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Appraisal of the pharmacokinetic profile, histopathology, and stability studies of a fast dissolving oral film based on natural polysaccharide

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Abstract: The present research is designed to evaluate the pharmacokinetic profile, histological evaluation, and stability studies of an orodispersible film (ODF) of tizanidine (TZ) and meloxicam (MX) prepared from a natural polysaccharide, i.e., xanthan gum. *In vivo* release study of TZ and MX was performed in rabbits and results indicated the better pharmacokinetics parameters and improved the oral bioavailability when compared to the oral aqueous suspension and solution of TZ and MX, respectively. The intermediate stability studies were performed at 30±2°C and 65±5% RH, whereas, the accelerated stability studies were carried out at 40±2°C and 75±5% RH, respectively for the duration of six months and results indicated that the ODF was stable for six months without any substantial difference in essential physico-chemical parameters, mechanical attributes, and morphological constraints. The toxicity profile of ODF was determined through histopathology of vital organs after administering the ODF to the rabbits. Histopathology revealed that the tissues of all vital organs are normal and did not exhibit any abnormalities, lesions, or hemorrhage. Therefore, the ODF prepared from xanthan gum exhibited a non-toxic and stable formulation with a better pharmacokinetics profile of MX and TZ.

Keywords: Polysaccharides, solvent casting method, polymeric film, fast dissolving film, orodispersible.

INTRODUCTION

Currently, different drug delivery systems (DDSs) and techniques are being used to enhance the bioavailability of the drugs and deliver the therapeutic agents at the desired site of action with minimum side effects (Li *et al*., 2019). Despite the development of advanced and novel DDSs, conventional dosage forms are still considered the effect DDSs to combat different diseases. As far as the oral DDSs are concerned, solid dosage forms, especially, tablets and capsules are given preference over liquid preparations mainly due to easy handling, unit dose packing and patient compliance (Kurczewska-Michalak *et al.*, 2020). However, in emergency conditions, such DDS are not suitable and have faced many problems including delayed response, non-cooperated behavior of the patients, and also the reduced efficacy due to first pass metabolism (Homayun *et al.*, 2019). Orodispersible DDSs, i.e., orodispersible tablets (ODTs) as well as orodispersible films (ODFs) are one of the better options to counter most of these issues. However, in comparison with ODTs, ODFs are considered the suitable option due to easy handling, feasibility, without choking hazards, and patient compliance (Slavkova and Breitkreutz, 2015).

Many excipients are being used in pharmaceutical industries depending upon their physical, chemical, and pharmaceutical properties. Based upon their origin, these excipients are usually classified as natural, synthetic and semi-synthetic. Mostly, naturally occurring materials are given preference due to their biocompatibility, biodegradability, non-toxicity, non-immunogenicity, and easy availability (Song *et al*., 2018; Haseeb *et al*., 2018; Hussain *et al*., 2020). These naturally occurring biomaterials are derived from plants, animals, and microorganisms (Song *et al*., 2018; Silvipriya *et al*., 2015; Farid-ul-Haq *et al*., 2020). Such materials are widely used for the development of immediate release as well as sustained or targeted DDSs (Fenton *et al*., 2018; Haseeb *et al*., 2019; Sivadasan *et al*., 2020). For ODFs, different naturally occurring biomaterials are investigated and used in the development of such formulations, i.e., pullulan,

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dextran, gelatin, starch, xanthan gum, guar gum and pectin (Nagar *et al*., 2011; Khan, 2020; Hussain *et al.*, 2011).

In our previous study, different polymers (natural, synthetic, and semisynthetic) were used to formulate an ODF for the delivery of MX and TZ. The physico-chemical analysis, mechanical properties and *in vitro* drug release study proved that the xanthan gum was a suitable polymer for the preparation of ODF (Sheikh *et al*., 2020). However, the *in vivo* profile of the formulated ODF is required to prove its effectiveness. Therefore, the focus of this research work is to evaluate the *in vivo* drug release profile and determine the stability of the prepared ODF through different pharmacokinetics parameters and intermediate and accelerated stability studies, respectively. Additionally, the assessment of any possible toxicity of the prepared ODF on the vital organs of the animal model through histopathology will be the focus of the present research.

