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Research Article

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Preparation and *In Vitro*/*In Vivo* Evaluation of Microparticle Formulations Containing Meloxicam

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Abstract. In this study, we have formulated chitosan-coated sodium alginate microparticles containing meloxicam (MLX) and aimed to investigate the correlation between *in vitro* release and *in vivo* absorbed percentages of meloxicam. The microparticle formulations were prepared by orifice ionic gelation method with two different sodium alginate concentrations, as 1% and 2% (*w*/*v*), in order to provide different release rates. Additionally, an oral solution containing 15 mg of meloxicam was administered as the reference solution for evaluation of *in vitro*/*in vivo* correlation (*ivivc*). Following *in vitro* characterization, plasma levels of MLX and pharmacokinetic parameters [elimination half-life ($t_{1/2}$), maximum plasma concentration (C_{max}), time for C_{max} (t_{max})] after oral administration to New Zealand rabbits were determined. Area under plasma concentration–time curve ($AUC_{0-\infty}$) was calculated by using trapezoidal method. A linear regression was investigated between released% (*in vitro*) and absorbed% (*in vivo*) with a model-independent deconvolution approach. As a result, increase in sodium alginate content lengthened *in vitro* release time and *in vivo* t_{max} value. In addition, for *ivivc*, linear regression equations with r^2 values of 0.8563 and 0.9402 were obtained for microparticles containing 1% and 2% (*w*/*v*) sodium alginate, respectively. Lower prediction error for 2% sodium alginate formulations (7.419±4.068) compared to 1% sodium alginate formulations (9.458±5.106) indicated a more precise *ivivc* for 2% sodium alginate formulation.

KEY WORDS: chitosan; *in vitro*/*in vivo* correlation; meloxicam; microparticle; sodium alginate.

INTRODUCTION

Meloxicam (MLX) is a member of non-steroidal anti-inflammatory drugs with its analgesic and antipyretic effects and commonly used for the treatment of rheumatoid arthritis and osteoarthritis. MLX is practically insoluble in water (12 μg/ml), but on the other hand, its solubility increases with the higher pH value of the medium (1–3). In this study, MLX was used for the investigation of suitability of Biopharmaceutics Classification System (BCS) class II drugs in *in vitro*/*in vivo* correlation (*ivivc*).

Microparticles containing MLX were prepared for oral administration. One of the most common polymers used in preparation of microparticle formulations is the chitosan, a natural polysaccharide, which is manufactured by the deacetylation process of the naturally existing chitin in the outer shells of the crustaceans (4). The solution–gel transition of the chitosan is between pH values of 6.5 and 7.0, which makes it a favorite excipient especially for dosage forms for oral drug delivery (5). In addition, non-toxic and biodegradability properties of chitosan make it popular for many formulation studies (4, 6). In this study, sodium alginate was used in combination with chitosan in order to modify the release rate of meloxicam from microparticle formulations. Sodium alginate has polyelectrolyte properties which make it suitable for cross-linking during formulation studies.

Various definitions for *ivivc* have been proposed in literature. The basic definition for *ivivc* is "a predictive mathematical model describing the relationship between an *in-vitro* property of an extended release dosage form (usually the rate or extent of drug dissolution or release) and a relevant *in-vivo* response, *e.g.*, plasma drug concentration or amount of drug absorbed" (7). The most significant property of an *ivivc* model is its ability to predict the *in vivo* performance of the drug depending on *in vitro* dissolution properties. The major aim in developing an *ivivc* for an extended release pharmaceutical product is proving a waiver for *in vivo* bioequivalence during the initial approval process or because of some certain pre- and/or post-approval changes such as formulation equipment, process, and site of manufacture (7).

Many investigators have previously attempted to develop such *ivivc* model using different types of formulations and active ingredients (8–11). There is no *in vitro* model that perfectly mimics the gastrointestinal conditions; thus, the dissolution tests are often determined case by case (12).

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There are many studies demonstrating *ivivc* for extended release dosage forms (9, 13–15).

