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ORIGINAL ARTICLE

Formulation and pharmacokinetics of multi-layered matrix tablets: Biphasic delivery of diclofenac

Ehab Mostafa Elzayat [a,*], Ali Abdelzaher Abdel-Rahman [c], Sayed Mohamed Ahmed [c], Fars Kaed Alanazi [a,b], Walid Abdulazim Habib [a], Hisham Suliman Abou-Auda [d], Adel Sakr [e]

[a] *Department of Pharmaceutics, Kayyali Research Chair for Pharmaceutical Industries, College of Pharmacy, King Saud University, Riyadh 11451, P.O. Box: 2457, Saudi Arabia*
[b] *Supervisor of Kayyali Research Chair for Pharmaceutical Industries, Riyadh, Saudi Arabia*
[c] *Department of Industrial Pharmacy, College of Pharmacy, Assiut University, Assiut, Egypt*
[d] *Department of Clinical Pharmacy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia*
[e] *Department of Industrial Pharmacy, College of Pharmacy, Future University in Egypt, Cairo, Egypt*

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Abstract The rapid availability of the drug at the site of action followed by maintaining its effect for a long period of time is of great clinical importance. Thus, the purpose of the present study was to prepare and evaluate multi-layered matrix tablets of diclofenac using Eudragit RL/RS blend to achieve both immediate and sustained therapeutic effects. Diclofenac potassium (25 mg) was incorporated in an outer immediate release layer to provide immediate pain relief whereas diclofenac sodium (75 mg) was incorporated in the inner core to provide extended drug release. Wet granulation was employed to prepare the inner core of the tablets that were further layered with an immediate release drug layer in the perforated pan coater. The in-vitro and in-vivo performance of the developed formulation was compared with the marketed products Voltaren® SR 75 mg and Cataflam® 25 mg. The in-vitro drug release of the prepared formulation showed similarity (f_2 = 66.19) to the marketed product. The pharmacokinetic study showed no significant difference ($p > 0.05$) in AUC_{0-24} and C_{max} between the test and reference formulations. The AUC_{0-24} values were 105.36 ± 83.3 and 92.87 ± 55.53 μg h/ml whereas the C_{max} values were 11.25 ± 6.87 and 12.97 ± 8.45 μg/ml, for the test and reference, respectively. The multi-layered tablets were proved to be bioequivalent with the commercially available tablets and were in agreement with the observed

* Corresponding author. Fax: +966 (1) 467 6295.
E-mail addresses: ezayat@ksu.edu.sa, ehab_zayat@hotmail.com (E.M. Elzayat).
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in-vitro drug release results. Stable physical characteristics and drug release profiles were observed in both long term and accelerated conditions stability studies.

1. Introduction

Diclofenac Sodium (DS) (sodium;2-[2-(2,6-dichloroanilino) phenyl]acetate) is a potent non-steroidal anti-inflammatory drug (NSAID) and one of the most useful pain killers, used clinically in treatment of rheumatic or non-rheumatic inflammatory diseases. Diclofenac inhibits the conversion of arachidonic acid to prostaglandins which are responsible for the inflammatory process (Fini et al., 2007; Kovala-Demertzi, 2006).

