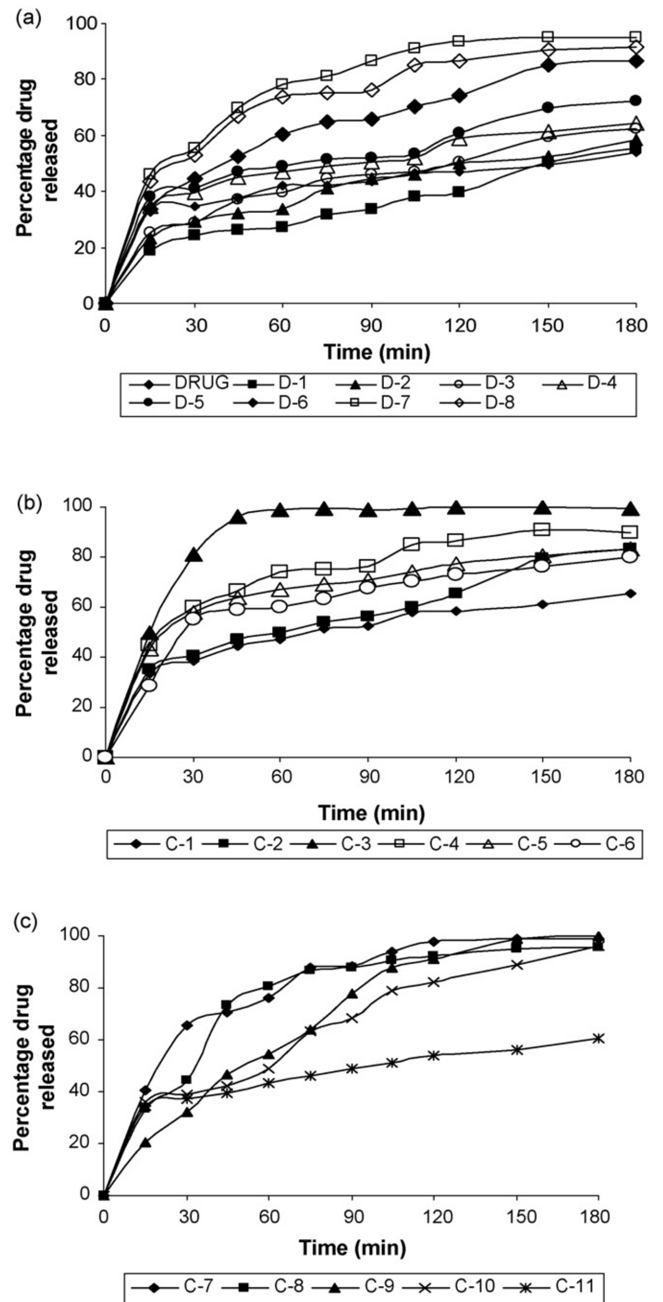

Fig. 5. *In vitro* release of aceclofenac from the prepared formulations in 0.1N HCl containing 2% Tween 80. The points represent mean values, $n = 3$.

chitosan (C-4 to C-6) did not show any significant improvement of the drug release when compared to that of C-3. Further, the crystals were prepared with 0.15% and 0.25% of chitosan (C-7 and C-8, respectively) in order for fine selection of chitosan concentration. The C-7 crystals showed a release of 70.70% in 45 min and 99.00% in 3 h; whereas C-8 crystals showed a release of 73.40% in 45 min and 95.00% in 3 h. The initial drug release was found to be less from these formulations when compared to that of C-3. Hence C-3 crystals with 0.2% chitosan were considered to be the optimum one. It was interesting to note that chitosan was able to increase the dissolution rate at lower concentrations when associated with sodium citrate. This could be due to efficient adsorption of chitosan on drug particles in the presence of sodium citrate. It has been reported that polymers with positively or negatively charged groups interact with molecules of opposite charges to form three-dimensional networks. The reaction of chitosan with multivalent anions like sodium citrate (anion cross-linker) allows the formation of bridges between the polymeric chains and results in cross-linking (by electrostatic interaction) between the chitosan molecules, which might have eventually resulted in efficient adsorption of chitosan on drug particles (Lu et al., 2005).