MATERIALS AND METHODS

Materials

Xanthan gum, polyvinyl pyrrolidone (PVP K30), polyethylene glycol (PEG) 400, methanol, and croscarmellose sodium were obtained from Riedel-de Haen, Germany. Aspartame and sodium hydroxide were procured from Sigma-Aldrich, Germany. Meloxicam and tizanidine used in this research are according to the specifications as mentioned in United States Pharmacopeia (USP). Analytical grade solvents, chemicals, and reagents were used during the research. Distilled water was also used for the preparation of different solutions.

Preparation of ODF

ODF was prepared according to the procedure mentioned in the literature and depicted in fig. 1 (Sheikh *et al.*, 2020). Briefly, xanthan gum (21mg) was allowed to mix in sufficient deionized water for 6 h at 50°C. After that, TZ (2 mg), PEG 400 (18.3mg), croscarmellose sodium (27.2 mg), aspartame (19.4mg) and PVP K30 (5.5mg) was added one by one. The mixture of all these ingredients was maintained at 50°C for 2h. Air bubbles were removed by placing this mixture in a sonicator for 30 min. The completely homogenized mixture was then poured into a petri dish. To get TZ loaded film, the petri dish was kept in a vacuum oven for 6h at 60°C. A mixture of methanol and 0.1 N NaOH (5:1) was used to dissolve the MX (7.5mg) and then poured on the TZ loaded film. Finally, the petri dish containing ODF was placed in a vacuum oven for another 8h at 80°C. The prepared dried film was detached from the petri dish, cut into desired pieces (2 × 3 cm), and placed in a desiccator until further use. The quantities of all ingredients of ODF were calculated according to the area of the petri-dish, i.e., 22.91 cm^2.

Characterization of ODF

Various physico-chemical (thickness, weight, pH of the film surface, disintegration time, content uniformity, loss on drying, moisture loss and moisture uptake) and mechanical (folding endurance, percent elongation, and tensile strength) parameters were used to evaluate the ODF (Al-Mogherah *et al.*, 2020; Sheikh *et al.*, 2020). The results of these parameters are the same as reported in our previous study (Sheikh *et al.*, 2020).

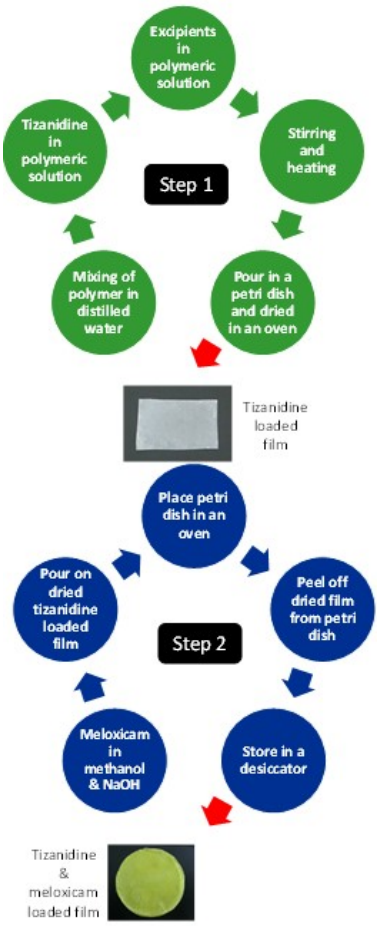

Fig. 1: Preparation procedure of the orodispersible film comprising of meloxicam and tizanidine

In vivo drug release study

Prepared ODF was evaluated for *in vivo* drug release study and different pharmacokinetics parameters (Zaman *et al.*, 2018b; Sheikh *et al.*, 2021).