The extended release (ER) tablets containing 75 mg of diclofenac sodium are administered as once daily to guarantee less frequent dosing and diminished occurrence of gastrointestinal side effects (Shaw et al., 2005). Different release matrices have been proposed for the preparation of diclofenac ER formulations (González-RodríGuez et al., 2003; Su et al., 2003) using different polymers including HPMC with various grades (Mourão et al., 2010) and Eudragits (González-Rodrí Guez et al., 2003; Pignatello et al., 2002). In most therapies, such ER preparations are desirable. However, in certain cases, rapid availability of the drug as a loading dose is needed to relieve the symptoms of the disease, followed by a maintenance dose of an effective drug plasma level for the continuation of the clinical effects. The combination of different drug delivery forms (immediate, enteric or sustained) of diclofenac in one pharmaceutical formulation has been explored (Lim et al., 2012; Sawayanagi and Otani, 1990; Shaw, 2012). Marketed pharmaceutical products, namely Diclofenac Duo® 75 mg (PharmaSwiss) or Divido® 75 mg (Tabuk Pharmaceuticals), contain two drug delivery forms of the drug (25 mg diclofenac sodium in gastro-resistant form and 50 mg diclofenac sodium in prolonged-release form) in each hard gelatin capsule. Fast release of the drug from such formulations is not substantial. Release of the drug from the prolonged release form may start in the stomach but the amount released is not adequate to achieve a rapid pharmacologic response because of the low acidic solubility of DS. In addition, the lag time associated with the gastro-resistant form of the drug will contribute to the delayed response. Such preparations are not suitable for initiating treatment of conditions where rapid onset of action is needed. Accordingly, in this study, development of multilayered tablet that combines two drug delivery forms (immediate and sustained) using two salt forms of diclofenac was pursued. Diclofenac potassium (DP), with a relatively higher water solubility and faster dissolution and absorption rate than observed with the sodium salt (Ahmad et al., 2010; Altman et al., 2015), was used to achieve prompt pain relief by incorporating it into an immediate release, fast dissolving outer layer, whereas the sodium salt with its lower solubility was incorporated into an insoluble matrix inner core to be released slowly and provide a sustained analgesic effect. This kind of formulation is expected to add a clinical and therapeutic value, especially for patients taking multiple drugs. The multi-layered tablets were prepared and assessed for their in-vitro behavior, in-vivo performance and stability in this research.

2. Materials and methods

2.1. Materials

DS and DP were kindly supplied by TABUK Pharmaceuticals (Tabuk, Saudi Arabia). Eudragit RL PO and Eudragit RS PO were obtained from Evonik (Germany). Granulac 200® (lactose monohydrate) was kindly supplied by Meggle Pharma (Wassenberg, Germany). Vivapur® PH 101 (microcrystalline cellulose or MCC) was purchased from JRS Pharma (Weissenborn, Germany). All other chemicals were of pharmaceutical grade.

2.2. Methods

2.2.1. Preparation of the inner core matrix tablets

A high shear mixer was used to prepare tablets by a wet-granulation technique. In our previous work (Elzayat et al., 2016b), the polymer concentration and compression force were optimized using a two factor, three level (3^2) full factorial design to obtain tablets matching the release profile of Voltaren® SR 75 mg and at the same time matching *USP* acceptance criteria for extended release diclofenac tablets. Briefly, each tablet of the optimized formulation (designated as Formulation E) contains 75 mg of DS, 52.5 mg of a 1:1 w/w blend of Eudragit RL and Eudragit RS, 3.5 mg of magnesium stearate, 1.75 mg of colloidal silicon dioxide and a sufficient quantity of a 70:30 w/w filler blend consisting of a mixture of lactose monohydrate and MCC to achieve a final tablet weight of 350 mg. Each 600 g of the powder blend specified in the experimental design was sieved through a 0.63 mm mesh screen and then mixed in the high shear mixer (chopper speed = 700 rpm, impeller speed = 200 rpm) for 20 min. A 20% w/v ethanolic solution of a 1:1 w/w mixture of Eudragit RL and Eudragit RS was used for granulation of the powder blend in the high shear mixer with the chopper speed at 2500 rpm, the impeller speed at 200 rpm, the spray rate at 46 ml/min, and the wet massing time set at 5 min to obtain the desired mass consistency. The amount of the polymer in the granulating solution was subtracted from the total amount of the polymer assigned for the formulation. The wetted mass was sieved through a 1.25 mm aperture mesh. Drying of the granules was carried out in a hot air oven (Memmert, Tv80ul, Germany) for 1 h at 40 °C. The percent of the residual solvent, 2–3% was calculated using an infrared dryer (Mettler, LP16 Greifensee, Switzerland). The dried granules were passed through a 0.8 mm mesh screen. A mixture of 1% w/w magnesium stearate and 0.5% w/w colloidal silicon dioxide was added and mixed in the high shear mixer (chopper speed = 400 rpm, impeller speed = 200 rpm for 5 min). The tablets were com-

pressed in RoTab T tablet press (Kg-Pharma, Germany) using standard concave punches of 10 mm diameter at 15 kN.

