AAPS PharmSciTech, Vol. 9, No. 2, June 2008 (© 2008)
DOI: 10.1208/s12249-008-9075-3

Research Article

Themed Issue: Oral Controlled Release Development and Technology
Guest Editors: Stephen A. Howard and Jian-Xin Li

Chitosan and Enteric Polymer Based Once Daily Sustained Release Tablets of Aceclofenac: *In Vitro* and *In Vivo* Studies

Srinivas Mutalik,[1,3,4] Krishnan Manoj,[1] Meka Sreenivasa Reddy,[1] Pralhad Kushtagi,[2] Achutha Nayak Usha,[1] Parambil Anju,[1] Averineni Kumar Ranjith,[1] and Nayanabhirama Udupa[1]

Received 19 July 2007; accepted 21 February 2008; published online 24 May 2008

Abstract. The purpose of this study was to develop a once daily sustained release tablet of aceclofenac using chitosan and an enteric coating polymer (hydroxypropyl methylcellulose phthalate or cellulose acetate phthalate). Overall sustained release for 24 h was achieved by preparing a double-layer tablet in which the immediate release layer was formulated for a prompt release of the drug and the sustained release layer was designed to achieve a prolonged release of drug. The preformulation studies like IR spectroscopic and differential scanning calorimetry showed the absence of drug–excipient interactions. The tablets were found within the permissible limits for various physicochemical parameters. Scanning electron microscopy was used to visualize the surface morphology of the tablets and to confirm drug release mechanisms. Good equivalence in the drug release profile was observed when drug release pattern of the tablet containing chitosan and hydroxypropyl methylcellulose phthalate (M-7) was compared with that of marketed tablet. The optimized tablets were stable at accelerated storage conditions for 6 months with respect to drug content and physical appearance. The results of pharmacokinetic studies in human volunteers showed that the optimized tablet (M-7) exhibited no difference in the *in vivo* drug release in comparison with marketed tablet. No significant difference between the values of pharmacokinetic parameters of M-7 and marketed tablets was observed ($p > 0.05$; 95% confidence intervals). However the clinical studies in large scale and, long term and extensive stability studies at different conditions are required to confirm these results.

KEY WORDS: aceclofenac; chitosan; matrix tablet; pharmacokinetics; sustained release.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are considered to be the first-line drugs in the relief of mild to moderate pain, acute and chronic inflammatory disorders such as rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. Aceclofenac, phenyl acetic acid derivative related to diclofenac, is one of the widely used NSAIDs (1,2). The short biological half-life (4 h) and frequent dosing make aceclofenac an ideal candidate for sustained release dosage forms (3). The use of controlled-release technology in the formulation of pharmaceutical products has become increasingly important in the past few years (4); but for drugs such as NSAIDs a prompt disposition of a fraction of the dose should be reached in the shortest time possible to relieve the symptoms of the disease and then the continuation of the drug effect should be prolonged to optimize the therapy. Such a biphasic release can be achieved by formulating a double-layer tablet in which the first layer is formulated to obtain a prompt release of the drug, so as to reach a high serum concentration in a short period of time. The second layer is a prolonged-release matrix, which is designed to maintain an effective plasma level for a prolonged period of time (5–7). For sustained release systems, the oral route of drug administration has, by far, received the most attention as it is natural, uncomplicated, convenient and safer route (6). Matrix tablets composed of drug and release retarding material offer the simplest approach in designing a sustained release system. In our earlier study, we have reported the formulation and evaluation of once daily sustained release tablets of aceclofenac using hydroxypropyl methyl cellulose as matrix material by direct compression method (7). Apart from hydrophilic swellable polymers like HPMC, the polymers with pH dependent solubility like chitosan, hydroxylpropylmethylcellulose phthalate (HPMCP) and cellulose acetate phthalate (CAP) can also be used in the formulation of matrix tablets. Chitosan is a nontoxic, biocompatible and biodegradable polymer (8). High molecular weight chitosan functions as matrix tablet retardant (9). Chitosan is widely used in the formulation of controlled release dosage forms (10), rapid release dosage forms (11), matrix tablets (12), buccal and vaginal tablets (13), etc. Hydroxypropyl methyl cellulose pthalate is insoluble in gastric fluid but swells and dissolves

[1] Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, 576104 Karnataka, India.
[2] Department of OBG, Kasturba Medical College, Manipal University, Manipal, 576104 Karnataka, India.
[3] School of Pharmacy, University of Queensland, St. Lucia, Brisbane, QLD 4072, Australia.
[4] To whom correspondence should be addressed. (e-mail: ssmutalik@yahoo.com)

rapidly in the upper intestine. It may be used alone or in combination with other soluble or insoluble binders in the preparation of granules with sustained drug-release properties; the release rate is pH-dependent. Cellulose acetate phthalate is also an enteric coating material insoluble in strongly acidic gastric fluid, but dissolves in mildly acidic or neutral intestinal environment. Sustained release matrix tablets are generally prepared by wet granulation method (14–15). Moreover there are no many reports on the combined usage of chitosan and enteric coating polymers to prepare sustained release matrix tablets (16–17). Hence in the present study, it was attempted to (1) use the combination of chitosan and an enteric coating polymer (hydroxylpropyl methyl cellulose phthalate or cellulose acetate pthalate) as a matrix material to formulate once daily sustained release tablet of aceclofenac and (2) to evaluate the prepared tablets with respect to preformulation studies, physicochemical parameters, *in vitro* drug release studies, stability studies, surface morphological studies and pharmacokinetic studies in human volunteers.

MATERIALS AND METHODS

Materials

Aceclofenac, hydroxylpropyl methyl cellulose phthalate-55 (HPMCP), cellulose acetate phthalate (CAP), polyvinyl pyrrolidone K-30 (PVP) and microcrystalline cellulose (MCC; Avicel PH102, FMC biopolymer, USA) were obtained as gift samples from Lupin Research Park, Pune, India. Chitosan was obtained from Sigma-Aldrich Laboratories, Germany. Magnesium stearate, talc, hydrochloric acid (HCl) and sodium lauryl sulphate (SLS) were purchased from S.D. Fine-Chem Ltd., Mumbai, India. All other chemicals used were of analytical grade.

Preformulation Studies

Micromeritic Properties

The angle of repose of aceclofenac and granules was determined by funnel method. The loose bulk density (LBD) and tapped bulk densities (TBD) were determined by using Density apparatus (Serwell, Bangalore, India). The Carr's index (%) and the Hausner's ratio were calculated (18).