Study design

Eighteen albino rabbits (1.6-1.8kg each) were selected and kept in a clean cage at 25°C and 40-60% humidity. Moreover, the rabbits were provided 12h photoperiod, standard laboratory food, and free access to ordinary clean and fresh water. One week before the start of the experiment, all rabbits were shifted to the laboratory for acclimatization to the conditions and kept fast overnight just before the day of the experiment.

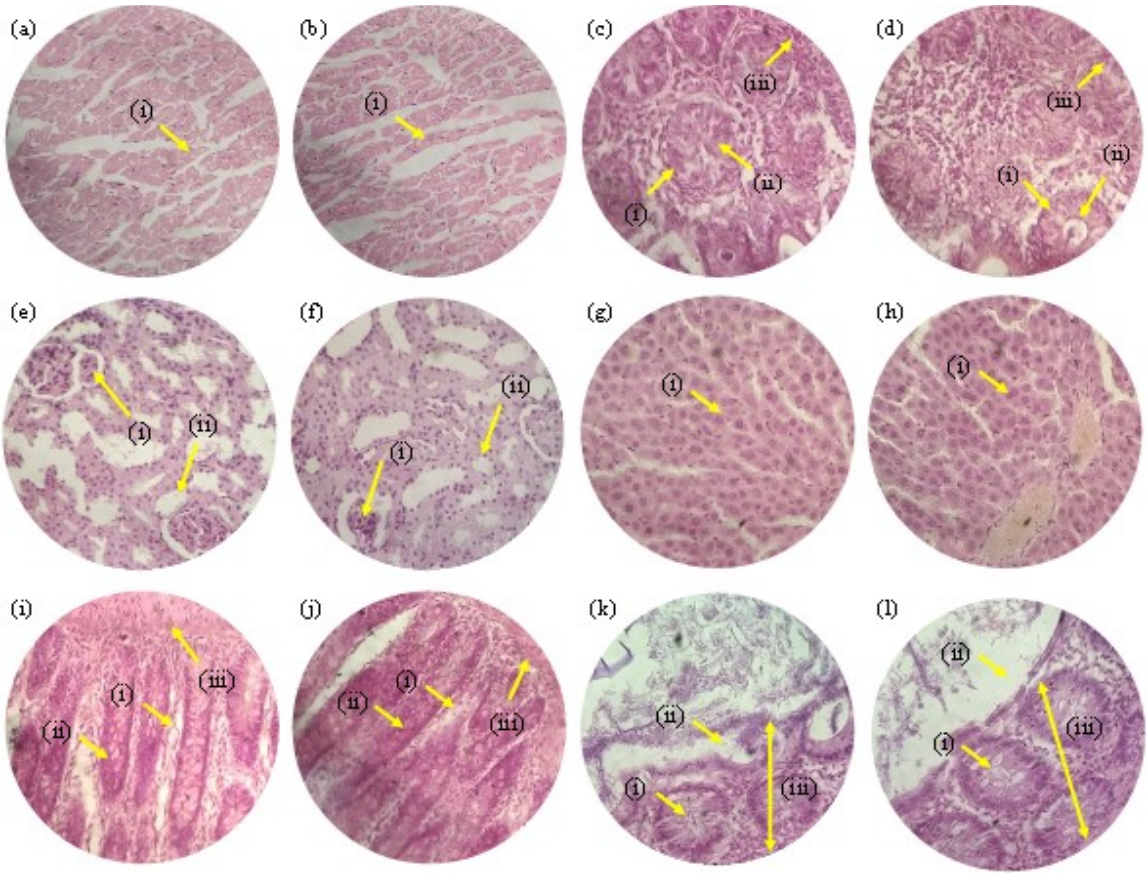

Fig. 2: Histopathology images of the heart tissues of (a) control and (b) sample group animals exhibiting the (i) striated heart muscles; (c, d) the tissues of the small intestine of control and sample group animals, respectively, expressing the (i) columnar epithelial cell with the basal nuclei, (ii) the acinous lumen and (iii) the muscularis mucosae; (e, f) the tissues of the kidney of control and sample group animals, respectively, presenting (i) the glomerulus and (ii) the renal tubules; (g, h) the tissue