A simple and rapid approach to evaluate the *in vitro in vivo* role of release controlling agent ethyl cellulose ether derivative polymer

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Abstract: Diclofenac sodium (DCL-Na) conventional oral tablets exhibit serious side effects when given for a longer period leading to noncompliance. Controlled release matrix tablets of diclofenac sodium were formulated using simple blending (F-1), solvent evaporation (F-2) and co-precipitation techniques (F-3). Ethocel® Standard 7 FP Premium Polymer (15%) was used as a release controlling agent. Drug release study was conducted in 7.4 pH phosphate buffer solutions as dissolution medium *in vitro*. Pharmacokinetic parameters were evaluated using albino rabbits. Solvent evaporation technique was found to be the best release controlling technique thereby prolonging the release rate up to 24 hours. Accelerated stability studies of the optimized test formulation (F-2) did not show any significant change ($p<0.05$) in the physicochemical characteristics and release rate when stored for six months. A simple and rapid method was developed for DCL-Na active moiety using HPLC-UV at 276nm. The optimized test tablets (F-2) significantly ($p<0.05$) exhibited peaks plasma concentration (C_{max}=237.66±1.98) and extended the peak time (t_{max}=4.63±0.24). Good *in-vitro in vivo* correlation was found (R^2=0.9883) against drug absorption and drug release. The study showed that once-daily controlled release matrix tablets of DCL-Na were successfully developed using Ethocel® Standard 7 FP Premium.

Keywords: Diclofenac sodium, Ethocel®, controlled release tablets, zero-order kinetics, anomalous release.

INTRODUCTION

Oral drug dosage forms are increasing exponentially on pharmacy shelves, and this growth is expected to continue in the years to come. The growth is due to many factors including patient convenience and compliance, reduced health care cost, unmet medical needs, market exclusivisity and the large scale at which these drugs are manufactured (Beldon, 2006, Orive *et al.*, 2004). All those products that can reduce dosing frequency provide convenience and satisfaction that can have a positive impact on patient fidelity and more positive outcomes (Lizheng *et al.*, 2007). Controlled release matrix tablets provide a drug moiety bio-available over a desired period of time thereby improving patient compliance (Collett and oreton, 2002, Evenstad and R, 1992). Diclofenac sodium is a widely used drug recommended to the patients with rheumatoid arthritis, osteoarthritis, ankylosing spondilitisand gout (Wortmann, 2005, Cannon *et al.*, 2006, Sidiropoulos *et al.*, 2008). Occasionally, it produces gastrointestinal ulceration and other serious side effects such as edema and abdominal pain (Cheng *et al.*, 2004). In order to minimize these adverse effects usually associated with pulse-type absorption from a conventional dosage forms, to improve the patient compliance, to maintain a constant therapeutic level of DCL-Na in blood and to overcome the problem associated with shorter half life (1-2 hours), once-daily DCL-Na controlled release matrix tablet is desired. Previously, *In vitro* studies of diclofenac sodium controlled-release from bio-polymeric hydrophilic matrices have been done to evaluate the role of hydrophilic polymers including hydroxylpropyl-methylcellulose and microcrystalline cellulose (Bravo *et al.*, 2002, Fassihi, 1987), thereby achieving sustained release for 12 hours. *In vivo* dissolution of a controlled release diclofenac sodium formulation in xanthan gum matrices, prepared by direct compression, has been evaluated and a higher plasma concentration was achieved after 10 hrs post dosing (Billa *et al.*, 2000). In another study pharmacokinetics parameters of sustained release DCL-Na tablets have been evaluated using a combination of hydroxypropylmethylcellulose and sodium carboxymethyl cellulose polymers, thereby achieving C_{max} and T_{max} of 538.00 and 2.30, respectively, after a mean retention time of 9.09 min (Madhusudan Rao *et al.*, 2001). Cellulose derivative polymers have often been used for drug delivery systems (Murthy, 1997). Ethylcellulose (EC) is an inert, stable(Rekhi and Jambhekar, 1995), non-toxic and easily compressible polymer with a number of pharmaceutical application such as binder, film and matrix forming agent (Shaikh *et al.*, 1987, Crowley *et al.*, 2004). Previously the effect of ethylcellulose, hydroxypropylmethylcellulose (HPMC), carboxymethylcellulose (CMC) and several other parameters have been investigated (Khan and Jiabi, 1998, Jan *et al.*, 2011, 2012, 2013a & 2013b, Shah *et al.*,2011 & 2012) and reported that EC possessed good capability of

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retarding the drug release rate from matrices tablets. Direct compression is the simplest and least expensive method of preparing controlled release matrices, avoiding various unnecessary steps required for wet-granulation and several other methods (Fachaux *et al.*, 1995, Ahrabi *et al.*, 2000, Fu *et al.*, 2004). By prolonging the residence time of drug carriers at the absorption site, delivery of DCL-Na can now precisely be controlled with respect to the time and interval of oral doses. The present work aims to design, formulate and evaluate once-daily controlled matrix tablets of DCL-Na both *in vitro in vivo*, using simple approaches including direct compression, solvent evaporation and co-precipitation techniques.

