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Original Article

Formulation, Characterization, *in vitro* and MTT Cytotoxicity Evaluation of Pioglitazone HCl Films for Topical Administration

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ABSTRACT

Aim: The main focus of the study was to create and analyze novel formulation to explore the potential of Pioglitazone HCl with Guar Gum, Chitosan and Sodium alginate for topical delivery and evaluate them. **Materials and Methods:** Pioglitazone HCl loaded films were formulated by solvent evaporation technique and were subsequently incorporated within the Guar gum and Chitosan. **Results:** For the investigation, various physicochemical parameters along with *in vitro* drug release studies, and accelerated stability study were performed. MTT assay with the drug-loaded films were also conducted. The thickness and weight uniformity were excellent in Chitosan and Gaur Gum films. The percentage moisture loss and absorption tests indicated that the films maintained balanced moisture content. The surface pH of the films was found to be within a range that is generally compatible with skin application, minimizing the risk of irritation. The *in vitro* drug release and kinetic investigations revealed that all batches exhibited a controlled and sustained release of Pioglitazone HCl over an 8-hr period. The release kinetics have been explored by using four different models: Zero-Order, First-Order, Higuchi, and Korsmeyer-Peppas. The Korsmeyer-Peppas models excellent fit across all batches. MTT assay shows that all formulations (F1 to F6) exhibited a similar trend of moderate cytotoxicity at higher concentrations, with % viability decreasing as the concentration increased. F3, F4, F5, and F6 demonstrated slightly higher cytotoxicity than F1 and F2, as reflected in their lower IC_{50} values. The accelerated stability investigation showed that drug-loaded polymeric films were stable for up to two weeks under accelerated situations.

Keywords: *In vitro* Studies, MTT Assay Higuchi kinetics and Korsmeyer-Peppas Analysis, IC_{50} value.

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INTRODUCTION

For past few decades, Topical drug delivery system considered more suitable delivery system over the conventional oral dosage forms. In the topical system skin is the major site of administration of dosage forms for the permeation. It provides the systemic bioavailability, controlled drug release rate and decline of the first pass metabolism of drug which reduces the efficacy of therapeutics of drugs.[1,2] In this research Pioglitazone HCl is used for designing, formulation and evaluation of the topical film. Pioglitazone HCl is type 2 anti-diabetic agents but has a strong potential for treating diabetic wounds during both the inflammatory and proliferating phases of healing, with good absorption and minimal systemic adverse effects.[3,4] Formulations of topical films containing Guar gum and chitosan matrices are novel medication delivery technologies that enhance the synergistic impact and bioavailability of drug at the administration site. This painless and non-invasive approach delivers the medicine to the circulation while controlling the therapeutic concentration. Composition of chitosan and guar gum films were accomplished by a simple solvent extraction method. Due to their ionic polysaccharide nature, sodium alginate can form films for formulation development. Propylene glycol (3% w/v) is used to increase the film's flexibility and mechanical strength. Tween 80 is a non-ionic surfactant that helps Pioglitazone HCl penetrates the skin. The examination parameters included thickness, weight uniformity, content uniformity, folding durability, % moisture loss and moisture absorption, pH, and *in vitro* drug release, MTT Cytotoxicity and accelerated study.[5]

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MATERIALS AND METHODS

Materials

Pioglitazone HCl was received as a gift from Sun Pharma Pvt. Ltd., Baddi, and Himachal Pradesh. Sodium alginate LV, extra pure, Rehsiff Scientific, Chitosan, Loba chemical. Guar Gum, Powder of Endosperm, Loba Chemical, Propylene Glycol, BRM Chemicals. Double distilled water had been utilized all through the investigation.

Development of Drug-Loaded Topical Films

Transdermal films were prepared by Solvent evaporation method. Firstly, chitosan was dissolved in 1% of glacial acetic acid mixture by continuous stirring at 500rpm for 1 hr by magnetic stirring. After that slightly heating at 40°C, if chitosan was not dissolved in the solution. A similar method was performed with Guar gum and sodium alginate as per the composition requirement. Propylene glycol (5 mL, 3%w/v) was incorporated in each polymeric solution then mixture was constantly stirring for 15 min for the uniformly distribution of each ingredients. Pioglitazone HCl was precisely weighed and added carefully to the polymeric solution while stirring at 500 rpm. Additionally, mixture was stirred for 30 min to ensure the complete dissolution and distribution of drug. The prepared drug -polymeric solution was poured into the 10cm diameter of dried, cleaned and flat surface, Glass petri-plates. To ensure the equally distribution of mixture (approximately 220 mL) with consistent film thickness across all batches while pouring the solution. Then petri-dishes were covered with an inverted glass funnel and avoid the air bubbles and contamination. After that, the films were gently removed from the petri-dishes; edges were trimmed to obtain uniform films of approximately 5 cm diameter. The trimmed films were individually wrapped in aluminum foil to protect them from light, moisture, and other environmental factors and stored in the desiccators to maintain moisture absorption (Table 1) (Scheme-1).[6]