2.2.2. Drug layering and coating of the prepared matrix tablets

Layering of tablets with a layer of DP was performed using a lab scale perforated pan mini coater (Glatt, Pratteln, Switzerland). Each of the 450 g batches of tablets was loaded into the coating pan. The drug layering suspension consisted of 10% w/w solid content of DP/Opadry II 3:1 in distilled water. The suspension was sprayed using the following conditions: air volume 25 m^3, inlet temperature 78 °C, outlet temperature 43 °C, spray rate 4 g/min, pump speed 25 rpm, atomization air 1.5 bar, air pattern 1.5 bar, pan speed 20 rpm and drying time 15 min. The spraying process continued until achieving a target tablet mass of approximately 383.3 mg, equivalent to a drug load of 25 mg DP. An extra outer protective film using a 20% w/w suspension of Opadry II was applied to add 5% w/w of the total tablet weight to each tablet to act as a taste masking and moisture protection layer. The coating conditions were the same as for the drug layering process except that the inlet temperature was 60 °C and the outlet temperature was 45 °C.

2.2.3. Assay for drug content

Drug content uniformity in the tablets was evaluated in accordance with USP guidelines. Ten tablets were taken individually, weighed, and crushed, and then the drug was extracted with methanol. The solution was filtered, and samples were injected into an ACQUITY™ UPLC system (Waters Corp., Milford, MA, USA). Briefly, separation was accomplished using reversed-phase isocratic elution using a 50:50 v/v mixture of 0.05 M acetate buffer (pH 2.5) and acetonitrile as the mobile phase at 0.5 ml min^{-1} and an injection volume of 1 µl. The photodiode array detector was set to acquire two-dimensional data from 210 to 280 nm; the second channel was recording at 254 nm. The column temperature was set at 50 °C while sample temperature was kept at 10 °C. This method was also used to measure DS content in DS-containing samples as well as dissolution samples (Elzayat et al., in press).

To determine the amount of DP alone, analysis for the amount of potassium present was carried out by flame photometry against a calibration curve of potassium chloride in aqueous solution. The exact amount of DP was back calculated from the flame photometry results.

2.2.4. In-vitro release studies

A USP Type II dissolution apparatus (LOGAN Instrument Corp., Somerset, NJ), was used to conduct the release studies. The dissolution medium was 900 ml 0.05 M phosphate buffer (pH = 7.5) kept at 37 °C for 24 h with paddle rotating at 50 rpm. Samples of 5 ml each were collected at predetermined time intervals and replaced immediately with freshly prepared dissolution medium. The collected samples were filtered and analyzed by injection into the UPLC (see Section 2.2.3). Release studies were conducted in triplicate and the average value of the cumulative percent of drug release was plotted against time. The release profiles for the matrix tablets before coating were compared to those of Voltaren® 75 mg. After drug layering and coating of these tablets, the release profiles obtained for these tablets were compared to Voltaren® 75 mg and Cataflam® 25 mg when these two commercial products were placed together in one dissolution vessel.

2.2.5. Modeling of the in-vitro release data

The mechanism of drug release from formulations was elucidated by fitting the data to zero and first order, Higuchi and Korsmeyer-Peppas models (Elzayat et al., 2016a).

The release efficiency (RE) was calculated from the fractional area under the curve at each time t, as determined using the trapezoidal rule (Khan, 1975). The mean dissolution times (MDT) were calculated according to the following equation (Costa and Sousa Lobo, 2001):

$$\mathrm{MDT} = \frac{\sum_{j=1}^{n} \hat{t}_j \Delta M_j}{\sum_{j=1}^{n} \cdot \Delta M_j} \tag{1}$$

where j refers to the number of the sample, n refers to the number of release time points, $\hat{t}_j$ refers to the time midway between $\hat{t}_{j-1}$ and $\hat{t}_j$, and ΔM_j refers to the extra amount of drug released between $\hat{t}_{j-1}$ and $\hat{t}_j$.

The RE and the MDT values were analyzed by one-way analysis of variance (ANOVA) using SPSS 18 software® to compare their mean values. Fisher's least significant difference procedure was used to test for significance and the level of confidence was set at 95%.

The similarity factor among formulations, f_2, was determined (Moore and Flanner, 1996):

$$f_2 = 50 \times \log\left(\frac{1}{\sqrt{\left[1 + \left(\frac{1}{n}\right)\sum_{t=1}^{n}(R_t - T_t)^2\right]}} \times 100\right) \tag{2}$$

where n refers to the number of samples, R refers to the drug release from the reference material at time t, and T refers to the drug release from the test sample at time t.