In order to optimize the concentration of sodium citrate, the optimized crystals (C-3) were then prepared with 1% and 3% of sodium citrate (C-9 and C-10, respectively). C-9 crystals showed 99.75% release in 3 h with a low initial drug release (46.68% in 45 min); whereas C-10 crystals showed a drug release of 95.89% in 3 h with 42.24% in 45 min. The results did not show any significant improvement in the drug release rate when compared to C-3 crystals (2% sodium citrate). Hence 2% of sodium citrate was considered to be suitable concentration for the preparation of crystals.

The increase in the aceclofenac solubility, although little, and dramatical increase in its dissolution rate from prepared crystals can be explained as following:

Chitosan has been proposed as a useful excipient for enhancing the bioavailability of poorly water-soluble compounds (Anal and Stevens, 2005). Chitosan and its derivatives have been reported as a good vehicle for enhancing the solubility and dissolution of poorly water-soluble drugs (Mura et al., 2003; Maestrelli et al., 2004; Kumar and Mishra, 2006). Chitosan dissolves readily in most of the acid solutions and upon dissolution, amine groups of the polymer become protonated, resulting in a positively charged polysaccharide (RNH^{3+}) and chitosan salts (chloride, glutamate, etc.) that are soluble in water (Rowe et al., 2006).

(1) Incredible decrease in the particle size of the prepared crystals during homogenization or micronization of drug (Perrut et al., 2005). Micronization is a commonly used enabling technology to improve the bioavailability of compounds where absorption is dissolution rate limited (Spence et al., 2005). Significant size reduction to micron or sub-micron level with narrow size distribution considerably enhances the dissolution due to the increased surface area (Shinozaki et al., 2006).
(2) The earlier literature reveals that dissolution rate not only depends on the surface area and particle size of the processed powder, but are greatly affected by crystal morphology and wettability (Perrut et al., 2005). So increased wettability of the drug by the adsorption of chitosan onto the hydrophobic surface of the drug is the second reason. In a previous study also, chitosan showed increased solubility and dissolution rate of naproxen due to adsorption on its surface (Maestrelli et al., 2004). As the concentration of polymer increased, dissolution rate was decreased (C-4 to C-6). This could be due to the formation of thick gel layer of the chitosan on the surface of the crystals.
(3) In order to clarify the causes of significant difference in the dissolution rate, the surface morphology of the crystals was examined by SEM. Thus the fine and fluffy physical state of C-3 crystals along with their porous and rough surface as supported by SEM photomicrographs might also have contributed to the enhanced solubility and dissolution rate of aceclofenac from these crystals.
(4) Decreased crystallinity or partial amorphozation of the drug in C-3 crystals as supported by XRD and DSC (enthalpy and melting point reduction) studies.

3.4. Stability studies

The results of accelerated stability studies carried out according to ICH guidelines, indicated that C-3 crystals did not show any physical changes during the study period and the drug content was found more than 96% at the end of 6 months in accelerated conditions The values for drug content ($n = 3$; mean ± S.D.) in C-3 crystals were: 0 day: 100.00 ± 0.00; 15 days: 99.55 ± 0.54; 30 days: 99.29 ± 0.75; 60 days: 98.36 ± 0.52; 90 days: 97.01 ± 0.21; 180 days: 96.05 ± 0.55. This indicates that the formulation C-3 is quite stable at accelerated storage conditions.

3.5. Preclinical studies

3.5.1. Anti-inflammatory activity

The anti-inflammatory activity of the optimized crystal formulation (C-3) in comparison with pure drug was evaluated on the basis of its ability to inhibit the edema produced in hind paw of rats after challenging with the carrageenan. The increase in paw volume in different groups was compared to assess possible improvement in activity of drug. The difference in paw edema volume values before and after drug administration was calculated and % inhibition of edema at each time point was calculated (Table 4). The C-3 crystals showed maximum inhibition of edema (83.38 ± 1.66% at 240 min); whereas pure drug provided maximum activity of 82.19 ± 1.44% at 300 min. Although the values corresponding to maximum effect were not significantly different from each other, C-3 crystals showed rapid

Table 4
Effect of aceclofenac-chitosan formulation on the paw edema induced by carrageenan in Wistar rats

Time (min)	Paw volume (ml)			Inhibition (%)	
	Control	Pure drug	C-3	Pure drug	C-3
60	0.200 ± 0.035	0.15 ± 0.05	0.133 ± 0.040*	25.00 ± 2.40	33.50 ± 1.54#
120	0.280 ± 0.056	0.16 ± 0.054*	0.143 ± 0.055*	42.85 ± 1.40	48.92 ± 2.63#
180	0.450 ± 0.066	0.19 ± 0.033*	0.180 ± 0.032*	57.77 ± 1.87	60.00 ± 2.26
240	0.680 ± 0.012	0.14 ± 0.041*	0.113 ± 0.041*	78.78 ± 2.54	83.38 ± 1.66#
300	0.730 ± 0.055	0.13 ± 0.056*	0.150 ± 0.038*	82.19 ± 1.44	79.45 ± 1.68#

All values are expressed as mean ± S.D., $n = 6$.
* Significant ($p < 0.05$) compared to control.
# Significant ($p < 0.05$) compared to pure aceclofenac.