Evaluation of Drug-Loaded Polymeric Films

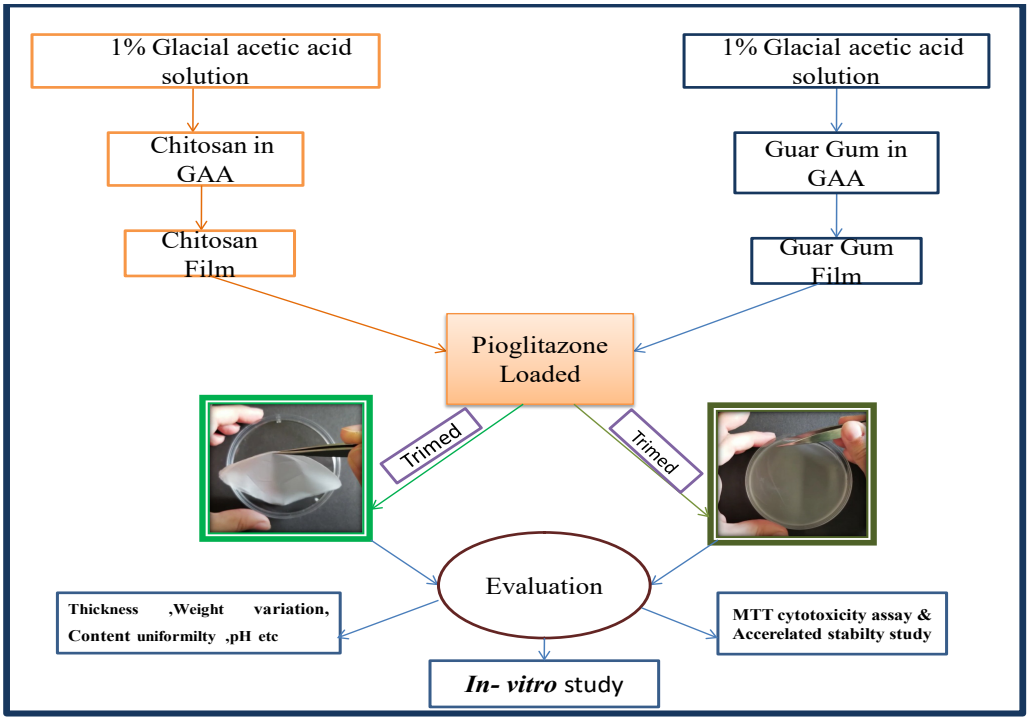
Thickness Measurement: The thickness of the drug-loaded polymeric films was measured using a digital micrometer at five different locations on each film to ensure uniformity. Readings were measured in triplicate to assess the consistency of the film thickness across different samples.[6]

Weight Uniformity: The weight uniformity of the drug-loaded films was assessed by weighing five individual samples from each batch.[6]

Content Uniformity: The Content uniformity was used to find out the accurate amount of the sample (drug) in each polymeric film. Five films from each batch were selected and dissolved individually in 10 mL of phosphate buffer (pH 7.4). The withdraw samples were analyzed at the wavelength of 269 nm of spectrophotometer.[7]

Folding Endurance: The films were repeatedly folded at the same point until they broke or developed visible cracks. The number of folds required for tearing of the film was recorded.[6, 7]

Percentage Moisture loss: For this experiment, films were accurately weighed and then placed in desiccators for 72 hr.[8]



Scheme 1: Schematic illustration of preparation of topical film and evaluation

Percentage Moisture Absorption: Topical films were accurately measured and then placed in a saturated solution of aluminum chloride, which maintains a Relative Humidity (RH) of approximately 79.5%. The films were left in the desiccators for 72 hr.[8]

pH Evaluation: A digital pH meter equipped with a flat-surface electrode was then gently placed in contact with the surface of the film. The pH value was recorded after stabilization. Each batch was tested in triplicate to ensure accuracy and consistency of the results.[4]

***In vitro* study:** A Franz diffusion cell was used for this investigation. The films were set in the donor compartment, and the receptor chamber was filled with 20 mL of phosphate buffer (pH 7.4) to simulate the physiological conditions. The receptor medium was maintained at 37°C±0.5°C and stirred continuously with a magnetic stirrer. At different time intervals (e.g., 1, 2, 3, 4, 5, 6, 7, and 8 hr), 1 mL samples were taken out from the receptor compartment and immediately refilled with fresh phosphate buffer to maintain a consistent volume. The amount of sample released was quantified using a UV-visible spectrophotometer at 269 nm.[6,8]

MTT Cytotoxicity Assay[3,10,11]