Regardless of the long plasma half-life ($t_{1/2}$) of MLX, which makes it unfeasible for a controlled release dosage form, the reason for choosing MLX as the model drug was because of its low-solubility and high-permeability properties. As stated before, these properties make it a member of class II drug according to BCS. In this study, one of the goals was to formulate controlled release microparticular dosage forms that would maintain a steady plasma concentration for MLX. In addition, among the BCS class I to IV drugs, the most possible *ivivc* models can be established in class II drugs. The rate-limiting step for the absorption is the solubility of the BCS class II drug molecule in the gastrointestinal tract after oral administration. Therefore, the secondary goal of this study was to model an *ivivc* for meloxicam modified release microparticle formulations containing chitosan and sodium alginate as the polymer combination. Hence, by preparing two different formulations with different release characteristics, the *ivivc* was investigated in this study.

MATERIALS AND METHODS

Materials

The model drug MLX was from Dr. Reddy's Laboratories (Mumbai, India). The polymers used in the preparation of microparticle formulations were purchased from Sigma Aldrich as chitosan (*medium molecular weight*) and sodium alginate (*medium molecular weight*) (Milwaukee, WI, USA). As the hardening agent, calcium chloride anhydride (Milan, Italy) was used in the preparation of microparticle formulations.

Preparation of Microparticle Formulations

Microparticle formulations were prepared by using the orifice ionic gelation method (16). Chitosan and sodium alginate were used as the matrix material. Two different types of microparticle formulations that have the ability to maintain different release profiles (*as fast and slow release*) were formulated. Briefly, 250 mg of MLX was homogeneously dispersed in the solution of sodium alginate at concentrations varying from 1% (*w*/*v*) (*fast-release formulation A*) to 2% (*w*/*v*) (*slow-release formulation B*) in water with Ultra Turrax T-25 homogenizer. In another container, the hardening agent calcium chloride anhydride (2.60 g) was dissolved in 1,000 ml of water and mixed with 25 ml of previously prepared chitosan solution (1%, *w*/*v*) in diluted acetic acid (1%, *v*/*v*). MLX dispersion in sodium alginate solution was added dropwise as 30 droplets per minute into the solution containing calcium chloride anhydride and chitosan. The resulting droplets were mixed for 12 h over a magnetic stirrer at 250 rpm, and afterward, resulting microparticles were filtered. They were washed with deionized water and dried at 30°C until they reach a constant weight.

Preparation Efficiency

The preparation efficiencies of the microparticle formulations were determined by calculating the ratio of the weight of the dried microparticles to the sum of polymer and MLX amounts. The batches were prepared over six replicates, and the resulting dried microparticles were collected in a single container.

Surface Methodology

The surface characteristics of the microparticle formulations were investigated with scanning electron microscopy (SEM). For this purpose, the microparticles were mounted on metal stubs and then sputtered with a 150-Å-thick layer of gold in a BIORAD apparatus. The SEM investigations were further evaluated with JSM 5600 device in 20 kV.

Mean Particle Size Distribution

The mean particle size for each formulation containing MLX was determined by the standard sieving method by using Endecotts sieves having mesh aperture size in a range of 125–810 μm. The amount of microparticles on each single sieve has been accurately measured, and the mean particle size was calculated over six replicates.

MLX Assay

In vitro quantification of MLX was carried out by using HP Agilent 1100 high-performance liquid chromatography (HPLC) equipped with an UV detector. In a previous study, Dülger *et al.* have developed a HPLC method for the determination of MLX from pharmaceutical formulations (17). Dülger *et al.* have used 50 mM phosphate buffer–acetonitrile–methanol mixture at the ratio of 50:15:35 (*v*/*v*/*v*) as the mobile phase, and the column used for separation was C_{18} reverse-phase column. The retention time for MLX was recorded as 11.1 min. In order to shorten the retention time of MLX, we have made some modifications in the mobile phase ratio. By keeping the components constant, the ratio was shifted to 50:25:25 (*v*/*v*/*v*). The injection volume and flow rate of the mobile phase were set as 15 μl and 1 ml/min, respectively. The UV detection of the samples was recorded at wavelength (λ) of 363.4 nm. The column used for separation was ACE C_{18} reverse-phase column with dimensions of 150×4.6 mm (length×internal diameter).