2.2.6. Bioavailability study

2.2.6.1. Administration program and blood sampling. The administration of the prepared matrix tablets and the marketed product to six dogs was completed according to a two way crossover design with a one-week washout period between each treatment phase. Dogs having weight of 12 kg, age of 12–14 months and sex of 4 females and 2 males, were divided randomly into two groups and allocated to one of the two treatments according to the study design shown in Table 1. Each dog received the same water and meal prior to treatment. A single oral dose equivalent to a 100 mg diclofenac dose in one of the formulations was administered after overnight fast-

Table 1 Study design of the crossover bioequivalence study.

Sequence	Dogs	Treatment	
		Period 1	Period 2
1	1, 3, 5	T[a]	R[b]
2	2, 4, 6	R	T

[a] Formulation E*.
[b] Voltaren® 75 mg SR, Batch number: T1339, and Cataflam® 25 mg IR, Batch number: Y0086, manufactured by Novartis Pharma AG, Switzerland.

ing for at least 10 h with free access to water. Multiple venous blood samples for analysis of diclofenac concentrations in plasma were collected prior to drug administration (0 h) and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h post-dose. The volume of blood taken for determination of diclofenac in plasma was 5 ml. The blood samples were placed in heparinized tubes, slightly shaken and centrifuged at 3000 rpm for 10 min. The plasma was then decanted and placed in coded polypropylene tubes and then stored at −70 °C until drug analysis. The protocol of the study for animal experiments was approved by the Institutional Animals Ethical Committee of the Department.

2.2.6.2. Plasma sample preparation and drug analysis. One milliliter of the plasma sample was spiked with 100 µl of ibuprofen (internal standard: 0.5 mg/ml dissolved in methanol). The sample was vortexed for 30 s, made acidic with 250 µl 0.15 M phosphoric acid, vortexed for another 30 s and extracted with 5 ml diethyl ether with vortexing for 1 min. The extract was centrifuged at 3000 rpm for 5 min, and then 3 ml of the supernatant was decanted to a glass tube. The separated quantity was evaporated to dryness at 50 °C and the remaining residue was dissolved with 1 ml of mobile phase and vortexed for 1 min. After centrifugation at 15,000 rpm for 2 min, ten microliters was injected into the UPLC–UV system for analysis.

The peak-area ratio of the drug to the peak area of the internal standard estimated by Masslynx Version 4.1 software (Waters, USA) allowed quantification of the DS present. The relationship between concentration and peak area ratio was linear, with an r^2 coefficient of not less than 0.99 within the range 0.1–30 µg/ml for diclofenac. The lower limit of quantification (LLOQ) for diclofenac from 1 ml of plasma was 0.5 µg/ml. Intra- and inter day accuracy of the method ranged from 97.04% to 107.8% and from 99.2% to 114.7% respectively, while the intra- and inter-day precision ranged from 0.29% to 9.3% and from 3.1% to 9.5%, respectively, for concentrations 0.5, 1, 8, 15 and 30 µg/ml. The average absolute analytical recovery of diclofenac ranged from 93.69% to 107.2%, and of the internal standard (ibuprofen) was 96.31%.

2.2.6.3. Pharmacokinetic analysis. WinNonlin software (Version 4.1, Pharsight Corp., Palo Alto, CA, USA) was used to determine the pharmacokinetic parameters. Non-compartmental models (Yamaoka et al., 1978), were employed for the evaluation of these parameters. The terminal log-linear portion of the concentration–time curves in plasma was analyzed by least squares regression analysis to determine the elimination rate constant and the half-life of the drug. The trapezoidal method was employed to determine the area under the concentration–time curve in plasma from zero time to the last quantifiable concentration (AUC_{0-t}) and to infinity ($AUC_{0-\infty}$). The ratio of $C_{max}/AUC_{0-\infty}$ was used as an estimate of the absorption rate constant (Endrenyi et al., 1991). The area under the moment curves from zero time to the last quantifiable concentration ($AUMC_{0-t}$) and to infinity ($AUMC_{0-\infty}$) was also calculated by the trapezoidal method. The following equation was used to estimate the mean residence time (MRT):

$$MRT = \frac{AUMC_{0-\infty}}{AUC_{0-\infty}} \quad (3)$$

The apparent volume of distribution at steady state (V_{ss}) was estimated using the following relationship (Perrier and Mayersohn, 1982):