Drug–Excipient Compatibility Studies

Infrared (IR) Spectroscopy

IR spectra were recorded by using a Shimadzu FT-IR 8300 Spectrophotometer (Shimadzu, Tokyo, Japan) in the wavelength region of 4,000 to 400 cm^{-1}. The procedure consisted of dispersing a sample (drug alone or mixture of drug and excipients) in KBr and compressing into discs by applying a pressure of 5 tons for 5 min in a hydraulic press. The pellet was placed in the light path and the spectrum was obtained.

Differential Scanning Calorimetry

DSC was performed using DSC-60 (Shimadzu, Tokyo, Japan) calorimeter. The instrument comprised of calorimeter (DSC 60), flow controller (FCL 60), thermal analyzer (TA 60) and operating software (TA 60). The samples (drug alone or mixture of drug and excipients) were heated in sealed aluminum pans under nitrogen flow (30 ml/min) at a scanning rate of 5°C/min from 24±1 to 250°C. Empty aluminum pan was used as a reference. The heat flow as a function of temperature was measured for the drug and drug–polymer mixture.

The physical mixtures of drug with different excipients for compatibility studies were prepared by triturating drug and additives in a dried mortar for 5 min.

Preparation of Tablets

The composition of the tablets is given in Table I. Different batches of prolonged release layer matrices of aceclofenac with chitosan and an enteric coating polymer (HPMCP or CAP) were prepared by wet granulation using various concentrations of PVP in isopropyl alcohol (IPA) as granulating agent. Dough mass was passed through sieve no. 12 (sieve opening: 1,680 μm). After drying, the granules were passed through sieve no. 20 (sieve opening: 840 μm) superimposed on sieve no. 40 (sieve opening: 420 μm). Then 20/40 fraction was blended with magnesium stearate and talc (1% *w*/*w* each) and compressed into tablets (single station tablet compression machine, Cadmach, Ahmedabad, India) using 9 mm flat-faced punches. Immediate release layer of the double-layer tablets was prepared in the similar way but contained only aceclofe-

Table I. Composition of Sustained Release Tablet Formulations

Ingredients (mg)	A	B	C	D	E	F	G	H	M-1	M-2	M-3	M-4	M-5	M-6	M-7	C-1	C-2
Aceclofenac	200	200	200	200	200	200	200	200	170	170	170	170	170	170	170	170	170
Chitosan	–	20	20	10	5	2.5	2.5	2	2	2	2	2	2	1.7	1.5	2	1.5
HPMCP	–	20	10	10	5	2.5	2.5	2	2	2	2	2	2	1.7	1.5	–	–
CAP	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	1.5
PVP	6.66	6.66	6.66	6.66	6.66	6.66	3.33	3.33	2.84	2.84	2.84	2.84	2.84	2.83	2.82	2.84	2.82
MS	2.07	2.47	2.37	2.27	2.17	2.12	2.08	2.07	1.77	1.77	1.77	1.77	1.77	1.76	1.75	1.77	1.75
Talc	2.07	2.47	2.37	2.27	2.17	2.12	2.08	2.07	1.77	1.77	1.77	1.77	1.77	1.76	1.75	1.77	1.75
Aceclofenac (IR layer)	–	–	–	–	–	–	–	–	30	30	30	30	30	30	30	30	30
MCC (IR layer)	–	–	–	–	–	–	–	–	–	5	10	20	30	30	30	30	30

HPMCP hydroxypropyl methylcellulose phthalate, *PVP* polyvinylpyrrolidone K-30, *MCC* microcrystalline cellulose; *MS* magnesium stearate, *IR layer* immediate release layer

nac, MCC and granulating agent. For the preparation of the two-layer tablets, the die of the tableting machine was filled with granules and a pre compression was done followed by filling of the immediate release constituents into the die cavity and then compressed to the final tablet.

Physicochemical Characterization of Tablets

The thickness and diameter of the tablets (n=3) were determined using digital vernier calipers. The hardness of the tablets (n=6) was determined by using Monsanto hardness tester. The friability (%) of the tablets (n=6) was determined using Roche Friabilator. Weight variation test of the tablets (n=20) was carried out as per the official method (19). For determining the drug content, 3 tablets were crushed and 100 mg of powder was dissolved in 100 ml of 6.8 phosphate buffer. The solution was then passed through a Whatmann (no. 1) filter and analyzed spectrophotometrically at 275 nm after sufficient dilution with phosphate buffer (pH 6.8).

Dissolution Studies

The *in vitro* dissolution study was carried out using USP Type 2 dissolution apparatus. The study was carried out in 900 ml of 2% SLS in 0.1N HCl for first 2 h and then 900 ml of phosphate buffer (pH 6.8) from 3 to 24 h (7). The dissolution medium was maintained at 37±0.5°C. The paddle was lowered so that the lower end of the stirrer was 25 mm above from the base of the beaker. The pre-weighed tablet was then introduced into the dissolution jar and the paddle was rotated at 75 rpm. At different time intervals, 5 ml of sample was withdrawn and analyzed spectrophotometrically at 275 nm for the drug release. At each time of withdrawal, 5 ml of fresh corresponding medium was replaced into the dissolution flask.

Scanning Electron Microscopy (SEM)

The surface morphology of the tablets at 0, 2 and 24 h of dissolution study was analyzed by SEM (JSM 6400, JEOL, Tokyo, Japan). Prior to analysis, the tablets were mounted onto double-sided adhesive tape that has previously been secured on copper stubs and coated with platinum.

Stability Studies

After determining drug content, the optimized tablets were charged for the accelerated stability studies according to ICH guidelines (40±2°C/ 75±5% RH) for a period of 6 months in stability chambers (Thermolab, Mumbai, India). They were placed in USP type-1 flint vials and hermetically sealed with bromobutyl rubber plugs and aluminum caps. The samples (n=3) were taken out at 15, 30, 60, 90 and 180 days and evaluated for the drug content and physical parameters like color change, friability and hardness.

Pharmacokinetic Studies in Healthy Human Volunteers

The pharmacokinetics of the optimized tablet in comparison with marketed tablet (Hifenac SR 200 mg Tablets; Intas Pharmaceuticals, India; Batch No.-ML635H) was carried out in healthy human volunteers. The study protocol was approved by Kasturba Hospital Ethics Committee, Kasturba Medical College, Manipal (Approval No. KHEC-57/2006).