MTERIALS AND METHODS

Material

Diclofenac sodium (DCL-Na) was received as gift from (Danas Pharmaceuticals, Pakistan). Ethocel® Standard 7 FP Premium (Dow Chemical Co., Midland USA). Voltral® SR 100mg tablets (Novartis, Jamshoro, Pakistan) was purchased from the local market. Acitonitrile and methanol (Merck, Germany) were purchased from the local market. All chemicals used were either of analytical or pharmaceutical grades. Albino rabbits were purchased from the local market.

Differential Scanning Calorimetery (DSC)

The physicochemical compatibility of DCL-Na and Ethocel® polymer was investigated by differential scanning calorimetric (DSC) analysis, using a DSC instrument with a thermal analysis data station system, computer and a plotter interface. The instrument was calibrated with an indium standard. The samples (5-10 mg) were weighed in aluminium pan and sealed with a lid using a punch. The samples were heated (50-300°C) at a constant scanning speed of 10°C/min in sealed aluminium pan using nitrogen as purging gas.

Infra-Red Absorption Spectroscopy

A computerized FTIR (Perkin Elmer, England) was used to study possible interaction of DCL-Na with Ethocel® polymer. Approximately 10mg of DCL-Na, F-1, F-2 and F-3 were placed on the plate, enough pressure was applied and sharp peaks were obtained at suitable intensity.

Preparation of tablets

Physical Blending Technique

Accurately weighed amount of Ethocel® Standard FP Premium polymer (15%) was blended with DCL-Na 100 mg powder (50%) and lactose (34.5%) to constitute 200 mg tablets. The blends were geometrically mixed in polythene bags for 20 min, and then magnesium stearate (0.5%) was added. Again geometric mixing technique was used to mix the blends thoroughly for 10 minutes. Physical mixtures were then passed through # 80 mesh and were directly compressed using a manually run Single Punch Machine (Erweka, Germany), keeping tablets weight fixed at 200 mg and hardness at 9 kg.

Solvent Evaporation Technique

Solid dispersions of DCL-Na was prepared by solvent evaporation technique as reported by Najib *et, al.* and Shivakumar *et al.* (Najib *et al.*, 1986, Shivakumar *et al.*, 2008). The required amount of DCL-Na (50%) was weighed accurately and dissolved in sufficient amount of chloroform. The solvent was evaporated using a rotary evaporator at 50°C and 100 rpm, till the complete evaporation of organic solvent. The residue was collected, sieved through # 100 mesh to get incorrigible uniform powder, mixed with Ethocel® (15%), lactose (34.5%) and then magnesium stearate (0.5%) (F-2) and was compressed by the above mentioned technique.

Co-precipitation Technique

Accurately weighed amount of Ethocel® polymer (15%) were impregnated with a little amount of hydroalcohalic solution to form a uniform paste. DCL-Na (50%) was then added to the above paste and kneaded for 20 minutes. The kneaded mixture was then dried and passed through sieve No 80.

Physicochemical Evaluation of Starting Materials and Tablets

Physicochemical characteristics of starting materials including bulk density, tapped density, hausner's ratio, angle of repose (AR) and compressibility index (CI), while those of tablets including thickness, diameter, hardness, weight variation, friability and drug content were studied and reported as their means and SDs. Briefly, angle of repose was determined by a fixed cone and funnel method, while hausner ratio and compressibility index were determined by cylinder method as per United States Pharmacopoeia, USPXXXI procedure. Hardness of the tablets was determined by hardness tester (Erweka, Germany) and friability-by-friability testing apparatus (Rosche friabilator, Erweka, Germany). Weight variation and drug contents were determined according to the standard procedures of United States Pharmacopoeia, USPXXXI.

Drug release studies, of test formulations (F-1, 2 & 3) and market-brand were conducted according to USP apparatus 1, using 8-station dissolution apparatus Pharma test PTWS-11/P, TPT (Hamburg, Germany). Each flask was filled with 900 ml dissolution medium (0.2 M phosphate buffer solution pH 7.4) maintained at 37±0.1°C. tablets were placed in different baskets and stirred at 100 rpm (perfect sink conditions). At predetermined time intervals of 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 18 and 24 hrs, a sample of 5 ml was taken with syringe using 0.45µm filter and was replaced with the same fresh medium. Samples were analyzed at 276 nm with the help of UV- Visible Spectrophotometer UV-1601 (Shimadzu, Japan). The

mean of three tablets was used to evaluate the drug release for each of the formulations.