of the liver of control and treated animals, respectively, depicting (i) the plates of hepatocytes; (i, j) the tissues of the colon of control and sample group animals, respectively, exhibiting (i) the lumen of crypt, (ii) the lamina propria and (iii) the muscularis externa; (k, l) the tissues of the gastric mucosa representing the control and sample group animals, respectively, showing (i) the gastric glands, (ii) the serosa and (iii) mucosa

Table 1: Pharmacokinetic data of the TZ and MX

Parameters	Meloxicam		Tizanidine	
	ODF	Oral suspension	ODF	Oral solution
AUC (h ng/mL)	6211.74	6109.18	1108.69	903.29
C_{max} (ng/mL)	519.49	481.83	105.67	63.28
t_{max} (h)	1.25	2	0.75	2
$t_{1/2}$ (h)	10.9	16.9	7.1	8.8

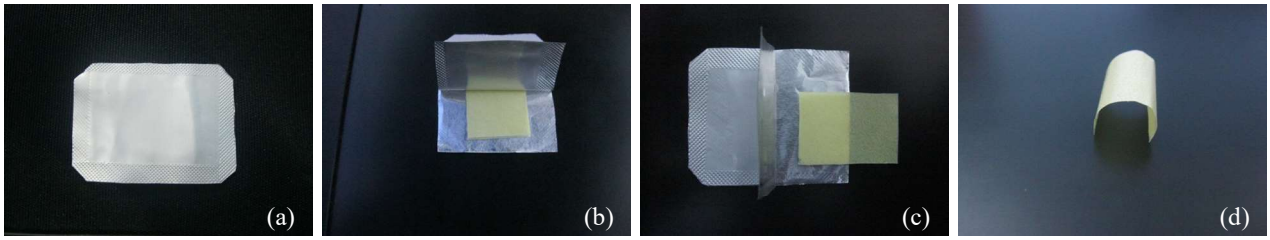

Fig. 3: Photographs of the packaging of the prepared orodispersible film: (a) Showing the packed and sealed pouch; (b) Placed between two layers of aluminum foil; (c) Removed from the aluminum foil; (d) Flexibility of the prepared film