Formulations. Optimized aceclofenac sustained release matrix tablets (M-7) and marketed tablets, both containing 200 mg of aceclofenac.

Subjects. Six healthy human volunteers.

Study design. Randomized, crossover, single blinded.

Study procedure. On the previous night of the study, the volunteers had a controlled diet and were continued on fasting upto 4 h after commencement of the study. The prepared tablets were administered to the volunteers and blood sample (2 ml) was withdrawn immediately and at 1, 2, 4, 6, 8, 12, 18, 24, 27 and 30 h post dose. After 4 h of dosing, the volunteers were given with controlled diet. After the last sampling, the volunteers were out of the study. After a time gap of 7 days the same procedure was performed on the same volunteers by administering marketed tablets. At each sampling point, the blood samples were collected in the vacutainers containing EDTA as anticoagulant. The plasma was separated immediately by using cold centrifuge at 5000 rpm for 10 min and stored at −70°C until analysis.

Analysis of Drug in Plasma

A sensitive high performance liquid chromatographic (HPLC) method was used to analyze the aceclofenac in plasma (7). 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 in the mixture of 80:20 *v*/*v* as a diluent.

Preparation of Calibration Standards in Plasma Curve

The studies were carried out using aliquots of plasma (95 μl) pipetted into a micro centrifuge tubes, spiked with 5 μl of the working standard solutions of drug (to give final concentration of 50, 100, 250, 500, 1,000, 1,500, 2,500 and 3,500 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. Diluent (675 μl) 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 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), 5μ; 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 to the internal standard) 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 10.26 and 18.26 min, respectively in human plasma.

Data Analysis

Student's *t*-test was employed to analyze the results (Graph Pad Prism Software). Difference below the probability level 0.05 was considered statistically significant. The pharmacokinetic parameters were calculated by using PK Solutions 2.0™ Noncompartmental pharmacokinetic data analysis software.

RESULTS AND DISCUSSION

Formulation Development

In the present study the advantage of the combination of pH dependent soluble polymers has been used to achieve sustained release of aceclofenac. The study was based on the assumption that enteric polymer (HPMCP/ CAP) being insoluble in acidic condition would control the drug release in stomach and chitosan being insoluble in alkaline condition would control the drug release in intestine.

Micromeritic Properties

The method employed for tableting in this study was wet granulation for which the granules should possess good flow and compacting properties. The optimum values for Carr's index (%) and Hausner's ratio are up to 15% and less than 1.20, respectively (18). Values for angle of repose (°) less than or equal to 30 generally indicate free flowing material. Plain aceclofenac exhibited angle of repose value of 40.12±0.13 indicating extremely poor flow property. It was further supported by high Carr's index value of 28.57±0.06 and Hausner's ratio of 1.40±0.01. All the prepared granules possessed good flow properties as indicated by low values of angle of repose (20.50±0.096–25.56±0.068), Carr's index (12.04±0.068–14.83±0.125) and Hausner's ratio (1.113±0.025–1.197±0.062).

Drug Excipient Compatibility Studies

Drug excipient compatibility studies were carried out by IR spectroscopy and DSC (20). The IR spectra of pure aceclofenac and its physical mixtures with other excipients are shown in Fig. 1. Pure aceclofenac showed 3,319.3, 2,970.2, 2,935.5, 1,716.5, 1,589.2, 1,506.3, 1,479.3, 1,344.3, 1,280.6, 1,255.6 and 665.4 cm^{-1} wave numbers as major peaks (7). There were no considerable changes in the IR peaks of aceclofenac, when mixed with excipients, indicating the absence of its interaction with excipients used. The results of DSC studies are shown in Fig. 2. Pure aceclofenac exhibited an endothermic peak of 154.49°C, corresponding to its melting point. There was no considerable change in the endotherm values of aceclofenac when it was mixed with other excipients compared to that of pure aceclofenac. This observation further supports the absence of the interaction between drug and excipients by IR spectra results. Hence the excipients selected in this study are inert with aceclofenac and are suitable for the formulation development.

Evaluation of Prepared Tablets

The tablets of different batches showed uniform thickness (2.10±0.08 to 2.60±0.04 mm), diameter (9 mm) and hardness (4.90±0.12 to 5.60±0.12 kg/cm^2). The friability (0.50±0.08 to 0.85±0.09%) and weight variation (% deviation: ±2.10 to ±4.02%) of different batches of tablets were found within the prescribed limits. The drug content was found to be uniform (>97%) within the batches of different tablet formulations.

Initially a formulation (A) was prepared by using PVP in IPA as a binding agent. But all the drug released in 7 h (Fig. 3). To sustain the drug release, two pH sensitive polymers, HPMCP and chitosan, were incorporated into the formulation. Formulation B containing 20 mg each of chitosan and HPMCP showed a cumulative drug release of 63.81% in 24 h. In order to achieve 100% release in 24 h, the polymer concentrations in the formulations were gradually reduced from 20 mg to 2.5 mg of each (formulations C–F). The results indicated an increase in drug release with decrease in the polymer concentrations. The cumulative drug release for these tablets was in the following order: C (68.38) <D (71.40)<E (76.34)<F (79.89). Even though the polymer concentration was reduced to 2.5 mg each of chitosan and HPMCP, only 79.89% release was observed in 24 h. Hence the quantity of binder (PVPK30) was reduced from 6.66 to 3.33 mg (Formulation G). The result revealed a satisfactory improvement in the drug release (89.16%). Formulation was further prepared by maintaining 3.33 mg of PVP and reducing the polymer concentration to 2 mg each of chitosan and HPMCP (Tablet H), which showed a release of 90% in 24 h,