Release Kinetics

To interpret DCL-Na release rate from matrix tablets, the data obtained from *in vitro* drug release studies was plotted in various kinetics models including Zero-order and First order, Hixson Crowell's Equation or Erosion Model, Higuchi's model and Korsmeyer-Pappas equation.

A simple model independent approach was used to compare the release profiles of test and market formulations (Shah *et al.*, 1998). The approach was also recommended by US Food and Drug Authority (FDA, 1997).

$$\text{Dissimilaity Factor} = \qquad f1 = \frac{\sum[R_t - T_t]}{\sum R_t} \times 100$$

Where n is the number of pull-points. R_t is the dissolution profile of the reference tablet while T_t is the dissolution profile of test tablet at time t.

Similarity in % dissolution curves are calculated by a similarity model/factor f_2. Following equation is used to calculate similarity factor.

$$\text{Similarity Factor} = \qquad f2 = 50 \cdot Log\left[\frac{1}{\sqrt{1 + \frac{1}{n}\sum (R_t - T_t)^2}} \times 100\right]$$

Where "n" is the number of data points collected, R_t and T_t are the percent drug dissolved at each time point for reference and test tablets, respectively. It has been suggested that if the similarity factor is close to 100 and difference factor close to zero, then the formulation release data could be consider as similar to each other (Shah *et al.*, 1998).

The optimized tablets (F-2) were stored in amber bottles at ambient conditions (temperature 25°C and relative humidity 65%) and accelerated condition temperature 40°C and relative humidity (RH) of 75±5%), in a stability chamber (Ti-Sc-THH-07-0400, Faisalabad, Pakistan) in accordance with International Commission for Harmonization (ICH) guidelines for a period of 0 (pre-storage), 1, 2, 3, 6 months. The aged samples were tested for appearance, thickness, diameter, friability, harness, weight variation, content uniformity and dissolution profiles at time intervals of 0 (pre-storage), 1, 2, 3, 6, 9, and 12. The *in vitro* drug dissolution studies were performed on aged tablets by the method described above.

In Vivo Studies Chromatographic Conditions and Method Development

In vivo studies were conducted on twenty-four healthy male albino rabbits, weighing about 3.0 kg, in accordance with the standard protocol by the research and Ethical Committee of Faculty of Pharmacy, Gomal University, D.I. Khan, KPK, Pakistan. The *in vivo* research proposal was approved by research and ethical committee. The whole *in vivo* experiments were conducted in accordance with the animal scientific procedure Act, 1986. In order to compare the pharmacokinetic parameters of the optimized controlled release test-tablet and the respective sustained release reference tablets (Market Brand) studies were conducted in two animal groups (each group comprising of 12 rabbits) in a parallel study design. The relative-bioavailability and *in vitro in vivo* correlation were determined using suitable pharmacokinetic parameters.

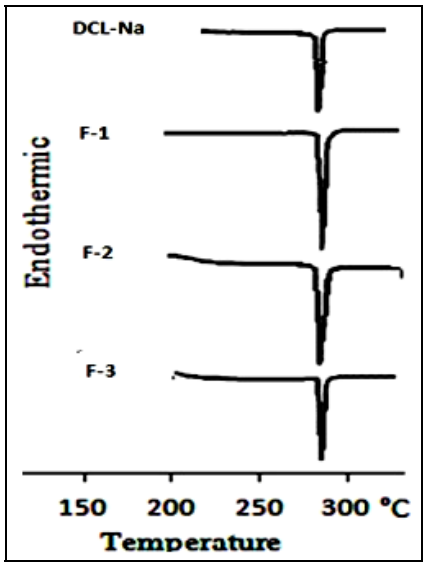

Fig. 1: Differential Scanning Calorimetric Thermograms of DCL-Na, F-1, F-2 and F-3

Briefly, At the time of dose administration, rabbits were shifted to placement restraints (wooden holder) and the tablets were given orally, using a 3ml syringe with its barrel smoothly cut at the needle end (care was taken to smoothen the edges of the cut end of the syringe to avoid damage to the oral mucosa of rabbit and to prevent any internal injury). Once tablet swallowing was confirmed, 10ml tape water was given to the rabbit, with the help of a 10ml syringe with oral tube in order of mime human dosing.