Table 5
Pharmacokinetic parameters from the plasma concentration–time curves

Parameters	Pure drug	C-3 crystals
C_{max} (μg/ml)	1.23 ± 0.09	1.65 ± 0.11*
T_{max} (h)	1.08 ± 0.49	1.00 ± 0.55
$t_{1/2}$ (h)	2.36 ± 0.09	2.12 ± 0.07*
AUC_{0-8} (μg-h/ml)	2.50 ± 0.05	4.20 ± 0.05*
K_e (h^{-1})	0.29 ± 0.01	0.33 ± 0.01*

All values are expressed as mean ± S.D., $n = 6$; C_{max}: maximum plasma concentration; T_{max}: time for maximum plasma concentration; $t_{1/2}$: biological half-life; AUC: area under the curve; K_e: elimination rate constant.
* Significant ($p < 0.05$) compared to pure drug.

anti-inflammatory effect when compared with that of pure aceclofenac.

3.5.2. Analgesic activity

The analgesic activity of C-3 crystals in comparison with pure drug was evaluated based on its ability to inhibit the pain produced after challenging with the i.p. injection of 1% acetic acid. The inhibition of contractions (%) by the C-3 crystals was higher (82.11 ± 6.55%) when compared to that of plain drug (65.15 ± 7.25%) at the end of 30 min. This clearly indicates the more rapid and effective analgesic activity of C-3 crystals than that of pure aceclofenac. The improved pharmacological response of C-3 crystals may be attributed to improved solubility and dissolution rate of aceclofenac, which in turn improved its rate of absorption.

3.5.3. Pharmacokinetic study

The pharmacokinetic parameters (Table 5) were calculated from the plasma concentration–time curves (Fig. 6). Aceclofenac absorption after oral administration was rapid with both groups as indicated by low T_{max} value of about 1.00 h. However, the C_{max} value was high with C-3 crystals indicating maximum absorption of drug. The elimination half-life ($t_{1/2}$) of aceclofenac with C-3 crystals was less indicating the drug is eliminated from the body rapidly. It was further supported by high elimination rate constant value (K_e) of formulation C-3 in comparison with pure drug. The prepared crystals showed high area under the curve (AUC) value indicating the greater bioavailability of drug than pure drug. This supports the higher values of C_{max} observed with aceclofenac chitosan crystals. Hence the pharmacokinetic study indicates rapid and higher absorption in addition to higher bioavailability of drug from C-3 crystals in comparison with pure drug. This could be due to improved solubility and dissolution rate of drug from prepared crystals.

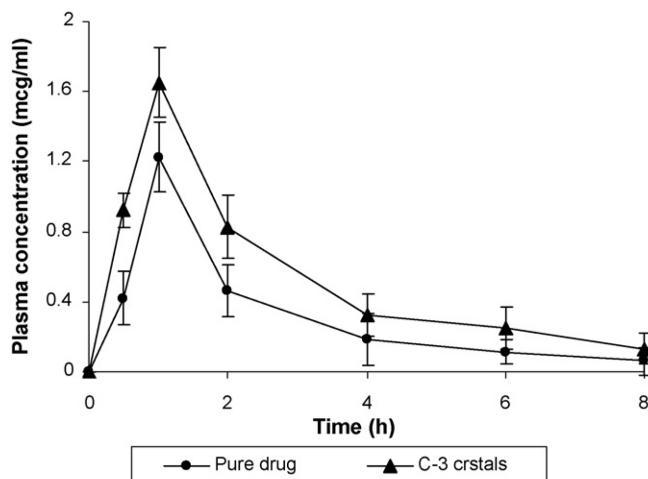

Fig. 6. Plasma drug concentration-time curve. The points represent mean ± S.D. values, $n = 3$.