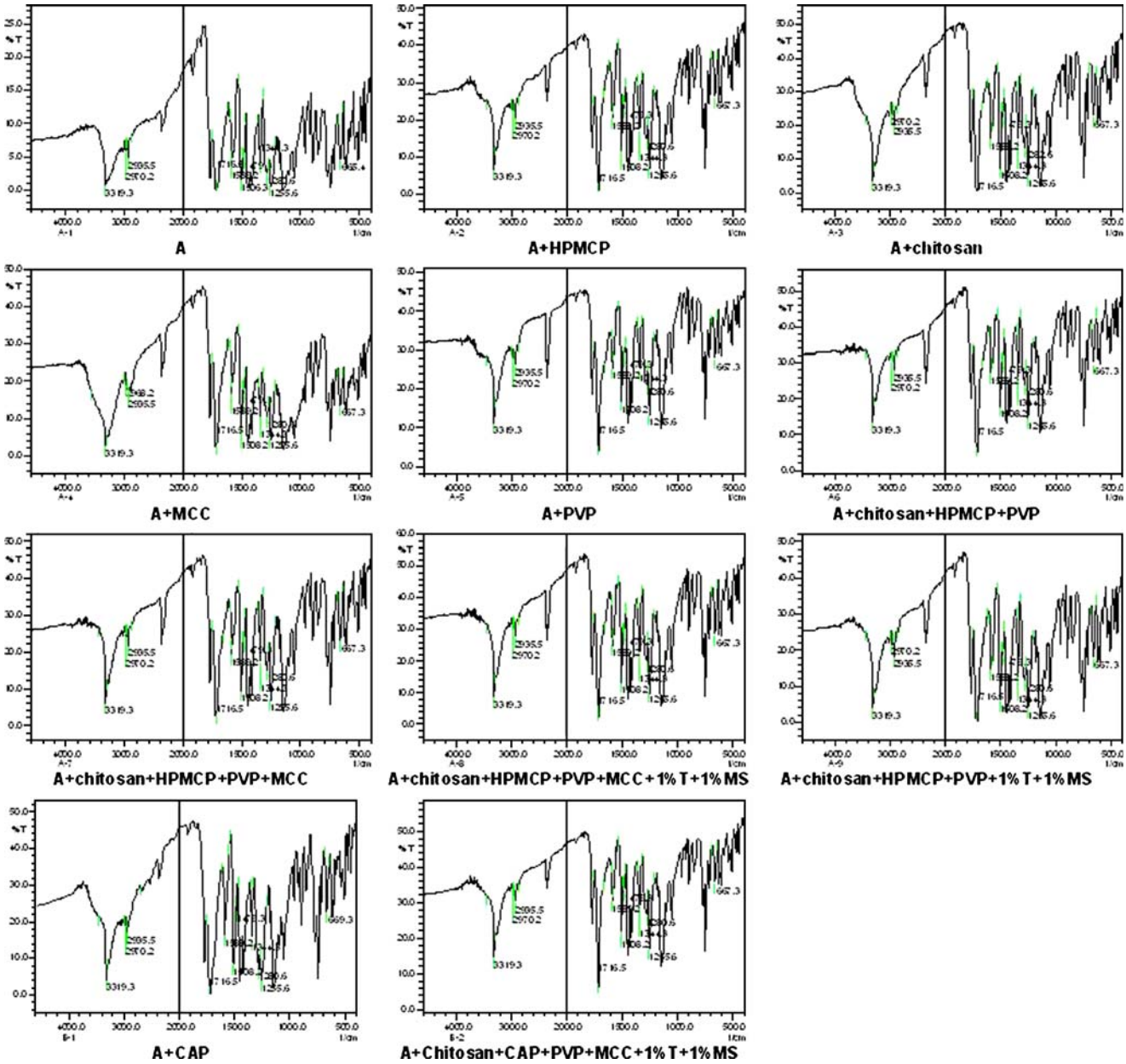

Fig. 1. IR spectra of aceclofenac (*A*) and its physical mixtures with different excipients. *A* aceclofenac, *MS* magnesium stearate, *T* talc

with an initial release of 2.76% in 0.1N HCl (in 2 h). The amount of drug released in first two hours of dissolution study was less than that of marketed tablet. At this moment it was speculated that if an initial drug release in first two hours is increased, then the cumulative drug release at the end of 24 h would be automatically improved.

Hence an additional immediate release layer of the drug was incorporated by keeping the total amount of the drug in the formulation as constant. Formulation M-1 was prepared by the modification of formulation H in such a manner that a layer of pure drug (30 mg) was compressed over the sustained release layer by keeping the total amount of the drug as 200 mg. Thirty mg of drug in immediate release layer was selected based on the in vitro drug release results of marketed tablet. Marketed tablet released about 14% (28 mg) in first 2 h and hence it was decided to incorporate 30 mg in immediate release layer. This formulation (M-1) showed a release of 2.38% in acid media and 91.54% drug was released at the end of 24 h. In order to further improve the drug release, and also the flow property (as pure drug possesses very low flowability), different ratios of MCC was incorporated into the immediate release layer. MCC is widely used as a binder/diluent in both wet granulation and direct compression. It also has lubricant and disintegrant properties that make it useful in tableting (21). Formulation M-2 having drug: MCC ratio of 1:0.16 showed a small increase in drug release (3.32% in 2 h). Further tablets were repeated by increasing the MCC concentration. Tablets M-3 and M-4 with drug: MCC ratios of 1:0.33 and 1:0.66, respectively, showed drug release of 11.39 and 12.40% respectively, in first 2 h. Formulation M-5, prepared with drug and MCC (1:1 in the immediate release layer), showed 15.80% drug release in first 2 h and 96.55% at the end of 24 h. Hence this ratio was considered optimum in immediate release layer.