Rabbits were fasted for 24 hours. First batch was fed with 100mg DCL-Na test tablets while the second batch was given 100mg DCL-Na market SR tablets. Water was given *ad libitum* during fasting and throughout the sampling time. Blood samples (0.7ml each time) were collected from the marginal ear vein into heparinized centrifuge tubes just before dosing and at intervals of 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 30, 36 & 42 hours, for DCL-Na test and market brands, during the study after dosing.

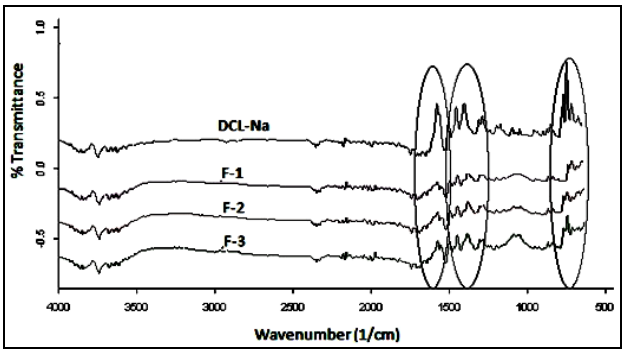

Fig. 2: FTIR spectroscopy of DCL-Na, F-1, F-2 and F-3

The collected blood was allowed to clot for about 30 minutes. The resulting clot was remimed with a sterile wooden stick and placed still in the original collection tube in the refrigerator for about one hour. Blood samples were centrifuged at 1500 rev/min and the plasma was separated. One undosed plasma sample was kept as blank. To 1ml each of the plasma samples, 5ml of diethyl ether was added and the tubes were then centrifuged at 2500 rev/min for 15 minutes. About 4ml of the supernatant was pipetted out which was evaporated at room temperature. The residue was reconstituted with 5ml of acetonitrile and drugs concentrations were determined by a rapid HPLC method (Joseph *et al.*, 2002).

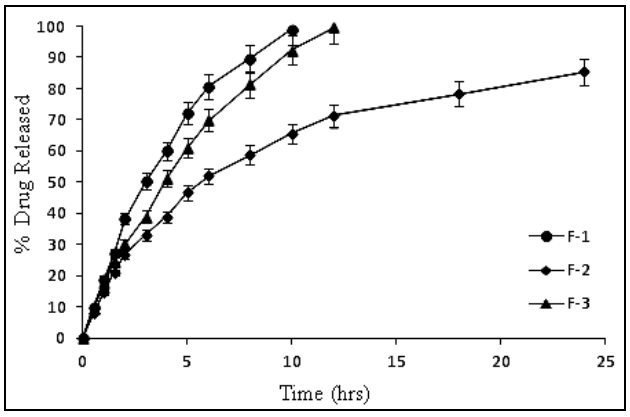

Fig. 3: Release profiles of DCL-Na from three different formulations, F-1, F-2 and F-3

An HPLC system (Agilent 1200, Agilent Corporation, Germany) comprising a quaternary pump, an automatic sampler and a photodiode array (PDA) detector was used with data acquisition by Chem Station® software (Agilent Corporation, Germany). The chromatographic separation was achieved using a phenomenex C18 column (5 µm; 4.6 mm×250 mm, Luna, USA) maintained at 25°C. The mobile phase was prepared and premixed. Fifty parts (by volume) of disodium hydrogen phosphate solution (30 mM) pH 7.0 were mixed with fifty parts (by volume) of acetonitrile (1:1); and the isocratic flow rate was 1.0 ml/min. An acetonitrile: water (50:50 v/v) mixture was used as a rinse solution for the injector, and the injection volume was fixed at 5µl. Detection was carried out using a wavelength of 276 nm. Linearity, precision, and accuracy of the method and percentage recovery of DCL-Na were determined using spiked serum of rabbits at concentrations of 5 to 50µg/ml. Intra-day and inter-day variabilities were determined by repeated injections of quality control (QC) samples. The QC samples were prepared at 8, 18 and 40µg/ml representing low, middle and high controls respectively.

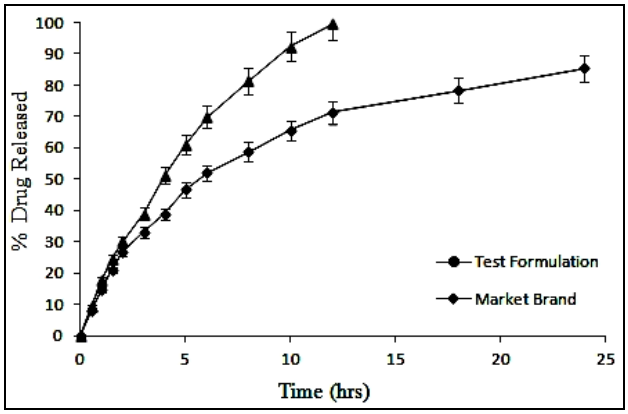

Fig. 4: Release profiles of DCL-Na from optimized test formulation F-2 and reference formulation