4. Conclusion

The present study demonstrated a successful and simple method to prepare aceclofenac-chitosan crystals to enhance its aqueous solubility and dissolution rate. The prepared crystals also exhibited exceptional stability and better *in vivo* performance in comparison with pure drug. If this process can be scaled-up to manufacturing level, this technique has the potential to develop into an invaluable technology in future.

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References

Agnihotri, S.A., Mallikarjuna, N.N., Aminabhavi, T.M., 2004. Recent advances on chitosan-based micro- and nanoparticles in drug delivery. J. Control. Rel. 100, 5–28.
Anal, A.K., Stevens, W.F., 2005. Chitosan–alginate multilayer beads for controlled release of ampicillin. Int. J. Pharm. 290, 45–54.
Babu, G.V.M.M., Prasad, C.D.S., Murthy, K.V.R., 2002. Evaluation of modified gum karaya as carrier for the dissolution enhancement of poorly water-soluble drug nimodipine. Int. J. Pharm. 234, 1–17.
Balakrishnan, A., Rege, B.D., Amidon, G.L., Polli, J.E., 2004. Surfactant-mediated dissolution: contributions of solubility enhancement and relatively low micelle diffusivity. J. Pharm. Sci. 93, 2064–2075.
Banakar, U.V., 1992. Pharmaceutical Dissolution Testing, vol. 49. Marcel Dekker, New York.
British Pharmacopoeia, 2005. The Stationary Office, MHRA, British Pharmacopoeial Commission Office, vol. 1, London.
Cai, K., Rechtenbachb, A., Haoa, J., Bosserta, J., Jandt, K.D., 2005. Polysaccharide-protein surface modification of titanium via a layer-by-layer technique: characterization and cell behaviour aspects. Biomaterials 26, 5960–5971.
Dupuis, G., LeHoux, J., 2007. Recovery of chitosan from aqueous acidic solutions by salting-out. Part 2. Use of salts of organic acids. Carbohydrate Polym. 68, 287–294.
Fukuda, M., Peppas, N.A., McGinity, J.W., 2006. Properties of sustained release hot-melt extruded tablets containing chitosan and xanthan gum. Int. J. Pharm. 310, 90–100.
Gowda, K.V., Rajan, D.S., Mandal, U., Selvan, P.S., Sam Solomon, W.D., Bose, A., Sarkar, A.K., Pal, T.K., Chattaraj, T.K., 2006. Evaluation of bioequivalence of two formulations containing 100 mg of aceclofenac. Drug Dev. Ind. Pharm. 32, 1219–1225.
Hel, P., Davis, S.S., Illum, L., 1999. Chitosan microspheres prepared by spray drying. Int. J. Pharm. 187, 53–65.
Imai, T., Shiraishi, S., Saito, H., Otagiri, M., 1991. Interaction of indomethacin with low molecular weight chitosan, and improvements of some pharma-

ceutical properties of indomethacin by low molecular weight chitosans. Int. J. Pharm. 67, 11–20.

Kapoor, A., Mujumdar, D.K., Yadav, M.R., 1998. Crystal forms of nimesulide-a sulfonanilide (non-steroidal anti-inflammatory drug). Indian J. Chem. B 37, 572–574.

Kim, T., Shin, J., Lee, B., 2001. Enhanced dissolution and bioavailability of poorly water-soluble aceclofenac using solid dispersion system. In: AAPS, Annual Meeting, Denver, Colorado, USA, October 21–25.

Kulkarni, S.K., 1997. Handbook of Experimental Pharmacology, second ed. Vallabh Prakashan, New Delhi.

Kumar, V.S.G., Mishra, D.N., 2006. Preparation, characterization and *in vitro* dissolution studies of solid systems of valdecoxib with chitosan. Chem. Pharm. Bull. 54, 1102–1106.

Lee, B., Jung, H., 1999. Enhanced bioavailability of poorly water-soluble aceclofenac using PEG-based solid dispersion in rats, beagle dogs and human subjects. AAPS Annual Meeting, New Orleans, LA, USA, November 14–18, Pharm. Sci., Supplement 1(4), S-614.

Lu, Z., Steenekamp, J.H., Hamman, J.H., 2005. Cross-linked cationic polymer microparticles: effect of *n*-trimethyl chitosan chloride on the release and permeation of ibuprofen. Drug Dev. Ind. Pharm. 31, 311–317.

Maestrelli, F., Zerrouk, N., Chemtob, C., Mura, P., 2004. Influence of chitosan and its glutamate and hydrochloride salts on naproxen dissolution rate and permeation across Caco-2 cells. Int. J. Pharm. 271, 257–267.