For *in vivo* quantification of MLX from plasma a validated HPLC method, developed by Velpandian *et al.*, has been used (18). For this purpose, tenoxicam (TNX) was used as the internal standard. We have added 75 μl of TNX solution (200 μg/ml) into 200 μl of plasma sample and vortexed for 15 s. After addition of 200 μl 1 M HCl solution for the precipitation of proteins, the solution was re-vortexed for 30 s. It was further extracted with 2 ml of chloroform for 3 min, and the samples were centrifuged at 4,500 rpm for 15 min. An aliquot (1 ml) of organic phase was transferred into a clear tube and evaporated under nitrogen gas. The residue was reconstructed with the mobile phase (100 μl), and 15 μl of this final solution was analyzed for quantification of MLX and TNX. The chromatographic conditions were set as follows: UV detection at wavelength (λ) of 363.4 nm, Nucleosil C_{18} reverse-phase column (length×internal diameter), mobile phase 50 mM diammonium hydrogen phosphate/methanol/acetonitrile (50:40:10 *v*/*v*/*v*), and flow rate 1 ml/min at ambient temperature.

Encapsulation Efficiency

Microparticles containing exactly 15 mg of MLX (theoretically) have been accurately weighed and transferred into 25 ml of HCl solution (0.1 N) in a volumetric flask (50 ml). They were mixed for 2 h and afterwards 8 ml of tribasic sodium phosphate was added. This solution was mixed for 4 h and sufficient amount of methanol was added up to 50 ml. The final solution was again mixed for 2 h over a magnetic stirrer. An aliquot of sample (5 ml) was withdrawn from the resulting solution and filtered through 0.45 μm polytetrafluoroethylene (PTFE) syringe filter. Finally, the MLX content was analyzed with the *in vitro* MLX assay method described in the previous section. The encapsulation efficiency was determined using triplicate determinations, and results were expressed with standard error (SE) of means.

In Vitro Release Studies

In vitro release tests were carried out with Sotax A7 Smart Dissolution Tester device by using the USP, Apparatus I. In order to simulate pH of the gastrointestinal conditions, microparticles containing exactly 15 mg of MLX were placed in the basket of Apparatus. The test was initialized in 750 ml of 0.1 N HCl solution (pH=1.2) and continued for 2 h at 75 rpm. After this period, 250 ml of 0.2 M tribasic sodium phosphate solution, which has been previously conditioned at 37±0.5°C, was added in order to adjust the pH of the medium to 6.8. At certain time intervals starting from the beginning of the experiment, samples of 5 ml were withdrawn and immediately replaced with fresh medium. The samples were filtered through 0.45-μm PTFE syringe filters, and the amount of MLX was quantified with the HPLC method, as previously described. *In vitro* data analysis of release profiles for two different microparticle formulations were compared with each other by using model-independent approach by calculating f_1 and f_2 values (19, 20).

In Vivo Studies

Six New Zealand rabbits weighing 2.5–3.5 kg were used in this study in accordance with an ethics protocol approved by the Hacettepe University Local Ethics Committee for Experimental Animals (*Protocol Number: 2006/40-5*). All animals were subjected standard housing conditions with the room temperature of 25±2°C, 55±10% relative humidity, and the lightening of the room was set as 12:12 h light/dark cycle.

Drug Administration and Blood Sampling

A single-dose pharmacokinetic study was conducted in New Zealand rabbits. The study was designed as open-label, three-way crossover study with a washout period of 10 days using formulations: oral solution (*reference*) (meloxicam solution in water containing meglumine as the pH-adjusting and solubilizing agent) and formulations A and B (*fast and slow release*, respectively). Animals were randomly assigned in equal numbers to three sequences of formulations so that they would receive all three formulations upon the completion of the study. Three sequences of the experiments consisted of the administration of (1) reference oral solution, (2) fast-release *formulation A*, and (3) slow-release *formulation B*. Following the administration of formulations containing 15 mg MLX to New Zealand rabbits by oral gavage, 10 ml of water was administered by the same route after each dose; 0.5 ml of blood samples was collected from the marginal ear vein into heparinized tubes prior to dosing (0 h) and at 1, 2, 3, 4, 5, 6, 8, 12, 16, 24, 36, 48, 60, and 72 h after dosing in each period. The samples were kept at −20°C until analysis.