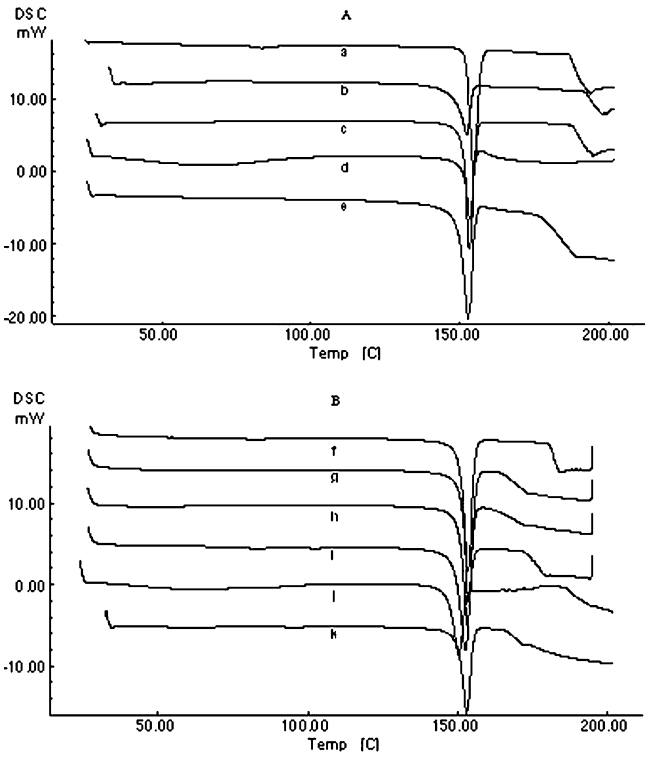

Fig. 2. DSC thermograms. *a*=154.49 (*A*); *b*=152.24 (*A*+HPMCP); *c*=153.02 (*A*+chitosan); *d*=153.28 (*A*+MCC); *e*=152.71 (*A*+PVP); *f*=153.40 (*A*+chitosan+HPMCP+PVP); *g*=153.56 (*A*+chitosan+HPMCP+PVP+MCC); *h*=153.14 (*A*+chitosan+HPMCP+PVP+MCC+1%T+1%MS); *i*=152.61 (*A*+chitosan+HPMCP+PVP+1%T+1%MS); *j*=150.38 (*A*+CAP); *k*=152.99 (*A*+chitosan+CAP+PVP+MCC+1%T+1%MS). *A* aceclofenac, *MS* magnesium stearate, *T* Talc

Another effort was made to further improve the drug release from M-5 by reducing the chitosan and HPMCP concentration to 1.7 mg each in the sustained release matrix (Tablet M-6). Since the cumulative drug release did not show considerable change, another formulation M-7 was prepared by still reducing the polymer concentration to 1.5 mg each of chitosan and HPMCP. The release profile showed a 98.77% release in 24 h with a good initial release of 16.75% in gastric pH (in first 2 h). There was no much difference in the drug release profiles of M-7 formulation (16.75% in 2 h; 98.77% in 24 h) and marketed tablet (14.03% in 2 h; 96.14% in 24 h). The similarity in the release profiles of marketed tablet and formulation M-7 was compared by making use of "Model independent approach". A simple model independent approach uses a difference factor (f_1) and a similarity factor (f_2) to compare dissolution profiles (http://www.fda.gov/cder/guidance). For M-7 formulation, when compared with marketed tablet, f_1 and f_2 values were found to be 9.65 and 64.68 respectively, indicating a good equivalence between these two formulations.

In order to check the performance of chitosan with another enteric polymer, tablets were prepared by using CAP instead of HPMCP. Formulation C-1 is modified formula of M-5 where CAP was used instead of HPMCP. Tablet C-1 showed an initial release of 14.53% in acid medium and 84.27% at the end of 24 h. Another formulation (C-2), prepared with 1.5 mg of CAP and 1.5 mg of chitosan showed a better drug release of 15.95% in 2 h and 95.61% in 24 h. Although there were no considerable changes in the overall performance of two enteric polymers, the tablets with CAP showed a less drug release than those prepared with HPMCP. This may be attributed to the lower solubility of CAP when compared to HPMCP (22). From all these results, it was envisaged that the formulation M-7 was found to be optimum and hence it was selected for in vivo studies in human volunteers.

SEM studies of the formulations M-7 and C-2 were carried out to assess the surface morphology and to confirm the mechanism of drug release. SEM photomicrographs of tablets at 0 h, 2 h and after 24 h of dissolution studies are