$$V_{ss}/F = \frac{D \cdot AUMC_{0-\infty}}{(AUC_{0-\infty})^2} \quad (4)$$

where F refers to the drug bioavailability of the drug and D refers to the dose administered. In addition, the oral clearance of the drug was calculated by the following equation:

$$CL/F = \frac{D}{AUC_{0-\infty}} \quad (5)$$

2.2.6.4. Statistical analysis. The Statistical Analysis System software package (SAS Institute, Cary, NC, USA) was employed. The Student's *t*-test for independent samples was employed to compare the calculated pharmacokinetic parameters between test and reference assuming homoscedasticity. It is well known that the time to peak plasma concentration (T_{max}) data do not follow a Gaussian distribution; therefore, the nonparametric Mann–Whitney test was used. The results were expressed as mean ± SD and comparisons were carried out at the 0.05 level of significance.

2.2.7. Effect of storage on physical properties and drug release from the tablets

The multi-layered tablet that demonstrated bioequivalence to the marketed product was assessed for the effect of long term and accelerated storage conditions on the physical characters and release profile, according to International Conference on Harmonization Guidelines (ICH, 2003). The tablets were stored in closed containers in stability cabinet (Binder Gmbh, Tuttlinger, Germany) at 25 ± 2 °C and 60 ± 5% relative humidity (RH) for 12 months and 40 ± 2 °C and 75 ± 5% (RH) for 6 months.

3. Results

3.1. Evaluation of the prepared tablets

The characteristics of the core matrix, drug layered and final coated tablets are listed in Table 2. The core tablets displayed acceptable weight variation, content uniformity and friability. Tablets were assessed after drug layering for the coating efficiency by determining the amount of diclofenac potassium loaded on each tablet. The flame photometry analysis showed an average DP content of 100.5 ± 3.54%. After the application of the final film coat of Opadry II, the average tablet weight was found to be 400 ± 3.72 mg, hardness 182.1 ± 6.33 N, thickness 4.83 ± 0.02 mm and overall diclofenac content 96.25 ± 1.64%.

3.2. In-vitro drug release

Fig. 1 shows that the release profile of formulation *E* (core matrix) and Voltaren® 75 mg are similar to each other with f_2 value of 79.2. The release efficiency (RE) and mean dissolution time (MDT) values were comparable where the RE was 80.15% and 83.5% and the MDT was 4.81 h and

Table 2 Physical characterization of biphasic release matrix tablets of diclofenac in the three manufacturing stages (before coat, drug layered and film coated).

Formulations	Formulation E (before coat[a])	DP layered[b]	Formulation E* (after final film coat)
Drug content (%) (RSD)	94.91 (1.86)	96.25 (1.7)	96.25 (1.7)
Tablet mass (mg) ± SD	351 ± 3.21	385 ± 4.32	400 ± 3.72
Hardness (N) ± SD	105.4 ± 5.17	129.3 ± 13.65	182.1 ± 6.33
Thickness (mm) ± SD	4.46 ± 0.02	4.78 ± 0.02	4.83 ± 0.02
Friability[c] (% loss) ± SD	0.1 ± 0.03	0.02 ± 0.01	0.02 ± 0.01

[a] Core matrix tablets before drug layering with DP.
[b] Content calculated based on total diclofenac content.
[c] Initial mass of 6.5 g.

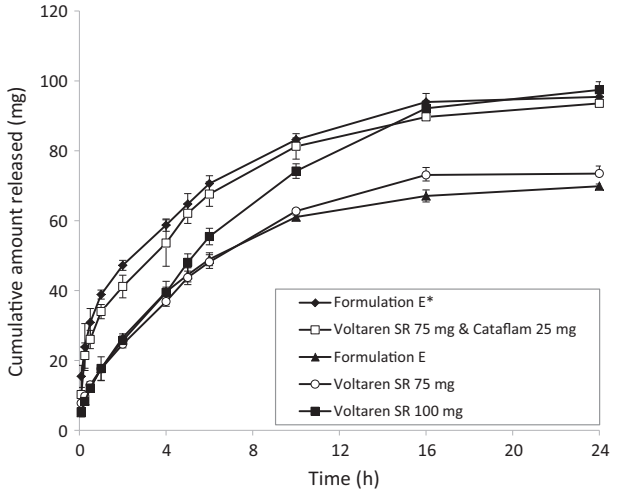

Figure 1 Comparative release profiles of diclofenac from different formulations at pH 7.5: Voltaren® 75 mg; Formulation E (before coat); Voltaren® 100 mg; Voltaren® 75 mg & Cataflam® 25 mg; Formulation E*. Each data point represents the average of a triplicate study.