In Vivo Data Analysis

The MLX concentration–time data were evaluated by the analysis of plasma samples by validated HPLC method (18). The measured plasma concentrations of MLX were used to calculate the area under the plasma concentration–time curve (AUC) from time zero to the last plasma sampling point (72 h). AUC_{0-72} was determined by the trapezoidal method, and the area under the plasma concentration–time curve from zero to infinity (∞) ($AUC_{0-\infty}$) was calculated by the addition of AUC_{0-72} value to the last log-linear concentration divided by the terminal elimination rate constant (k_e) ratio, which was estimated by fitting the logarithm of the plasma concentration *versus* time to a straight line over the observed exponential decline. The Wagner–Nelson method was used to calculate the percentage of MLX dose absorbed (21):

$$\text{Dose Absorbed } \% = [(C_t + k_e \cdot AUC_{0-t})/k_e \cdot AUC_{0-\infty}]$$

where C_t is the concentration at any time point

In Vitro/In Vivo Correlation

The data obtained from the bioavailability study with New Zealand rabbits were used in the evaluation of *ivivc*. The possible correlation was investigated between the percent of dissolved MLX from formulations using the appropriate dissolution method and the fraction of MLX absorbed by the Wagner–Nelson method. The deconvolution procedure was used to obtain *in vivo* input profiles of MLX using the oral solution as the reference standard. Linear regression analysis was used to investigate the relationship between percent of MLX dissolved and percent of MLX absorbed. The assessment of the predictability and validity of the correlations was evaluated by the comparison of model predicted and observed $AUC_{0-\infty}$ values for each formulation. The percent prediction errors (PE) for $AUC_{0-\infty}$ were calculated by using the following formula (7):

$$PE(AUC_{0-\infty})\% = \left[\left(AUC_{0-\infty(\text{Observed})} - AUC_{0-\infty(\text{Predicted})}\right) / AUC_{0-\infty(\text{Observed})}\right] \times 100$$

The *ivivc* model is assumed to be valid if the prediction error percentage is <10% for AUC and if the prediction error for each formulation does not exceed 15% (22).

RESULTS

Preparation Efficiency

The preparation efficiencies of the microparticle formulations were calculated as 92.64±2.57% and 95.12±1.92% for the formulations prepared by using 1% (*w*/*v*) and 2% (*w*/*v*)

sodium alginate, respectively. The results were expressed as the mean percentages of six different batches with SE.

Surface Methodology

The investigation of the surface morphologies of the microparticle formulations prepared by using sodium alginate at different concentrations showed that the surface structures of these two formulations were similar. The particles neither had perfect circular shape nor smooth regular surface. When the two formulations were compared, microparticles prepared by 2% (*w*/*v*) sodium alginate solution were more spherical in shape and larger in size. In addition, pores on the surface of the microparticles have been investigated for both of the formulations (Fig. 1). The fast release of MLX from microparticles prepared with 1% sodium alginate may also depend on the smaller particle size, which was also confirmed by this surface characterization.

Mean Particle Size Distribution

The mean particle sizes for microparticle formulations prepared with 1% (*w*/*v*) and 2% (*w*/*v*) sodium alginate polymer solutions were determined as 505±1.38 and 740 ±1.39 μm, respectively (mean±geometric standard deviation).