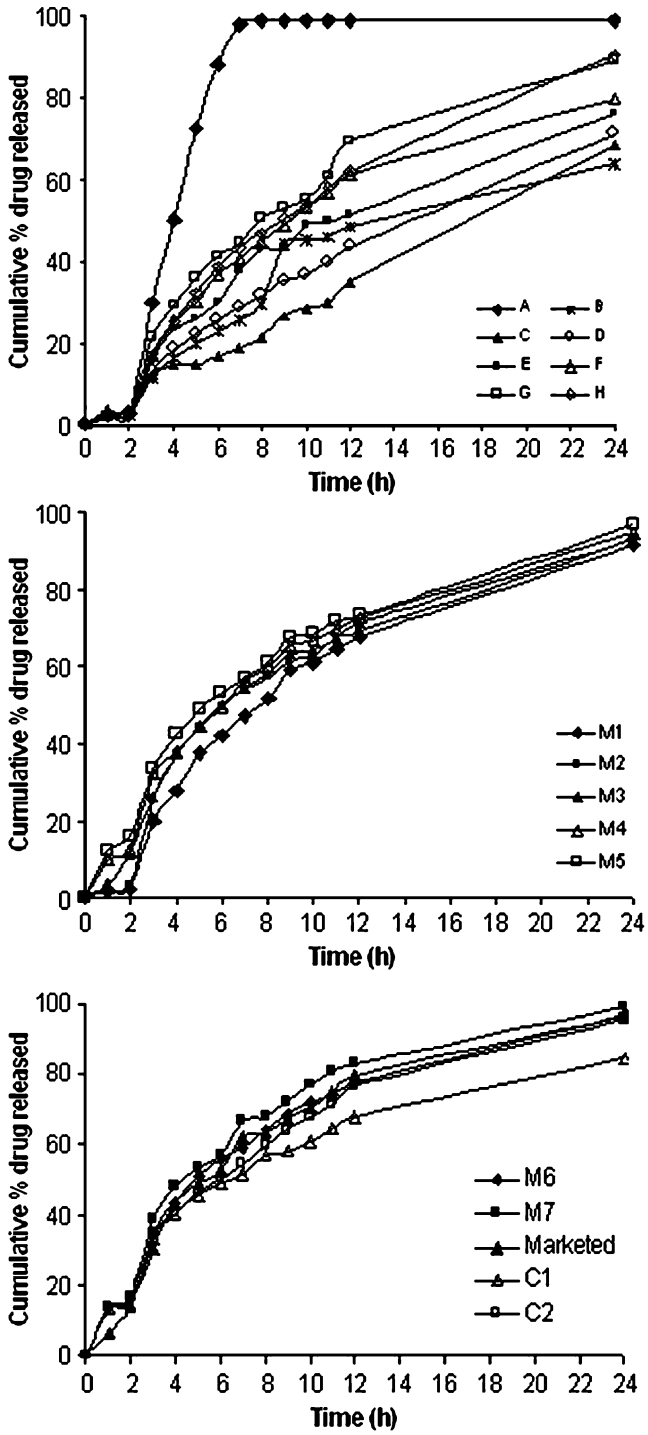

Fig. 3. *In vitro* release of aceclofenac from various formulations

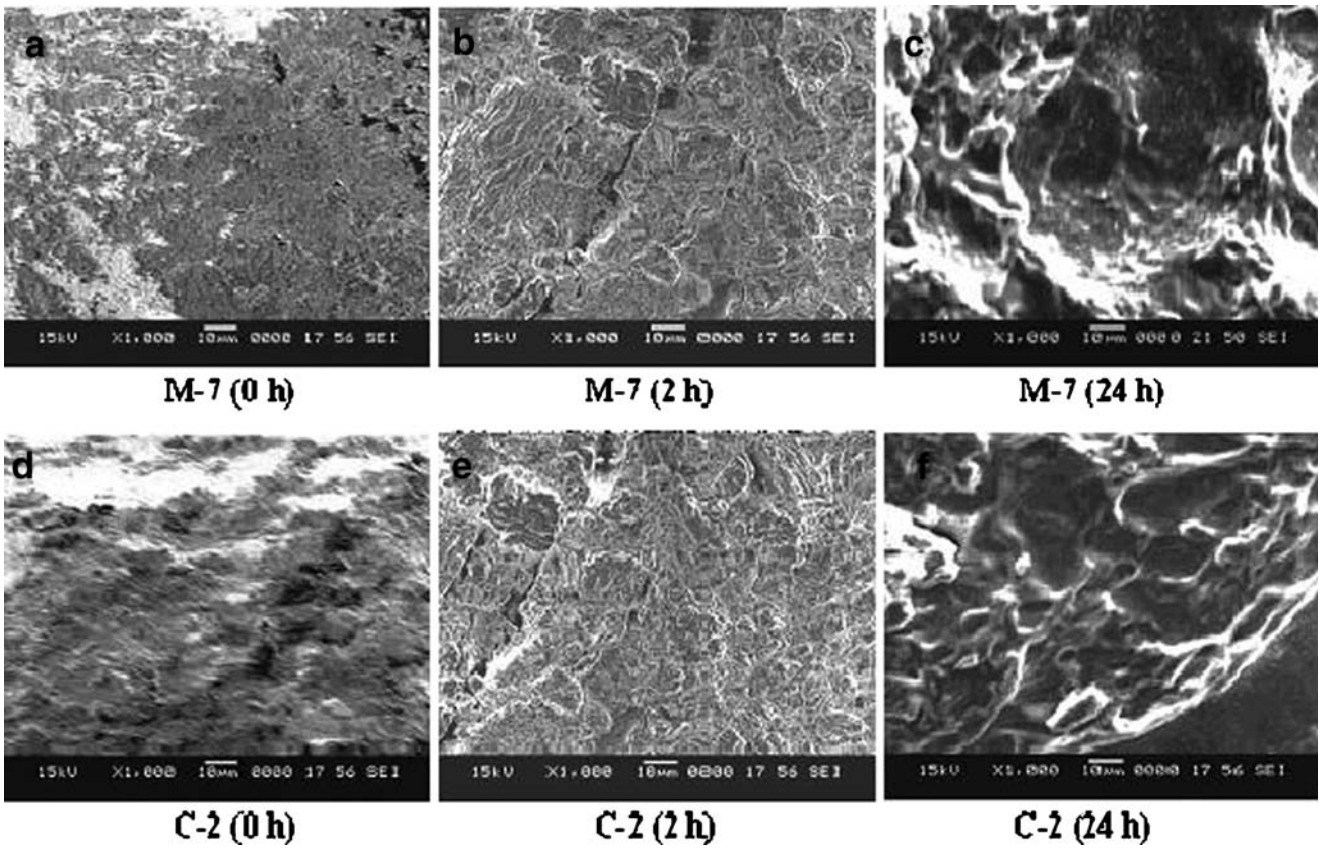

Fig. 4. SEM photomicrographs (1,000×) of matrix tablets showing surface morphology at different time intervals in dissolution study. **A** M-7 tablet at 0h; **B** M-7 tablet at 2h; **C** M-7 tablet at 24h; **D** C-2 tablet at 0h; **E** C-2 tablet at 2h; **F** C-2 tablet at 24h

shown in Fig. 4. The surface of the fresh tablets did not show any pores at ×1,000 magnification; but the surface showed pores and cracks at 2 and 24 h of dissolution. In case of formulation M-7, more number of pores was formed at 2 h, and the cracks were more prominent, which might have resulted in the superior drug release of M-7 in 2 h. At the end of 24 h, a very small amount of intact matrix was present and pores were formed throughout the matrix. It confirms that the erosion of matrix increased with respect to time. It also showed the formation of gelling structure, especially at the end of 2 h, indicating the possibility of swelling of matrix tablets. At all the time intervals, the pores and gelling structure were less with tablet C-2 than those of with tablet M-7. The formation of pores and gelling structure on tablet surface together with decrease in total mass of tablet with time, indicate the involvement of both erosion and diffusion mechanisms behind the drug release from the prepared matrix tablets.

In matrix tablets, drug release is controlled by extraction of the medicament by a simple diffusional process through an enveloping homogenous matrix and by leaching of the medicament by the bathing fluid, which is able to enter the drug matrix phase through pores, cracks and inter granular spaces. Modified release dosage forms may actually be complicated due to one or a combination of factors such as, partial dissolution of matrix substances, simultaneous break up of matrix, drug on the surface of matrix being released more rapidly than the drug in the matrix, one fraction of the dose being in a different, non matrix, readily available form (23). Chitosan has a substantial ability to swell and form a hydrogel in gastric medium. Hence the initial drug release in first 2 h might have achieved due to the presence of chitosan and also due to the drug release from the immediate release layer containing MCC; whereas the enteric polymers remain insoluble in the gastric pH, controlling the release of drug within the desired range. When the pH increases the solubility of chitosan was decreased, whereas the solubility of enteric polymers increased maintaining the drug release upto 24 h in the required range. The drug release at lower pH was accompanied with the dissolution of chitosan, where as at higher pH by the enteric polymers such as HPMCP and CAP (24,25). At pH values higher than their p*K*a, enteric polymers like CAP, cellulose acetate trimellitate, HPMCP, Eudragit L and Eudragit S could behave as hydrocolloids and so be used the same as classic hydrocolloids in the formulation of hydrophilic matrix tablets for drug controlled release (24).