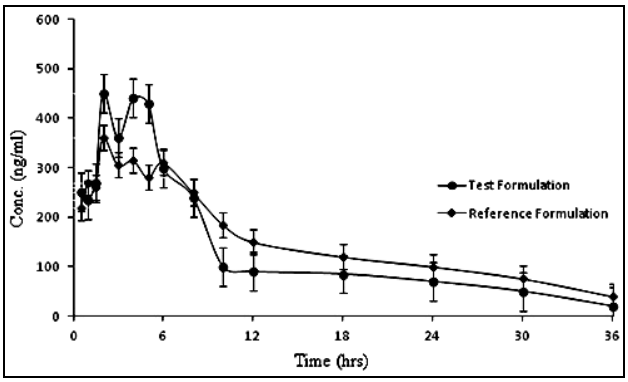

Fig. 5: Mean plasma DCL-Na concentration versus time cures of optimized test formulation

Pharmacokinetic parameters, such as area under the curve (AUC), peak concentration (C_{max}), time to attain peak concentration (T_{max}) and elimination half-lives ($t_{1/2}$) were derived from the plasma concentration versus time data using Kinetica ver 5.0. For the computation of the above pharmacokinetic parameters, a non-compartmental approach implemented in Kinetica was used. The values of the rate constant for elimination (k_{el}) were used to calculate the absorbed and unabsorbed fractions of drugs using Wegnar-Nelson method given in Pk-Fit ver 2.01.

The *in vitro in vivo* correlation of the optimized formulation (F-2) was investigated by plotting the percent drug absorbed (F_a) *in vivo* against percent drug released (F_r) *in vitro*.

Unpaired *t*-tests were conducted using SPSS14 software to compare the pharmacokinetic parameters of CR test tablets and reference tablets. *P* value of <0.05 were considered as significant.

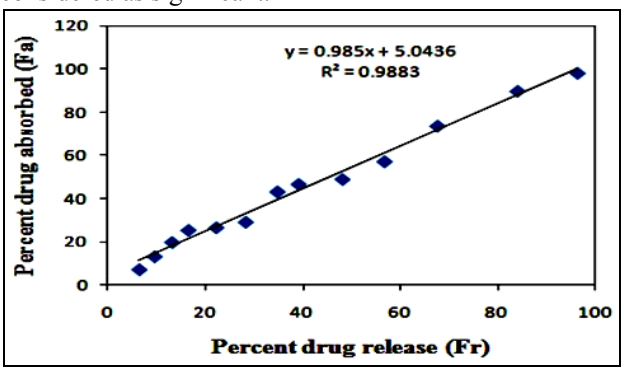

Fig. 6: Percent drug absorbed (Fa) plotted against percent drug released (Fr) to show *in vitro in vivo* correlation of DCL-Na test formulation

RESULTS

Fig. 1 shows DSC curves of DCL-Na, F-1, F-2 and F-3. Analytical grade of DCL-Na an all the three formulations showed single well-defined endothermic peak at 280°C corresponding to its melting temperature (Petar *et al.*, 2001).

FTIR spectra analysis

FTIR studies of DCL-Na, F-1 F-2 and F-3 are presented in fig. 2. The spectroscopy was conducted to investigate any possibility of occurring drug-polymer interaction. In case of pure DCL-Na drug and all the three formulations, the spectra showed principal peaks at 1572, 756 due to chlorine bonding (C-Cl). Smaller peaks at 1504 (C=C), 775 and 1586 (C=C, aromatic) could be due to aromatic ring (Adeyeye and Li, 1990).

Physicochemical evaluation of starting materials and tablets

Active DCL-Na drug showed poor flowability and compressibility as compared to their physical mixtures prepared by three different techniques (physical blending, solvent evaporation and co-precipitation) (Table 1). Angle of repose was measured as 32.64° for DCL-Na powder, while that for F-1, 2 & 3 ranged from 20° to 28° indicating fair flow properties. HF for DCL-Na powder was found to be 2.630, which might be contributed to the poor flow properties because of interparticular friction of powdered DCL-Na. While HF for F-1, F-2 & F-3 was found to be in the range of 1.017±0.02 to 1.032±0.11, indicating good flow properties of powder with reduced friction. Percent compressibility of DCL-Na powder was found to be 51.20%, which was in close agreement with angle repose, while % compressibility for all the three formulations ranged 17.21±0.21 to 19.32±0.09. The % drug content was found to be in the range of 100.3±0.01 to 102.1±0.21.