MLX Assay

The linearity of the calibration curve has been established within the concentration range of 1–40 μg/ml for *in vitro* determination of MLX (r^2=0.99), and the retention time for MLX was recorded as 2.62 min. The recovery values for all concentration points (1–8–40 μg/ml) were found between 102.60% and 98.87%. For the specificity parameter, no interfering peak was recorded after the analysis of the dissolution medium. The limit of detection (LOD) and limit of quantitation (LOQ) values were calculated as 0.07 and 0.16 μg/ml, respectively. The calibration range for the *in vivo* HPLC method was between 66.66 and 2,133.33 ng/ml (r^2=0.99). The retention time for determination of MLX from plasma was recorded as 6.11 min. The recovery values of all samples (66.66–1,230.76 and 2,133.33 ng/ml) varied between 99.52% and 102.97%. The MLX peak, having a good symmetry, was well separated from the medium for both *in vitro* and *in vivo* methods. Also, in *in vivo* method, internal standard (TNX 15 μg/ml) was well separated from MLX with retention times of 3.96 and 6.11 min for TNX and MLX, respectively. The LOD and LOQ values for *in vivo* determination of MLX from plasma samples were calculated as 41.37 and 66.66 ng/ml, respectively (23).

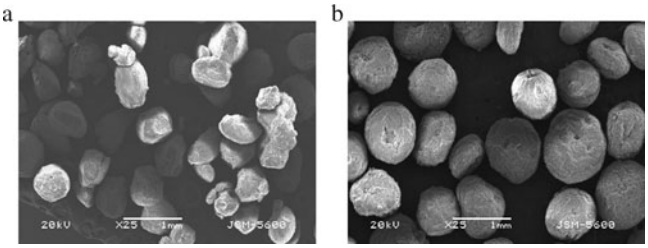

Fig. 1. Scanning electron microscopy images of the microparticle formulations. **a** Microparticles prepared by 1% (*w*/*v*) sodium alginate polymer solution. **b** Microparticles prepared by 2% (*w*/*v*) sodium alginate polymer solution

Encapsulation Efficiency

MLX contents of the microparticle formulations were determined as 99.51±1.38% and 100.83±2.46% for the formulations prepared with sodium alginate polymer solution at the concentrations of 1% (*w*/*v*) and 2% (*w*/*v*), respectively.

In Vitro Release Studies

The *in vitro* release profiles for microparticle formulations were determined over six replicates for each formulation. The release profiles for both formulations clearly indicated a negligible release over the first 2 h with release medium at pH 1.2. On the other hand, as the pH was shifted to 6.8, release of MLX from microparticle formulations was started depending on the increase in the solubility of chitosan coating. After the first 2 h, discriminatory release profiles were observed (3). The increase in sodium alginate polymer concentrations from 1% (*w*/*v*) to 2% (*w*/*v*) governs the release pattern of MLX from microparticle formulations as soon as the pH of the release medium is increased (Fig. 2). As stated in the guideline, the dissolution curves are considered as similar when f_1 values are in the range of 0–15 and f_2 values greater than 50 (50–100) (20). The comparison of the results of these microparticle formulations revealed that these profiles were not similar with the values of f_1=16.67 (>15) and f_2=45.08 (<50).

In Vivo Studies

The plasma profiles after administration of all formulations have been determined, and the pharmacokinetic parameters were calculated (Table I). The t_{max} for the oral solution of MLX was 2.33±1.21 h. Microparticle formulations, as the concentration of sodium alginate used in the preparation was increased, had an increased t_{max} value (9.66±3.88 h and 16.33±12.02 h for *formulations A* and *B*, respectively). On the other hand, C_{max} values for MLX in plasma significantly decreased in microparticle formulations with respect to the oral solution. The total plasma profiles of the New Zealand rabbits after administration of MLX containing formulations are shown in Fig. 3. After the determination of the coefficients for equations defining the plasma curves of the MLX formulations, the absorbed% of MLX was calculated as a function of time. The results for each New Zealand rabbit and the average results are summarized in Table II and Fig. 4. The *in vitro*/*in vivo* modeling results indicated a good correlation between *in vivo* absorbed percentages and *in vitro* released amount of MLX from microparticle formulations with r^2 values of 0.8563 (fast-release *formulation A*) and 0.9402 (slow-release *formulation B*), which show a better correlation for slow-release *formulation B* depending on the r^2 values. The prediction errors were calculated as within the acceptable limits for both formulations (9.458±5.106 and 7.419±4.068 for *formulations A* and *B*, respectively).