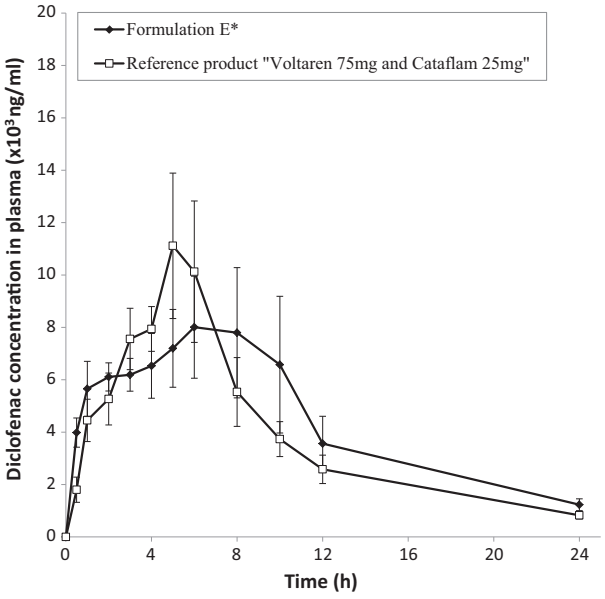

Figure 2 Mean diclofenac plasma profiles from a single dose bioavailability study compared with the reference product (n = 6). Voltaren® SR 75 mg & Cataflam® 25 mg; Formulation E*. Each point represents the mean ± S.E.

4.91 h for formulation E and Voltaren® 75 mg; respectively. Formulation E* (after DP layering and coating) showed an increased amount of drug released at each time point compared with previous formulations due to the additional drug load. This was also the case when monitoring the simultaneous release from the two tablets Voltaren® 75 mg and Cataflam® 25 mg as the reference product. The release efficiency values were comparable 86.38% vs 82.72% and the mean dissolution times were 4.05 h vs 4.52 h for formulation E* and the reference, respectively.

3.3. Pharmacokinetic study

The plasma concentration–time curves for the two formulations, formulation E* and the reference (Voltaren® 75 mg & Cataflam® 25 mg) are shown in Fig. 2. Table 3 lists the pharmacokinetic parameters of diclofenac in both formulations. No significant difference between any of the mean values of the pharmacokinetic parameters calculated for the treatments was found at a 95% confidence interval (CI). The C_{max} values were 11.29 ± 6.87 and 12.97 ± 8.45 μg/L and the $AUC_{0\text{-}24}$ values were 105.36 ± 83.3 and 92.87 ± 55.53 μg h/L for formulation E* and the reference, respectively. This indicates that

Table 3 Pharmacokinetic parameters (mean ± SD) for formulation E* and the reference (Voltaren® 75 mg and Cataflam® 25 mg) in 6 dogs.

	Formulation E*	Reference	p-value
k_{el} (h^{-1})	0.121 ± 0.05	0.12 ± 0.05	0.95
$t_{1/2}$ (h)	6.63 ± 2.69	7.46 ± 5.34	0.74
T_{max} (h)	4.83 ± 3.76	4.33 ± 0.82	0.94
C_{max} (μg/L)	11.29 ± 6.87	12.97 ± 8.45	0.71
C_{last}/C_{max}	0.13 ± 0.1	0.1 ± 0.12	0.65
$AUC_{0\text{-}t}$ (μg h/L)	105.36 ± 83.3	92.87 ± 55.53	0.77
$AUC_{0\text{-}\infty}$ (μg h/L)	118.12 ± 85.8	104.05 ± 52.05	0.74
AUC ratio	0.88 ± 0.1	0.88 ± 0.19	0.95
$C_{max}/AUC_{0\text{-}\infty}$ (h^{-1})	0.124 ± 0.1	0.127 ± 0.05	0.91
$AUMC_{0\text{-}\infty}$ (μg h^2/L)	1357 ± 960	1199 ± 809	0.77
MRT (h)	11.19 ± 4.12	12.12 ± 8.93	0.81
V_{ss}/F (L/kg)	0.89 ± 0.54	1.08 ± 0.94	0.67
CL/F (L kg^{-1} h^{-1})	0.096 ± 0.06	0.094 ± 0.04	0.94