Hardness was found to be suitable to reduce the tendency to cap; ranging 6.4±0.28 to 6.9±0.46kg/cm^3. Friability and weight variation tests were also in the acceptable range of 0.21±0.06 to 0.75±0.06w/w and 200±0.5 to 203±0.8 mg respectively. Content uniformity test fell in the best suitable range of 98.10±0.04 to 99.20±0.05%. Thickness and diameter ranged from 2.1±0.1 mm to 2.3±0.4 mm and 4.2±0.1 mm to 4.4±0.3 mm.

Formulations F-1, F-2, F-3 and standard reference released 98.98, 85.43, 99.58 and 99.99% drug within 10, 24, 12 and 12 hours, respectively (fig. 3 & 4). The drug release profiles might show that the tablets remained intact during the dissolution. It could also be observed that the pH of the dissolution medium did not affect the drug release profile.

Release kinetics

Sample formulations F-1, F-2 and F-3, tested for release kinetics exhibited drug release periods of 10, 24 and 12 hours, respectively (fig. 3). The release data showed that hardness of the tablets and pH of the dissolution medium had no significant effect ($p<0.05$) on the release mechanism. The values of release exponent showed good linearity with correlation coefficient r^2=0.981 for F-2 indicating zero-order release kinetics. Effect of pH of the dissolution medium (1.5, 4.5, 6.8, 7, 7.2 & 7.4 pH) was also evaluated via similarity factor *f*2 (79.89, 82.81 and 83.32 for F-1, F-2 & F-3, respectively). The tablets prepared by solvent evaporation technique (F-2) were selected for further studies. Statistical evaluation proved that no significant difference was observed for drug contents of the test tablets. Aging also revealed that storage conditions did not significantly affect the drug content, friability, weight variation, hardness and physical appearance of the optimized test tablets when studied for six months. Aging did not show any significant alteration in the release behavior of the drug and revealed maximum stability of matrix tablets designed fig. 4. Similar results were obtained by San Vicente et al, and Gupta VK while studying the aging of solid unit dosage form (San Vicente *et al.*, 2000, Gupta *et al.*, 2001). The best release exponent was found to be n=0.785 followed by the linearity r^2=0.984, which indicated anomalous release (non-Fickian Case-1) coupled diffusion and erosion, when the release data (F-1) was fitted to korsemeyer-peppas equation (Dabbagh *et al.*, 1996).

In vivo studies

A simple, rapid and reproducible method was developed using rabbit serum collected at zero-time, showing no peak, while spiked serum exhibited single peak of DCL-Na. The retention time and sample recovery were found to be 5.1 min and 95%. The calibration curve was found to linear in the range of 10-50 µg/ml with good linearity of R^2=0.9996. Percentage recovery ± % RSD ranged from 98.08±0.007 to 102.87±0.009. Limit of detection and

limit of quantification were analyzed to be 0.155 µg/ml and 0.657µg/ml, respectively. Inter-day and intra-day precision for three samples were found to be ranging from 0.001 to 0.029% and 0.002 to 0.042%, respectively. All the above said parameters comply with the ICH guidelines (ICH, 1996).

The mean plasma diclofenac sodium concentration versus time profiles of F-2 and reference formulations are shown in fig. 5. The profiles showed that the absorption patterns of drug from the matrix tablets were effectively controlled and the concentration level was detectable even at 36 hours after dosing.

Various pharmacokinetic parameters, including T_{max}, C_{max}, and $AUC_{0-\infty}$ for F-2 and reference formulations were evaluated. T_{max} and $AUC_{0-\infty}$ are related to the respective rate and extent of drug absorption, while C_{max} is related to both of the processes. There was no significant difference between T_{max} values of F-2 and reference formulations ($p \geq 0.05$). Similarly, no significant difference was observed between the values of $AUC_{0-\infty}$ (p=0.1564) and the values of C_{max} (p=0.05129) of the two preparations. It could also be observed that 90% confidence interval was estimated, for the ratios of $AUC_{0-\infty}$ values of test formulation (F-2) over those of reference formulation, to be between 0.85 and 1.07, which could be considered within the acceptable bioequivalence interval of 0.80 to 1.25. Multiple peaks were observed in the plasma profiles and for the reason 90% confidence interval could not reliably be estimated for the parameter C_{max} (table 1).