DISCUSSION

The analytical method for the quantification of MLX was applied with slight modifications in the previously developed

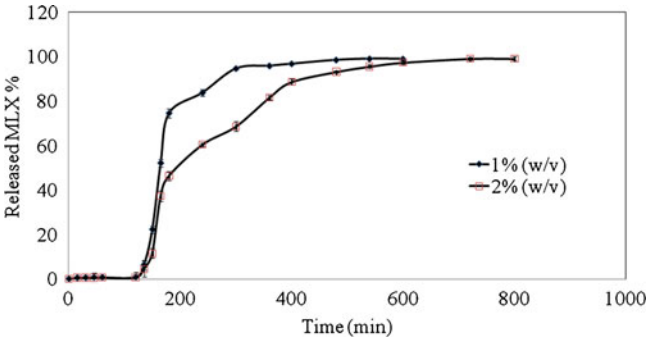

Fig. 2. The release profiles of MLX from microparticle formulations prepared by two different sodium alginate concentrations as 1% (*w/v*) and 2% (*w/v*). No significant release was recorded within the first 2 h; however, as the pH of the release medium is increased to 6.8, release of MLX from both of the microparticle formulations starts to dominate the release profiles. The results at sampling time points are represented over the average values of six replicates with standard error bars

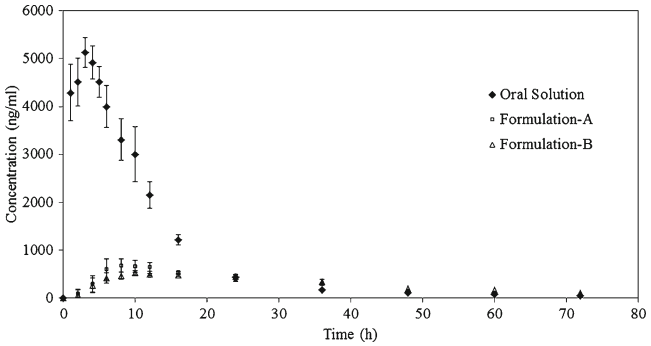

Fig. 3. Plasma profiles for MLX after administration of oral solution, *formulation A* and *formulation B* to New Zealand rabbits

method (17). The reason for this modification was to attenuate the long retention time (11.1 min) in the reference method. For this purpose, the ratio of the mobile phase components was modified while keeping the other parameters constant. The ratio of the mobile phase consisting of 50 mM phosphate buffer/acetonitrile/methanol was fixed as 50:15:35 (*v/v/v*) resulting in the retention time for MLX as 2.62 min, which was a fair good one without any interfering peaks with the other components existing in the method.

Microparticle formulations were prepared by using two naturally derived polymers. The main reasons for this choice were the non-toxic, biocompatible, and biodegradable properties of both chitosan and sodium alginate (6, 24). Also, these polymers have polyelectrolyte structures, which make them good candidates for controlled drug delivery systems. Chitosan has positively charged amino groups, and sodium alginate has negatively charged carboxyl functional groups. The hardening agent used in the preparation of microparticles was selected as calcium chloride anhydride. The positively charged Ca^{+2} ion binds to the α-L-glucuronic structure existing in alginate and creates a network-like structure. However, these network structures are easily broken and the desired release time of the active drug substances may not be achieved. This explains the reason for incorporating chitosan in microparticle formulations. The outer surfaces of the microparticles were coated with cationic chitosan in order to tighten the network structure.