Stability Studies

The results of accelerated stability studies, carried out according to ICH guidelines, indicated that M-7 tablets did not show any changes in physical parameters (colour, friability and hardness) during the study period and the drug content (n=3; mean±SD) was found above 96% at the end of 180 days (0 day: 99.96±0.38%; 15 days: 99.15±0.42%; 30 days: 98.44±0.56%; 60 days: 98.02±0.32%; 90 days: 97.25±0.65%; 180 days: 96.05±0.88%). This indicates that M-7 tablet

Table II. Pharmacokinetic Parameters from the Plasma Concentration–Time Curves

Parameters	Tablet M-7 (test)	Marketed tablet (reference)	Deviation (*T*/*R* %)
C_{max} (mg/ml)	6.46±1.03	6.14±1.13	105.14
T_{max} (h)	5.67±1.51	5.00±1.67	113.33
AUC_{0-30} (mg h/ml)	123.30±10.22	130.30±12.52	94.63
$AUC_{0-\infty}$ (mg h/ml)	128.00±12.69	132.00±11.25	96.97

All values are expressed as mean±SD, n=6; confidence interval=95%
T/*R* test/reference, C_{max} maximum plasma concentration, T_{max} time for maximum plasma concentration, *AUC* area under the curve

exhibited good physical stability and acceptable potency at accelerated storage condition for 6 months.

Pharmacokinetic Study in Human Volunteers

The pharmacokinetic parameters were calculated (Table II) from the plasma concentration–time curve (Fig. 5). The absorption of aceclofenac from both tablets was observed within 1 h and drug was detected in plasma at this time point. The C_{max} values were found to be 6.46±1.03 and 6.14±1.13 μg/ml for M-7 and marketed tablets, respectively and T_{max} values were about 5 h for both tablets (5.00±1.67 and 5.67±1.51 h for M-7 and marketed tablets, respectively). There was no significant (p>0.05; 95% confidence intervals) difference between the C_{max} and T_{max} values of both tablets. The values of AUC_{0-30} were found to be 123.30±10.22 and 130.30±12.52 μg h/ml and $AUC_{0-\infty}$ values were found to be 128.00±12.69 and 132.00±11.25 μg h/ml for M-7 and marketed tablets, respectively. These results showed no significant (p>0.05; 95% confidence intervals) difference between M-7 and marketed tablets with respect to AUC. The % deviation (Test/Reference %) observed for M-7 (Test) and marketed (Reference) tablets was within the range of 80–120% with respect to C_{max}, T_{max} and AUC values (95% confidence intervals), which is the general regulatory requirement for the tablets to be bioequivalent (http://www.fda.gov/cder/guidance/3616fnl.htm). To further assess the *in vivo* release of test and marketed tablets, the *in vitro–in vivo* correlation (IVIVC) was performed by deconvolution method (26–28) and corresponding R^2 (linear regression fit) values were compared (Fig. 6). Nearly equivalent R^2 values (0.9612 and 0.9651 for M-7 and marketed tablets, respectively) confirm the bioequivalence of M-7 and marketed tables.

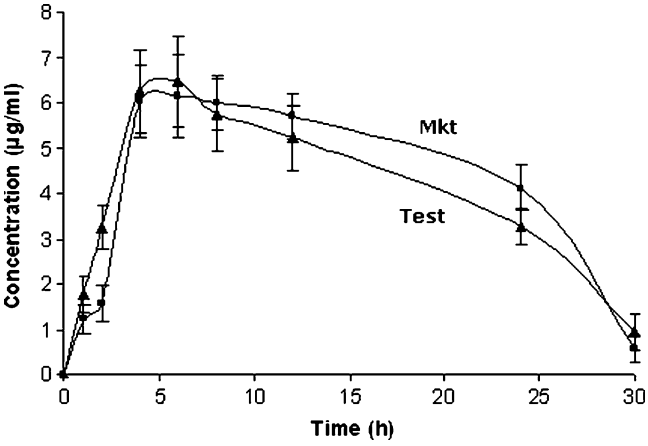

Fig. 5. Plasma drug concentration–time curve. *Mkt* marketed tablet, *Test* M-7 tablet; each point is presented as mean±SD, n=6

CONCLUSION

The present study demonstrated the successful application of the combination of chitosan and an enteric polymer to sustain the aceclofenac release upto 24 h at different pH conditions. The prepared tablet (M-7) showed good physical stability and acceptable potency in the stability studies. The pharmacokinetic studies in human subjects revealed no significant difference between the values of pharmacokinetic parameters of M-7 and marketed tablets (p>0.05; 95% confidence intervals). However clinical studies in large scale and, long term and extensive stability studies at different conditions are required to confirm these results.

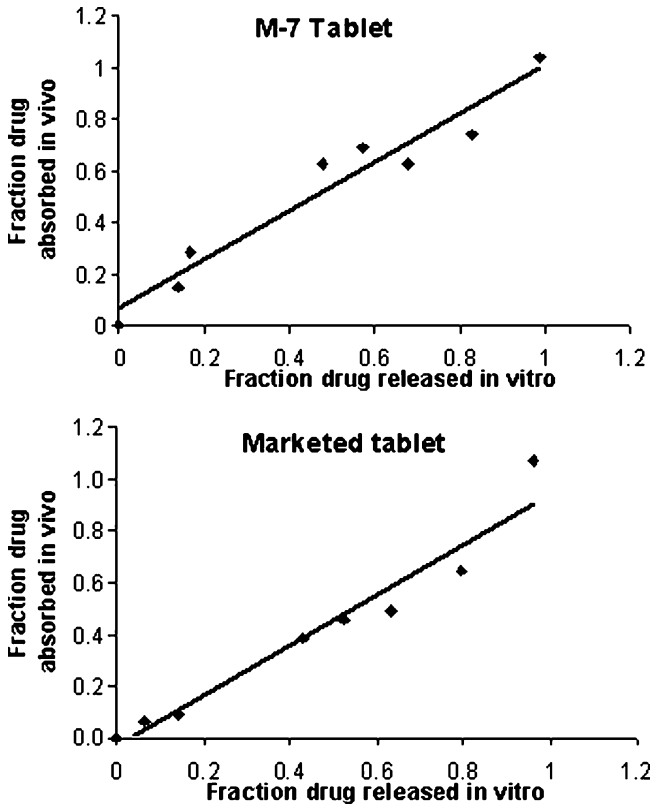

Fig. 6. *In vitro–in vivo* correlation plots

ACKNOWLEDGEMENTS

Authors are thankful to Lupin Research Park, Pune, India for the gift samples of aceclofenac and other excipients. They are grateful to Dr. K. Narayana Prabhu, Asst Professor, NITK, Suratkal for his kind help and co-operation in SEM studies.