DISCUSSION

To overcome the problems associated with phenylacetic acid derivative (DCL-Na) conventional dosage form, once-daily controlled release matrix tablet is desired to optimize the blood level and ultimately to lesser the side effects and improving patient compliance. During pilot studies lactose was tried alone to investigate the release mechanism from a designed matrix tablets, which showed a simple burst release with 10 minutes thereby releasing 99.99% drug all at once. Later, ethylcellulose ether derivative polymer Ethocel® Standard Premium 7-FP was added (30%), using 10:3 drug to polymer ratio. Drug to polymer ratios, 10:1 and 10:2, using Ethocel® polymer have already been investigated in our previous studies (Akhlaq *et al.*, 2011, Akhlaq *et al.*, 2010). DSC chromatograms obtained for pure DCL-Na drug, F-1, F-2 and F-3 might indicate that there is no conclusive interaction of DCL-Na with polymer and/or other excipients used. The effects of processing were also investigated and it was found that the size and shape of melting endotherms were not significantly different for each of the parameters (mixing and compression) investigated. The single endothermic peaks demonstrated that no impure polymorph of DCL-Na was present and that no inter-conversion from one form to the other took place during the melting process. The FTIR spectra of pure DCL-Na drug, F-1, F-2 and F-3 also showed principal peaks in the same region, which could be ascribed as simple addition of Ethocel® polymer and other excipients to DCL-Na drug. Other minor changes might be due to variations in the resonance structure, stretching and bending, rotation of a part of molecule or certain bonds or minor distortion of bond angles. Similar study was conducted by the other researchers (Kulkarni *et al.*, 2007, Gokulakumar and Narayanaswamy 2008). Bulk and tapped densities results might indicate that there is no conclusive effect of physical mixing upon particle size. The tapped density for DCL-Na powder drug was improved after addition of excipients in case of physical blending, solid dispersions and co-precipitation techniques. After mixing both of the densities fell in the acceptable limit, which might indicate the formation of uniform mixture with better flow ability and compressibility. Angle of repose indicated that the frictional force between the particles in the loose powder was acceptable but the addition of polymer and excipients improved the powder flow properties and reduced the cohesive force among the particles up to negligible extent during compression (Sharma, 2008). Higher value of hausner's factor in case of DCL-Na powder might be contributed to the poor flow properties because of interparticular friction. In case of F-1, F-2 and F-3 HF reduced enough indicating good flow properties of the powder mixture with reduced interparticular friction. Lower values of percent compressibility might indicate

Table 1: Pharmacokinetic parameters determined for 100mg DCL-Na controlled release test and market formulations in Rabbit sera (Mean ± SD)

Pharmacokinetic Parameter Calculated for Flurbiprofen	Test Tablets	Reference Tablets
Half Life t1/2 (hours)	10.48±0.02	6.13±0.11
Time of maximum plasma concentration T_{max} (hours)	4.63±0.24	3.43±1.11
Maximum plasma concentration C_{max} (µg/ml)	237.66±1.98	246.24±2.81
Area under the curve AUC_0 (µg.hour/ml)	4882.19±3.45	3202.12±3.28
Area under the curve $AUC_{0\text{-}inf}$ (µg.hour/ml)	83501.7±2.61	33563.3±4.21
Mean residence time $MRT_{0\text{-}48hrs}$ (hours)	15.12±1.19	8.80±1.62
Clearance (Cl) (ml/min)	0.004±0.02	0.62±0.18

Note: Values are significantly different (P<0.05, 0.001) between means of test and reference formulations