The *in vitro* release test that simulates the intestinal conditions plays a major role for the development of *ivivc* model. The conditions were kept as close as possible in order to reflect the *in vivo* conditions. For this purpose, the release medium for the initial 2 h was 0.1 N HCl solution (pH 1.2), and for the rest of the experiment, pH was shifted to 6.8 by addition of 250-ml 0.2-M tribasic sodium phosphate solution as stated in United States Pharmacopoeia 24 for enteric coated formulations. In acidic pH conditions, the alginate structure is protonated, and it is transformed into insoluble form alginic acid. The positively charged groups of chitosan interact with the alginate ions resulting in the prevention of release (25, 26). Incorporation of higher concentration of alginate in microparticle formulations extends the release of MLX. The release profiles of two microparticle formulations were compared with each other by model-independent approaches as stated in FDA guideline titled "Guidance for Industry: Dissolution testing of immediate release solid Oral Dosage forms" which was published in 1997 (20). The comparison of these two *in vitro* release profiles by model-independent approaches revealed that these profiles were not similar. As well-known if the difference factor, f_1, is between values of 0 and 15, the profiles are considered as similar. On the other hand, similarity factor, f_2, must be calculated as greater than 50 in order to consider two profiles as similar. For *ivivc*, at least two formulations with different release rates must be used in *in vivo* studies. That is the reason why we made this similarity comparison. We aimed to clarify that these two formulations are different in release characteristics. The results of this comparison proved that these formulations are different from each other in with respect to *in vitro* release profiles with f_1=16.67 and f_2=45.08 values (19, 20).

Table I. Pharmacokinetic Parameters for MLX in New Zealand Rabbits (n=6)

Parameter and unit	Oral solution	Formulation A (1%, *w/v* sodium alginate)	Formulation B (2%, *w/v* sodium alginate)
$AUC_{0-\infty}$ (μg h/ml)	63.68±14.49	19.84±12.70	20.85±3.53
Min–max	46.73–82.92	8.39–44.74	17.25–27.56
t_{max} (h)	2.33±1.21	9.66±3.88	16.33±12.02
Min–max	1–4	6–16	4–36
$t_{1/2}$ (h)	10.48±10.04	23.37±10.47	34.75±28.44
Min–max	5.5–32.32	13.47–43.04	10.67–87.72
C_{max} (μg/ml)	5.46±0.77	0.78±0.44	0.68±0.33
Min–max	4.212–6.58	0.44–1.66	0.47–1.29

Data are mean±standard deviation
$AUC_{0-\infty}$ area under the concentration–time plasma curve extrapolated to infinity, t_{max} time for maximum plasma concentration, $t_{1/2}$ elimination half-life, C_{max} maximum plasma concentration

Table II. The Overall View of the Equations for *Formulation A* (1%, *w/v* Sodium Alginate) and *Formulation B* (2%, *w/v* Sodium Alginate) After the Administration to New Zealand Rabbits Defining the Relation Between Absorbed% (*In Vivo*) and Released% (*In Vitro*) with Coefficients, where Oral Solution of MLX was Accepted as the Reference Formulation

Animal no.	Formulation	Equation	Determination coefficient (r^2)	Correlation coefficient (r)
1	A	Absorbed% = (0.0666 × Released%) + 1.8808	0.8778	0.9369
	B	Absorbed% = (0.1467 × Released%) + 6.4303	0.9687	0.9842
2	A	Absorbed% = (0.0834 × Released%) + 13.918	0.8909	0.9438
	B	Absorbed% = (0.0417 × Released%) + 17.526	0.9801	0.990
3	A	Absorbed% = (0.2251 × Released%) + 25.843	0.9087	0.9532
	B	Absorbed% = (0.1654 × Released%) + 25.85	0.9273	0.9629
4	A	Absorbed% = (0.0784 × Released%) + 2.4331	0.861	0.9279
	B	Absorbed% = (0.1021 × Released%) + 1.9078	0.9140	0.9560
5	A	Absorbed% = (0.0946 × Released%) + 5.5501	0.9068	0.9522
	B	Absorbed% = (0.0787 × Released%) + 2.1304	0.9141	0.9560
6	A	Absorbed% = (0.1039 × Released%) + 42.507	0.8672	0.9312
	B	Absorbed% = (0.1643 × Released%) + 14.421	0.9194	0.9588
Average	A	Absorbed% = (0.1102 × Released%) + 11.687	0.8563	0.9253
	B	Absorbed% = (0.1393 × Released%) + 5.5696	0.9402	0.9696