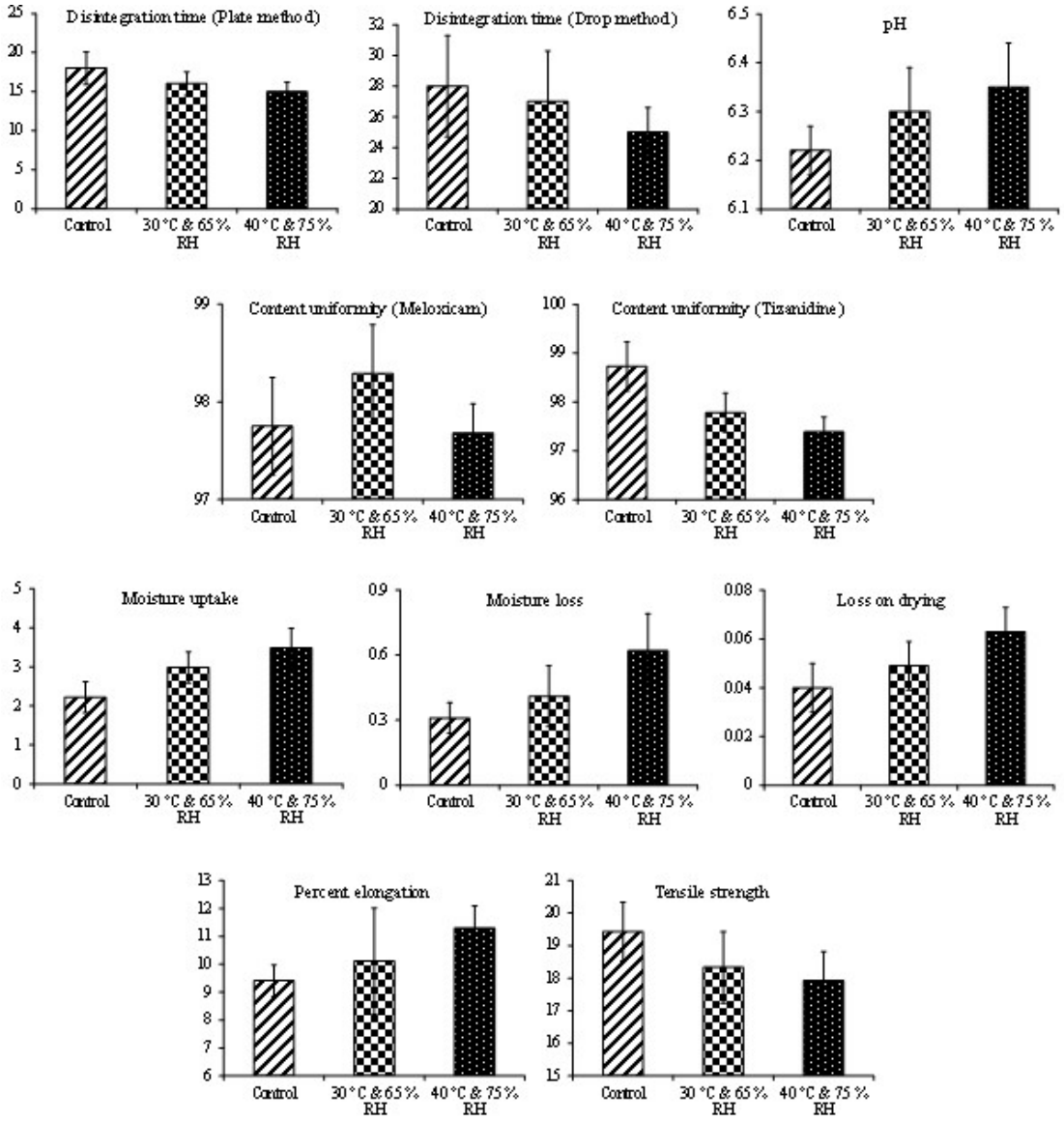

Fig. 4: Results of different parameters of ODF after completing the stability studies performed at ambient temperature (control), intermediate conditions (30 °C and 65% RH) and accelerated conditions (40°C and 75% RH) performed after six months