improved compressibility and flow ability (Lachman *et al.*, 1987). Polymer viscosity and concentration might have increasing effect upon the size of tablet, which might affect the hardness of compressed tablet. Therefore, to omit the effect of hardness and compressibility variation upon drug release profiles (Abdelkader *et al.*, 2007). Thickness and diameter might affect the internal stress of the tablet and could be considered during handling. Friability, thickness, and diameter were also kept constant. Aging did not reveal any sort of degradation and reduction in the drug content so it might prove the physical stability of the prepared matrix tablets when stored for a longer time. The prolonged dissolution profiles might indicate that the test formulations released the drug in well-controlled manner especially in case of F-2, thereby achieving a prolonged release till 24 hours. Hydrophobic nature of ethyl cellulose ether derivative polymer effectively retarded the release of the drug and prolonged it up to 24 hrs thereby achieving a maximum release of 98.98, 85.43 and 99.58% after 24 hrs for F-1, F-2 and F-3, respectively. This effect might be ascribed to an increase in the extent of gel formation in the diffusion layer when the dissolution medium came in contact with tablet surface (Alderman, 1984), with more compressibility, swell ability and slow diffusion and erosion. The Ethocel® might act as barrier to the drug release due to its hydrophobic nature. The matrices might have formed uniform channels for water to diffuse, dissolve and release the drug in controlled manner. The idea could be in line with the previously reported studies (Howard and Timmins, 1988). A constant and uniform release rate also indicated that Ethocel® possesses binding and matrix forming properties and uniform tablets were produced with optimum friability and desired hardness level. Zero order-dug release provides a constant and uniform amount of drug available for absorption and maintains plasma concentration required for a desired therapeutic action. To evaluate the release mechanism of DCL-Na the release data was fitted in the Korsmeyer-Peppas model. The release exponent "n" was calculated through the slope of the straight line (Korsmeyer *et al.*, 1983). The value of "n" <0.45 shows Fickian release, while 0.45<0.89 shows anomalous transport (coupled diffusion and erosion), while the value of "n" ≥0.89 shows typical zero order release (Ritger and Peppas, 1987). It has been observed that in case of tablets Fickian diffusion refers to the diffusion of a drug through the channels formed in the polymer matrix, while zero-order usually describes drug release from a polymer matrix by a combined process of diffusion and erosion (Peppas, 1985). Release exponent "n" was used to select the most appropriate model to evaluate the release mechanism. Based on the release exponent "n" and/or goodness of fit test (i.e. coefficient of determination "R^2"), the drug release mechanism showed a typical zero order release pattern for F-2 only. The release rate and mechanism remained unaffected as the concentration of polymer (30%), hardness (5.0-10 kg/cm^2) and pH of the dissolution medium (7.4 pH) were kept constant throughout the study. A typical zero order release is the indication of uniform channels and porosity of the test tablets after hydration. Similar findings were observed by Rekhi *et al.* (Rekhi *et al.*, 1999). Ethocel® a cellulose derivative polymer (with ethoxyl substitution on anhydroglucose ring backbone) is essentially insoluble in water. The viscosity grade plays a vital role in controlling the release of a drug, so only Ethocel® Standard 7 FP Premium was selected for the study. Also, variation in viscosity grade also produce changes in tablet hardness during compression. Ethocel® was found to be relatively stable and pH did not affect the release rate. Therefore, it could be assumed that pH change in the stomach may not affect the typical zero order release of DCL-Na *in vivo* (Fukui *et al.*, 2004). The current study shows the lower values of dissimilarity factor $f1$ (i.e. <5) and the higher values of similarity factor $f2$ (>80.0) which are the indications of negligible difference of the dissolution profiles, when studied for test and market brand tablets. It could also be observed that the pH variations had no significant effect on the drug release from the matrix tablets when studied for pH range of 1.2-7.4. Rabbit has been chosen as the model units since there have been many bioavailability studies of NSAIDs available using this animal as model (Fara and Myrback, 1990) maintaining a fairly constant but optimum serum concentration of the drug for a longer period of time when compared with reference formulation. DCL-Na is rapidly absorbed when given orally. The usual oral dose of DCL-Na is 75 to 150 mg daily in individual doses. It has been investigated that, although, orally administered DCL-Na is almost completely absorbed, it is subjected to first pass metabolism, so the about 50% of the unchanged drug reaches the systemic circulation (Martindale, 1996).

Fig. 5 shows the plot of plasma concentration of the drug versus time for DCL-Na test and market formulations. Minor peaks fluctuations and multiple peaks in plasma profile were observed after oral administration. The multiple peaks may indicate the changes in the rate of drug release from the dosage form with changes in the pH of the environment within gastrointestinal tract. The same results were reported by Hasan *et al* (Hasan *et al.*, 2003). Significant extension in time for peaks plasma concentration (T_{max}) of DCL-Na from test formulation is indicative of drug release at a slower rate for a longer period of time. At the same time no significant difference was observed between the mean AUC of the test and reference formulations, which indicates that the test tablets were bioequivalent to the reference formulation. The *in vitro* release data of DCL-Na test formulation was also observed to be related with the *in vivo* parameter area under the curve at respective time points. Under the FDA guidelines, different levels of correlations between *in vivo* and *in vitro* parameters, such as A, B, C, D & E have been

introduced to achieve *in vitro in vivo* correlation (FDA, 1997). To achieve a best *in vitro in vivo* correlation of DCL-Na test tablets, the percent drug release *in vitro* (Fr) was plotted against percent drug absorbed *in vivo* (Fa) (Wagner and Nelson, 1964), as shown in fig. 6. As indicated by R^2 value of 0.9883 for DCL-Na test tablet, the correlation was found to be high. These results showed that the test formulation was suitable enough to be used for further therapeutic evaluation.

CONCLUSION

It could be concluded that solvent evaporation technique using ethylcellulose ether derivative polymer Ethocel® Standard 7 FP Premium was found to be the successful method to effectively control the release rate of DCL-Na up to 24 hours both *in vitro in vivo*. Due to inherently cross-linked structure, Ethocel® could form uniform channels to release the drug in a well-controlled manner. Solid dispersions technique helped in controlling the drug release rate both *in vitro in vivo*. Optimized test formulation exhibited good stability and was found to be bioequivalent to the reference formulation.

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