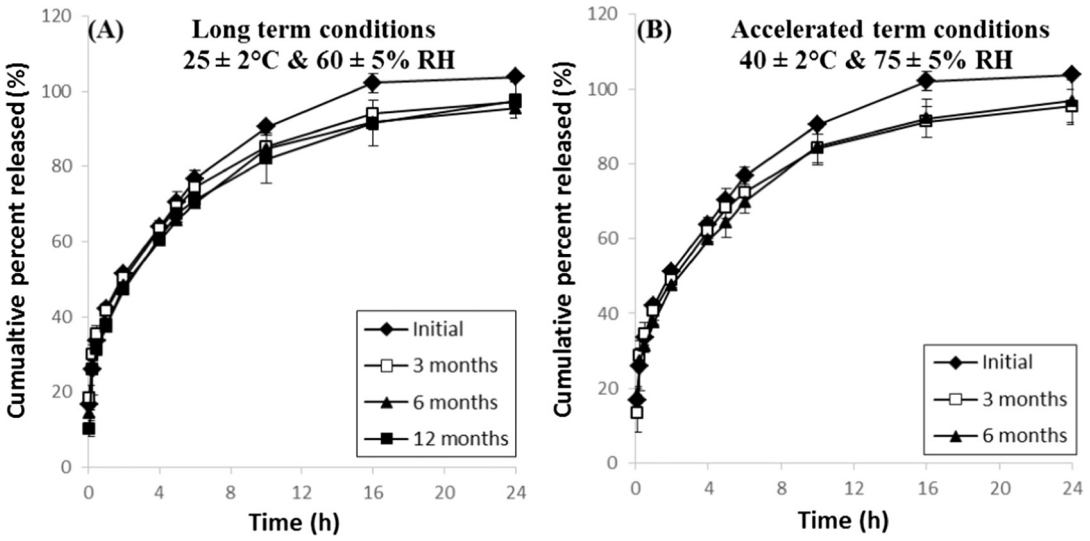

Figure 3 Release profiles after stability studies in long term (A) and accelerated (B) conditions for formulation E* according to ICH guidelines.

the rate and extent of absorption were comparable between these two formulations. These results are in agreement with the observed in-vitro release data.

3.4. Stability study

The moisture content as well as the rate of moisture uptake is affected by humidity and temperature and should be considered. Fig. 3 shows the release profiles for the formulation E* after storage under long term and accelerated conditions. Table 4 shows the physical characteristics of formulation E* and the release profile similarity factor f_2 after exposure to the long term and accelerated conditions as compared to the initial release profile. There was no significant increase in tablet mass or hardness. The f_2 values for drug release, after long term and accelerated conditions, ranged from 62 to 71 suggesting similarity to the initial release results.

4. Discussion

4.1. In-vitro drug release

Both formulation E* tablets and the commercially available formulations (Voltaren® SR 75 mg and Cataflam® 25 mg) exhibited higher initial concentrations when compared to results of formulation E or Voltaren® 75 mg alone due to the rapid dissolution of the immediate release layer of DP from formulation E* or the contribution of the rapid disintegration followed by dissolution of diclofenac by the Cataflam® 25 mg tablet. The remainder of the drug is released over a 16 h period; however, the core matrix did not lose its integrity even after 24 h. The drug release from formulation E* reached 42% in 1 h, which suggests that the pharmacologic effect can be attained rapidly. Analysis of drug release data revealed that release from formulation E* or the reference material occurred by diffusion (Higuchi model fit with $r^2 = 0.99$) where the release of diclofenac was dependent on the square root of time. The Korsmeyer-Peppas exponent (n) was 0.34 and 0.405 for formulation E* and the reference, respectively after fitting the dissolution profiles into the exponential equations. It can be inferred from $n < 0.45$ that diffusion is the dominating mechanism in the release process from these matrix type formulations (Su et al., 2003). After exposure of the inner matrix to the aqueous medium, solvent penetrates into the voids between macromolecular chains of Eudragit RL more easily allowing solvation of the polymer chains. This might suggest polymer relaxation as a result of increased polymer dimensions due to the hydrostatic pressure build up by the entered solvent (Apu et al., 2009). The similarity factor (f_2) value was found to

Table 4 Effect of long term and accelerated storage conditions on the physical properties of formulation E* (mean ± SD) and the similarity factor f_2.