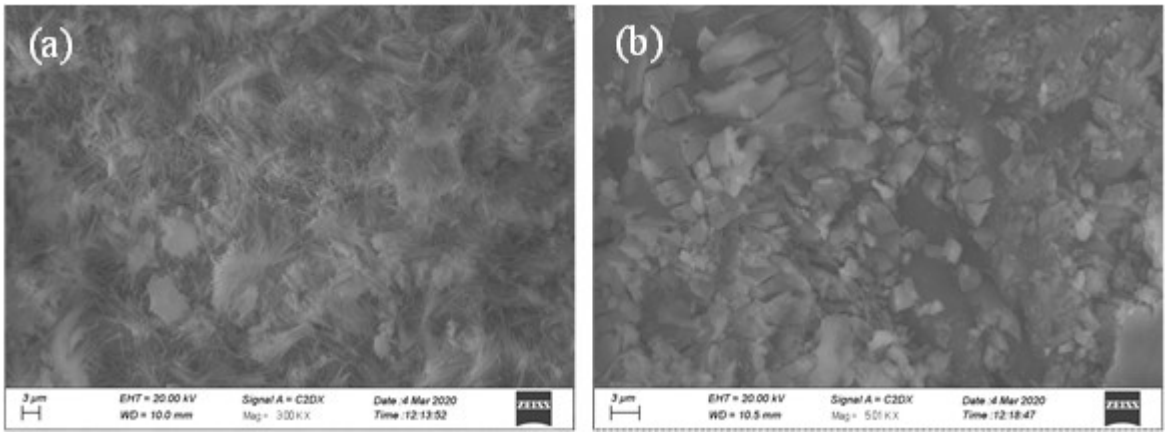

Fig. 5: SEM images of the ODF: (a) Before and (b) After stability studies placed at 40°C and 75% RH for six months

Rabbits were divided into three equal groups having six rabbits in each group. Rabbits of the sample group were provided the prepared formulation according to the weight of the animals (Zaman *et al*., 2018b). For the rabbits of the standard group, an aqueous solution of TZ and an aqueous dispersion of MX were administered. The study protocol was approved by Institutional Review Board, GC University, Faisalabad through letter no. 284, study no. 019284, dated 10th October 2017. Moreover, the study design was followed according to the NIH guidelines, "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985).

Pharmacokinetic evaluation

For the evaluation of pharmacokinetic parameters, the prepared ODF was administered to the rabbits. Before administration of the ODF, the rabbits were slightly anesthetized for easy handling during the whole experimentation. A mixture of ketamine and diazepam in a dose of 10mg/kg and 1mg/kg, respectively were given through intramuscular injection. After that, the ODF was placed on the tongue of the rabbit. The jaws of the rabbits were closed and held firmly to ensure the complete disintegration of the administered ODF in the mouth. After periodic time intervals, the blood sample was collected from the marginal ear vein of the rabbits in the heparinized tubes. The blood sample was processed through centrifugation and collected the supernatant followed by the treatment with methanol and diethyl ether to precipitate the protein. The purified supernatant was carefully separated and dried at 40°C in a water bath. The dried sample was dissolved in a 200µL mobile phase and then processed through HPLC. The mobile phase is composed of a mixture of HPLC water and methanol in a ratio of 2:8. The pH of the resultant mixture was adjusted at 3.0 with the help of orthophosphoric acid (Zaman *et al*., 2018a). Different pharmacokinetics parameters, i.e., plasma half-life ($t_{1/2}$), peak plasma concentration (C_{max}), area under curve (AUC), peak time (t_{max}), and bioavailability were calculated through PKSolver software.

Histopathological evaluation

Newly developed ODF formulations were investigated for the possible toxicity of the ingredients. ODF was administered to the rabbits and the effect on vital organs was observed through histological study.

Study design

Two groups of male albino rabbits were used in this study. Six rabbits (1.4-1.5kg, each) in each group (control and sample) were placed and kept fast overnight before the start of the experiment. All rabbits were given the ODF in a dose of 1g/kg body weight of the rabbits (OECD 420). After 24 h, the rabbits were sacrificed to get the vital organs. Formalin solution (10%, v/v) was used to preserve the kidney, liver, small intestine, gastric mucosa, heart and colon. The experimental conditions of animal housing and the detail of study protocol were the same as described earlier.

Histopathology

For histological examination of the vital organs, a rotary microtome was used to cut the piece of organ and placed it on a glass slide. Haematoxylin and eosin were used to stain the piece of the organ (Ali *et al*., 2022) and observed the tissue structure under the binocular microscope (XSZ 107 BN).

Packaging of ODF

For the packing of ODF, a specially designed aluminum pouch to protect the ODF from harsh environmental conditions, i.e., moisture, sunlight, heat, etc. The photographs of the packaging of ODF were taken through a good quality digital camera. This packed ODF was also used during the stability studies.