Moneghini, M., Kikie, I., Voinovich, D., Perissutti, B., Filipovic-Grcic, J., 2001. Processing of carbamazepine-PEG 4000 solid dispersions with supercritical carbon dioxide: preparation, characterization and *in vitro* dissolution. Int. J. Pharm. 222, 129–138.

Mura, P., Zerrouk, N., Mennini, N., Maestrelli, F., Chemtob, C., 2003. Development and characterization of naproxen-chitosan solid systems with improved drug dissolution properties. Eur. J. Pharm. Sci. 19, 67–75.

Mutalik, S., Usha, A.N., Reddy, M.S., Ranjith, A.K., Pandey, S., 2007a. Improved bioavailability of aceclofenac from spherical agglomerates: development, *in vitro* and preclinical studies. Pak. J. Pharm. Sci. 3, 218–226.

Mutalik, S., Naha, A., Usha, N., Ranjith, A.K., Musmade, P., Manoj, K., Anju, P., Prasanna, S., 2007b. Preparation, *In vitro*, preclinical and clinical evaluations of once daily sustained release tablets of aceclofenac. Arch. Pharm. Res. 30, 222–234.

Nijlen, T.V., Brennan, K., Mooter, V.G., Blaton, N., Kinget, R., Augustijns, P., 2003. Improvement of the dissolution rate of artemisinin by means of supercritical fluid technology and solid dispersions. Int. J. Pharm. 254, 173–181.

Nokhodchi, A., 2005. The effect of type and concentration of vehicles on the dissolution rate of a poorly soluble drug (indomethacin) from liquisolid compacts. J. Pharm. Pharmaceut. Sci. 8, 18–25.

Paradkar, A.R., Pawar, A.P., Chordiya, J.K., Patil, V.B., Ketkar, A.R., 2002. Spherical crystallization of celecoxib. Drug Dev. Ind. Pharm. 28, 1213–1220.

Parfitt, K., 1999. Analgesics anti-inflammatory and antipyretics. In: Reynolds, J.E.F. (Ed.), Martindale: The Complete Drug Reference, 32nd ed., Massachusetts, pp. 2–12.

Park, S., Choi, H., 2006. The effects of surfactants on the dissolution profiles of poorly water-soluble acidic drugs. Int. J. Pharm. 321, 35–41.

Pennings, F.H., Kwee, B.L.S., Vromans, H., 2006. Influence of enzymes and surfactants on the disintegration behavior of cross-linked hard gelatin capsules during dissolution. Drug Dev. Ind. Pharm. 32, 33–37.

Perrut, M., Jung, J., Leboeuf, F., 2005. Enhancement of dissolution rate of poorly-soluble active ingredients by supercritical fluid processes. Part I. Micronization of neat particles. Int. J. Pharm. 288, 3–10.

Portero, A., Remunan-Lopez, Vila-Jato, J.L., 1998. Effect of chitosan and chitosan glutamate enhancing the dissolution properties of the poorly water soluble drug nifedipine. Int. J. Pharm. 175, 75–84.

Rawlinson, C.F., Williams, A.C., Timmins, P., Grimsey, I., 2007. Polymer-mediated disruption of drug crystallinity. Int. J. Pharm. 336, 42–48.

Rowe, R.C., Sheskey, P.J., Owen, S.C., 2006. Handbook of Pharmaceutical Excipients. Pharmaceutical Press, London, United Kingdom.

Shinozaki, H., Oguchi, T., Suzuki, S., Aoki, K., Sako, T., Morishita, S., Tozuka, Y., Moribe, K., Yamamoto, K., 2006. Micronization and polymorphic conversion of tolbutamide and barbital by rapid expansion of supercritical solutions. Drug Dev. Ind. Pharm. 32, 877–891.

Shu, X.Z., Zhu, K.J., 2002. Controlled drug release properties of ionically cross-linked chitosan beads: the influence of anion structure. Int. J. Pharm. 233, 217–225.

Spence, J.K., Bhattachar, S.N., Wesley, J.A., Martin, P.J., Babu, S.R., 2005. Increased dissolution rate and bioavailability through comicronization with microcrystalline cellulose. Pharm. Dev. Technol. 10, 451–460.

Zhang, M., Li, X.H., Gong, Y.D., Zhao, N.M., Zhang, X.F., 2002. Properties and biocompatibility of chitosan films modified by blending with PEG. Biomaterials 23, 2641–2648.