Stability status		Drug content (%)	Tablet weight (mg) ± SD	Hardness (N) ± SD	Thickness (mm) ± SD	Friability[a] (% loss)	f_2
Initial		96.3 (1.7)[b]	400 ± 3.7	182 ± 6.3	4.83 ± 0.02	0.02	N/A
Long term	3 M	96.4 (1.64)	402 ± 3.2	166 ± 7.2	4.87 ± 0.02	0.02	71.74
	6 M	96.5 (1.64)	400 ± 4.7	167 ± 6.6	4.87 ± 0.02	0.03	64.14
	12 M	96.8 (1.37)	406 ± 3.6	189 ± 4.9	4.86 ± 0.03	0.01	62.02
Accelerated conditions	3 M	96.5 (1.2)	408 ± 4.3	194 ± 6.6	4.92 ± 0.01	0.01	66.2
	6 M	96.4 (1.21)	408 ± 4.5	192 ± 5.8	4.92 ± 0.01	0.01	63.31

[a] Amount of tablets weighed equivalent to 6.5 g.
[b] Values in parentheses are the RSD of the mean.

be 66.19 indicating comparable release profiles for diclofenac from the two formulations.

4.2. Pharmacokinetic study

The bioavailability study presents strong evidence for bioequivalence between the test and comparator formulations. Being a highly permeable drug, diclofenac is found to be absorbed throughout the gastrointestinal tract (Gleiter et al., 1985). After dosing of the two formulations, the plasma concentration of diclofenac was easily detectable in the blood after 30 min from each of the formulations. This corresponded well with the observed pulse effect of drug release that occurred due to the fast dissolution of DP layer in formulation E* and the rapid drug release of DP from Cataflam® 25 mg. The in-vivo profiles can be explained on the basis of the three main stages. The first stage takes place from 0 to 2 h where the extent and rate of absorption of formulation E* were higher than those of the reference. This can be attributed to the rapid dissolution of the immediate release coat containing DP. On the other hand, the Cataflam® 25 mg tablet tends to disintegrate first and then dissolution begins, and this decreased the absorption rate relative to formulation E*. The second stage is from 2 to 7 h at which point the drug reached the small intestine which has a high effective surface area that facilitates absorption. Even though diclofenac solubility is higher, with greater extent of absorption, in the small intestine than in the stomach, the absorption rate in case of formulation E* is controlled by the core matrix which, according to Fig. 1, releases drug at a slower rate than did the outermost coat with its DP. And that is what is reflected in the 2–7 h time frame in Fig. 2. Although the release from the core of Formulation E* is slightly slower than observed with the rate provided by the reference combination of the commercial products, there is still no statistical difference between the two formulations at each point of the drug release during this stage which may be attributed to the wide scatter in the reference data. In the third stage from 8 to 24 h, a higher level of the drug in the blood was detected from formulation E* than the reference combination where the drug in formulation E* continued to be released from the tablets. Formulation E*, hence, keeps an elevated diclofenac concentration in the plasma for a longer time which is good for the patient.

4.3. Stability study

The drug release and the physical characteristics of the tablets remained unchanged after storage for 1 year under long term conditions and 6 months at accelerated conditions suggesting that DS is stable in the multi-layered tablets. Noticeably, there was an insignificant decrease in the drug release after 10 h in both long and accelerated conditions. This effect can be linked with the slight increase in hardness of the tablets. The long exposure of the tablets to elevated humidity/temperature might promote fusion of the polymer particles due to increased mobility with possible redistribution of the pores within the matrix system (Grund et al., 2014). This resulted in a small retardation in the drug release in accordance with similar findings that have been reported previously (Grund et al., 2014). However, the overall assessment of the release profile indicates stability of the tablets according to ICH guidelines.

5. Conclusion

Modification of Eudragit-based matrix systems made it possible to achieve biphasic drug release patterns. A first pulse was obtained by incorporation of a percentage of the drug in an outer immediate release layer applied onto a release controlling matrix which would provide the sustained pharmacologic effect. The clinical significance of such formulations would be profound with patients suffering from acute or long lasting pains. Based on its in-vitro, in-vivo and stability profiles of formulation E*, it is a system that can accomplish both immediate and sustained release of diclofenac for extended pain relief.

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