REFERENCES

1. K. Parfitt. Analgesics, anti-inflammatory and antipyretics. In J. E. F. Reynolds (ed.), *Martindale: The Complete Drug Reference*. 32nd ed, Massachusetts, 1999, pp. 1–12.
2. A. E. Kay, and A. Alldred. Rheumatoid arthritis and osteoarthritis. In R. Walker, and C. Edwards (eds.), *Clinical Pharmacy and Therapeutics*, 3rd ed., Churchill Livingstone, London, UK, 2003, pp. 791–807.
3. A. C. Moffet, M. D. Osselton, B. Widdop. *Clarke's analysis of drugs and poisons*. 3rd ed., Pharmaceutical Press, London, UK (2006).
4. M. Y. Rao, J. K. Veni, and G. Jayasagar. Formulation and evaluation of diclofenac sodium using hydrophilic matrices. *Drug. Dev. Ind. Pharm*. **27**:759–766 (2001).
5. L. Maggi, E. O. Machiste, M. L. Torre, and U. Conte. Formulation of biphasic release tablets containing slightly soluble drugs. *Eur. J. Pharm. Biopharm*. **48**:37–42 (1999).
6. Y. W. Chien. Regulatory considerations in controlled release medication. In Y. W. Chien (ed.), *Novel Drug Delivery Systems*, Marcel Dekker, New York, NY, 1982, pp. 577–578.
7. S. Mutalik, A. Naha, A. N. Usha et al. Preparation, *in vitro*, Preclinical and clinical evaluations of once daily sustained release tablets of aceclofenac. *Arch. Pharm. Res*. **30**:222–234 (2007).
8. M. George, and E. T. Abraham. Polyionic hydrocolloids for the intestinal delivery of protein drugs: alginate and chitosan—a review. *J. Controlled. Rel*. **114**:1–14 (2006).
9. M. Fukuda, A. P. Nicholas, and J. W. McGinity. Properties of sustained release hot-melt extruded tablets containing chitosan and xanthan gum. *Int. J. Pharm*. **310**:90–100 (2006).
10. K. Wang, and H. Zhimin. Alginate–konjac glucomannan–chitosan beads as controlled release matrix. *Int. J. Pharm*. **244**:117–126 (2001).
11. R. Barreiro-Iglesias, R. Coronilla, A. Concheiro, and C. Alvarez-Lorenzo. Preparation of chitosan beads by simultaneous cross-linking/insolubilisation in basic pH. Rheological optimisation and drug loading/release behaviour. *Eur. J. Pharm. Sci*. **24**:77–84 (2005).
12. J. Akbua. Use of chitosonium malate as a matrix in sustained-release tablets. *Int. J. Pharm*. **89**:19–24 (1993).
13. J. Knapczyk. Antimycotic buccal and vaginal tablets with chitosan. *Int. J. Pharm*. **88**:9–14 (1992).
14. K. R. Reddy, S. Mutalik, and S. Reddy. Once-daily sustained-release matrix tablets of nicorandil: formulation and *in vitro* evaluation. *AAPS PharmSciTech*. **4**:E61 (2003).
15. A. M. Agrawal, S. H. Neau, and P. L. Bonate. Wet granulation fine particle ethylcellulose tablets: effect of production variables and mathematical modeling of drug release. *AAPS PharmSci*. 2: Article 13 (2003).
16. R. Chen, H. Takahashi, H. Okamoto, and K. Danjo. Particle design of three-component system for sustained release using a 4-fluid nozzle spray-drying technique. *Chem. Pharm. Bull*. **11**:1486–1490 (2006).
17. C. M. Silva, A. J. Ribeiro, D. Ferreira, and F. Veiga. Insulin encapsulation in reinforced alginate microspheres prepared by internal gelation. *Eur. J. Pharm. Sci*. **2**:148–159 (2006).
18. M. E. Aulton, and T. I. Wells. *Pharmaceutics-The Science of Dosage Form Design*. Churchill Livingstone, London, UK, (1988).
19. Indian Pharmacopoeia. Ministry of Health and Family Welfare. Government of India, Vol 2, Delhi, India: Controller of Publication; 1996.
20. B. V. Joshi, V. B. Patil, and V. B. Pokharkar. Compatibility studies between carbamazepine and tablet excipients using thermal and non-thermal methods. *Drug Dev. Ind. Pharm*. **6**:687–694 (2002).
21. H. A. Kibbe. *Hand Book of Pharmaceutical Excipients*. 3rd ed. American pharmaceutical association, Pharmaceutical press, London, (2000).
22. J. A. Seitz, S. P. Mehta, and J. L. Yeager. Tablet coating. In L. Lachman, H. A. Liberman, and J. L. Kanig (eds.), *The Theory and Practice of Industrial Pharmacy*, 3rd ed., Varghese Publishing House, Delhi, India, 1987, pp. 346–373.
23. U. V. Banakar. Dissolution of modified release dosage forms. In J. Swarbrick (ed.), *Pharmaceutical Dissolution Testing*, Marcel Dekker, New York, NY, 1992, pp. 299–312.
24. G. F. Palmieri, S. Michelini, P. D. Martino, and S. Martelli. Polymers with pH-dependent solubility: possibility of use in the formulation of gastro resistant and controlled-release matrix tablets. *Drug Dev Ind Pharm*. **26**:837–845 (2000).
25. C. Yomota, T. Miyazaki, and S. Okada. Sustained-release effect of the direct compressed tablet based on chitosan and Na alginate. *Yakugaku Zasshi*. **114**:257–263 (1994).
26. J. S. Grundy, K. E. Anderson, J. A. Rogers, and R. T. Foster. Studies on dissolution testing of the nifedipine gastrointestinal therapeutic system. II. Improved *in vitro–in vivo* correlation using a two-phase dissolution test. *J. Control Release*. **48**:9–17 (1997).
27. C. M. Negrin, A. Delgado, M. Llabres, and C. Evora. In vivo–in vitro study of biodegradable methadone delivery systems. *Biomaterials*. **22**:563–570 (2001).
28. X. Yang, G. Zhang, W. Li, B. Peng, Z. Liu, and W. Pan. Design and evaluation of Jingzhiguanxin monolithic osmotic pump tablet. *Chem. Pharm. Bull*. **4**:465–469 (2006).