Stability studies

The stability of the ODF was evaluated under the influence of high temperature and increased humidity levels. Therefore, intermediate and accelerated stability studies were performed at 30±2°C and 65±5% relative humidity (RH) and 40±2°C and 75±5% RH, respectively for six months and compared with control, i.e., placed at ambient temperature. After regular and prefixed time intervals, i.e., 1, 2, 3 and 6 months, ODFs were thoroughly characterized through physico-chemical and mechanical aspects and also for SEM analysis (Shen *et al*., 2013). The stability studies were performed according to the guidelines of ICH (CPMP/ICH/2736/99).

SEM analysis

The morphology of the prepared ODF before and after the completion of stability studies was observed through SEM analysis. A small piece of the film was placed on an aluminum stub followed by sputter coating using gold. To get the image, SEM (S-2380N, Hitachi, Japan) was operated at a voltage of 8kV.

STATISTICAL ANALYSIS

The results of the pharmacokinetic analysis and stability studies were analyzed through one-way analysis of variance (ANOVA) (Shen *et al*., 2013) using Minitab 11 software. Data were expressed as a mean ± standard deviation (SD). The value of $p<0.05$ was considered significantly different.

RESULTS

In vivo drug release studies and pharmacokinetics evaluation

HPLC was used to determine the concentration of TZ and MX in the blood sample. Results of the pharmacokinetic parameters are shown in table 1. The values of the t_{max} of

MX and TZ were determined as 1.25 h and 0.75 h for ODF, respectively. Similarly, the t_{max} values of MX and TZ were recorded as 2h for both drugs in oral suspension and oral solution. The half-life ($t_{1/2}$) of MX was noted as 10.9h and 16.9h for ODF and oral suspension, respectively. Likewise, the half-life ($t_{1/2}$) of TZ was recorded as 7.1h and 8.8h for ODF and oral solution, respectively. The C_{max} of MX and TZ from ODF were recoded as 519.49ng/mL and 105.67ng/mL and from their respective solvents are 481.83ng/mL and 63.28ng/mL, respectively. Moreover, the bioavailability of the MX and TZ from the ODF was calculated as 101.68% and 122.74%, respectively.

Histopathological studies

Histopathology of the different vital organs, i.e., liver, kidney, gastric, intestine, heart, and colon are represented in fig. 2. Tissues of the vital organs of treated animals have shown the normal texture without any sign of lesions, degeneration, inflammation, hemorrhage, and necrosis. These results are comparable with the tissues structures of the normal organ (Farid-ul-Haq *et al*., 2020; Lodhi *et al*., 2020).

Packaging of ODFs

The packaging of the ODF was designed to protect it from environmental factors. The packaging was easily torn off to remove the ODF. The photographs of the packed ODF, removed from the aluminum foil and the flexibility are expressed in fig. 3.

Stability studies

Results of the stability studies are represented in fig. 4. The values of the disintegration time recorded through the drop method were noted as 28s, 27s and 25s and from the plate method was determined as 18s, 16s and 15 s for control, intermediate and accelerated stability studies, respectively. The percent elongation of control, intermediate and accelerated stability studies were found as 9.41%, 10.11% and 11.3%, respectively. The value of tensile strength after stability studies was found to be 19.43N/m^2, 18.33N/$m^{2,}$ and 17.92N/m^2 for control, intermediate and accelerated studies, respectively. After the intermediate and accelerated stability studies and at ambient temperature, MX and TZ contents were found as 98.29% and 97.78%, 97.68% and 97.39% and 97.75% and 98.73%, respectively. The pH of the ODF after completing the stability studies at ambient temperature for six months and also the intermediate and accelerated conditions were noted as 6.22, 6.3 and 6.35, respectively. Moisture uptake recorded after completing the stability studies at ambient temperature and intermediate and accelerated conditions are calculated as 2.22%, 2.98%, and 3.48%, respectively. The values of moisture loss were recorded as 0.31%, 0.41% and 0.62% for the ODF placed at ambient temperature and intermediate and accelerated conditions. Similarly, the loss on drying was determined for the ODF sample placed at ambient temperature, intermediate and accelerated stability studies were 0.04%, 0.049% and 0.063%, respectively.