The *in vivo* experiments were conducted by using New Zealand rabbits. The drug formulations were administered by the oral route with gastric gavage that guarantees the proper dose of delivery (15 mg). Plasma profiles clearly indicated that release of MLX was successfully extended from the microparticle formulations. As the concentration of the sodium alginate was increased, similar to the *in vitro* release tests, the released amount, and so as the plasma concentration of MLX, was decreased. The washout period in animal studies was determined as 10 days that ensures seven elimination half-life of MLX in plasma for prevention of any possible interaction between two different dosage forms.

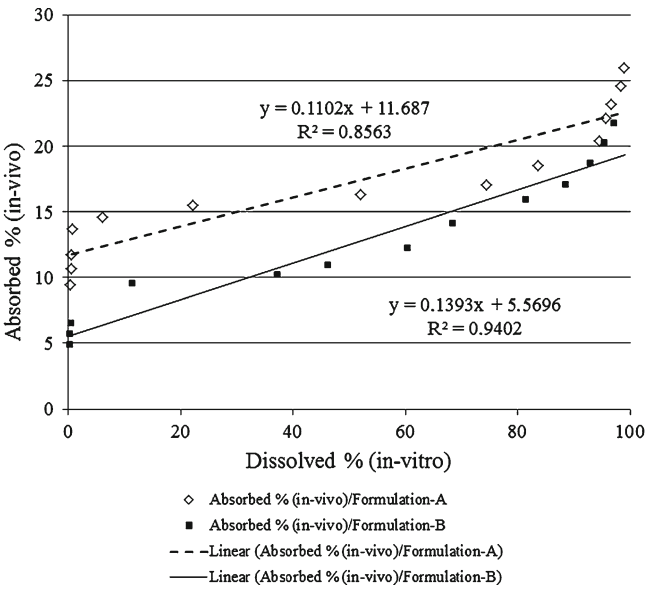

Fig. 4. The correlation profiles for the *formulation A* and *formulation B* between absorbed% (*in vivo*) and released% (*in vitro*) for New Zealand rabbits, where oral solution is considered as reference

MLX was chosen as the model drug depending on its low-solubility and high-permeability properties. By this way, the rate-determining step in *in vivo* absorption of the drug in gastrointestinal tract would be the release rate of the active ingredient from different formulations. Therefore, changes in formulation parameters (sodium alginate concentration) would result in different release rates of MLX which will lead us for modeling of a possible *ivivc* modeling. The pharmacokinetic parameters, listed in Table I, are in good correlation with our *in vitro* findings. The increase in sodium alginate concentration resulted in the extension *in vivo* release of MLX from microparticle formulations. Briefly, t_{max} and $t_{1/2}$ values were increased in *formulation B* (2% sodium alginate) with respect to oral solution and *formulation A* (1% sodium alginate). On the other hand, this increase in polymer concentration resulted in insignificant increase in $AUC_{0-\infty}$ values with respect to *formulation A* ($p > 0.05$).

The *ivivc* modeling was established by the correlation of *in vitro* released% with *in vivo* absorbed% after the calculation of *in vivo* absorption with deconvolution procedure. As the sodium alginate concentration in microparticle formulations was increased, the release of MLX also decreased. At this point, the rate-limiting step for MLX absorption became *in vivo* drug dissolution, but not the low solubility of MLX. At this point, higher r^2 value (0.9402) and lower prediction error (7.419) supported our expectation for achieving a better ivivc model for formulation B.

CONCLUSIONS

The microparticle formulations prepared by using 1% and 2% (*w/v*) sodium alginate extended the release of MLX for a period of 10 up to 13 h. According to the overall experimental results, it can be concluded that a better *ivivc* modeling has been established with the *formulation B* in which the polymer ratio was set as 2%

(*w*/*v*). However, additional internal and external validation studies are needed to establish the further validation this *ivivc* modeling.

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