SEM analysis

The surface morphology of the prepared ODF before and after stability studies (accelerated) was evaluated through SEM and the captured images are depicted in fig. 5. Well distributed particles on the surface of ODF were observed in the image capture before the stability studies (fig. 5a) and after completing the accelerated stability studies (fig. 5b).

DISCUSSION

ODF was prepared using the solvent casting method and considered an efficient method for the simultaneous administration of both MX and TZ in which one drug is hydrophobic and other is hydrophilic. The presence of NaOH in the formulation increased the solubility of the MX in the solvent during preparation as well as in the saliva which possibly enhanced the bioavailability of the drug as indicated in the pharmacokinetics parameters. The pharmacokinetics profile revealed that the t_{max} value of MX and TZ was reduced after the incorporation of these drugs in the ODF as compared with their respective suspension and solution. Similarly, the increased values of C_{max} of both drugs from ODF also supported the enhanced absorption from the film formulation. A more than 100% increase in the bioavailability of both drugs was observed as compared to the oral suspension and oral solution of MX and TZ, respectively. Therefore, an enhanced bioavailability of both drugs was achieved through ODF formulations (Van Nguyen *et al*., 2022). However, in the case of ODF, the $t_{1/2}$ of both drugs was decreased as compared with oral suspension and solution which was accepted as the elimination of both drugs started earlier due to earlier absorption.

Histopathology of the vital organs indicated that the developed formulations and all of their ingredients are safe and have not imparted any toxicity to the vital organs (Sheikh *et al*., 2021). Therefore, the method and composition of ODF are considered safe for formulation design.

Proper packing of the ODFs prevents them from environmental hazards and maintains the physical and chemical integrity and therapeutic efficiency. The results of stability studies proved the efficiency of the aluminum packing (Cupone *et al*., 2020).

Results of intermediate and accelerated stability studies indicated that all essential parameters remain almost the same and comparable with the control confirming a good formulation design (Serrano *et al*., 2019; Swain *et al*., 2015). However, insignificant changes in the values of some parameters were observed. A slight decrease in the

disintegration time of all ODF formulations was observed which might be due to the absorbance of water present in the atmosphere by the polymer resulting in the reduction of disintegration time. Similarly, the percent elongation of ODF formulations was slightly increased and also the tensile strength was decreased. Moisture uptake of all formulations was marginally increased and hence moisture loss and loss on drying were also increased due to the absorbed moisture. Content uniformity of the active ingredients was the same and within the standard range. Statistically insignificant changes in the values of all other parameters were observed and also comparable with the control.

The SEM image of the ODF captured before the start of stability studies has shown the well-distributed small particles of the ingredients embedded in the fibrous network of the xanthan gum (fig. 5a). Similarly, after the stability studies, the small particles of the ingredients were also observed in the ODF (fig. 5b). Moreover, all ingredients were uniformly scattered throughout the polymeric matrix. Therefore, SEM analysis of the prepared ODF revealed the insignificant difference of the surface of the ODF before and after stability studies (accelerated). Hence, the prepared ODF incorporated with both drugs (hydrophilic and hydrophobic) exhibited the appropriateness of the preparation method and showed an insignificant difference in the morphology of the film.

CONCLUSION

The pharmacokinetics studies of the MZ and TZ loaded ODF proved the enhanced bioavailability of both drugs. The method adopted for the preparation of ODF did not exhibit any toxic effect on the vital organs as revealed through histopathology. Stability studies also supported the suitability of this preparation method as no significant difference in stability parameters was observed. Therefore, ODF prepared through xanthan gum using a modified solvent casting method proved to be a safe method of preparation with enhanced bioavailability and resulted in a non-toxic and stable product.

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