MTT Assay Protocol for Test Items F1 to F6

Firstly, prepared the stock solution of each test item (F1 to F6) by dissolving them in a DMSO at a concentration of 5 mg/mL. The solution was gradually diluted at concentration of 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL, 0.3125 mg/mL, and 0.039 mg/mL. Fibroblast cells strain was used for the experimental setup. Four control measure were set as Negative Control (No test item), Solvent Control: Cells culture medium (Dulbecco's Modified Eagle Medium, Fetal Bovine Serum 10% and other growth factors)+DMSO (same concentration of DMSO as in test samples), Positive Control: Sodium Lauryl Sulfate (SLS) as per ISO EN 10993, Part-5:2009. A plate map for a 96-well microtitre plate was prepared, designating the wells for blank, negative control, and test items. Cells were inserted at a density of 1−3×1051−3 \times 10^51−3×105 cells/mL in the designated wells, and 100 µL of the cell suspension was received by each well. The culture vessel was incubated at 37°C with 5% CO_2 and >90% humidity for 24 hr, preparing them for subsequent experimental analyses. The absorbance was monitored at 550 nm with Biorad Microplate reader to determine cell viability. The OD or optical density, at this wavelength reflects the count of viable cells based on the transformation of MTT to formazan.

Accelerated Stability Study

The samples were preserved in a stability chamber set at 40°C±2°C and 75%±5% Relative Humidity (RH) to check the drug content, absorbance, physical appearance and film integrity. The samples films were endured under these accelerated circumstances for 1 month to evaluate their stability.[9]

RESULTS

Development of Transdermal Films

F1 to F3: These batches incorporate Chitosan and Sodium alginate was prepared successfully (Figure 1a).

F4 to F6: These batches use Guar Gum and Sodium alginate with various concentrations was fabricated successfully (Figure 1b).

Thickness Measurement

The mean thickness values for the batches F1 through F6 were 0.228 mm, 0.236 mm, 0.247 mm, 0.216 mm, 0.226 mm, and 0.236 mm, respectively. The standard deviation across all batches was minimal, ranging from 0.0015 mm to 0.0019 mm, indicating high reproducibility and uniformity (Table 2 and Figure 2).

Weight Uniformity

The results explain that the films across different batches displayed 0.15 mg, 0.16 mg, 0.17 mg, 0.13 mg, 0.16 mg and 0.16 mg respectively F1 to F6 batches and demonstrating that the film preparation process was consistent and reproducible (Table 2 and Figure 2).

Content uniformity

The batch F1 showing an average content of 22.04 mg, batch F2 at 22.10 mg, batch F3 at 22.14 mg, batch F4 at 21.97 mg, batch F5 at 22.03 mg, and batch F6 at 22.12 mg. The standard deviation was minimal across all batches, ranging from 0.05 mg to 0.07 mg, which indicates excellent consistency in the size of sample (drug)

Table 1: Formulation table of PIG loaded polymeric films.

Batch No	Drug (mg)	Chitosan (mg)	Guar Gum (mg)	Sodium Alginate (mg)	Propylene Glycol (3% w/v)	Tween 80 (mL)	Glacial Acetic Acid (1% w/v)
F1	22.04	100	-	75	5	5	5
F2	22.04	150	-	50	5	5	5
F3	22.04	200	-	25	5	5	5
F4	22.04	-	100	75	5	5	5
F5	22.04	-	150	50	5	5	5
F6	22.04	-	200	25	5	5	5

present in each film. The drug was evenly dispersed throughout the polymer matrix (Table 2 and Figure 2).

Folding Endurance

The results revealed that the folding duration of the films varied slightly between the different batches, with F4 showing the highest average folding strength of 332 folds, and F3 showing the lowest average 314 fold numbers. The Standard Deviation (SD) was ranging from 2.65 to 4.16, indicating consistent performance across the samples within each batch (Table 2 and Figure 2).

Percentage Moisture Loss

The results stated that the percentage moisture loss for all batches ranged from 3.89% to 4.33%. The mean moisture loss was lowest for batch F6 at 3.90% and highest for batch F4 at 4.31%. The standard deviation values were laid between 0.09% and 0.13%, indicating that the results were consistent across the replicates within each batch (Table 2 and Figure 2).

Percentage Moisture Absorption

The results displayed that the % moisture absorption for trial ranged from 5.50% to 6.58%. Batch F4 exhibited the highest moisture absorption with a mean value of 6.55%, while batch F3 showed the lowest with a mean of 5.56%. The standard variance has been determined to be between 0.08% and 0.12% in every batch (Table 2 and Figure 3a).

pH Evaluation

The results suggest that the surface pH of the films across different batches ranged from 6.7 to 7.2. Batch F6 exhibited the highest average pH at 7.1, while batch F4 had the lowest at 6.8 (Table 2 and Figure 3b).

In vitro Study

The *in vitro* drug release study was designed to test the rate and extent of Pioglitazone HCl release from the drug-loaded

Figure 1: (a) and (b) Transdermal Films.

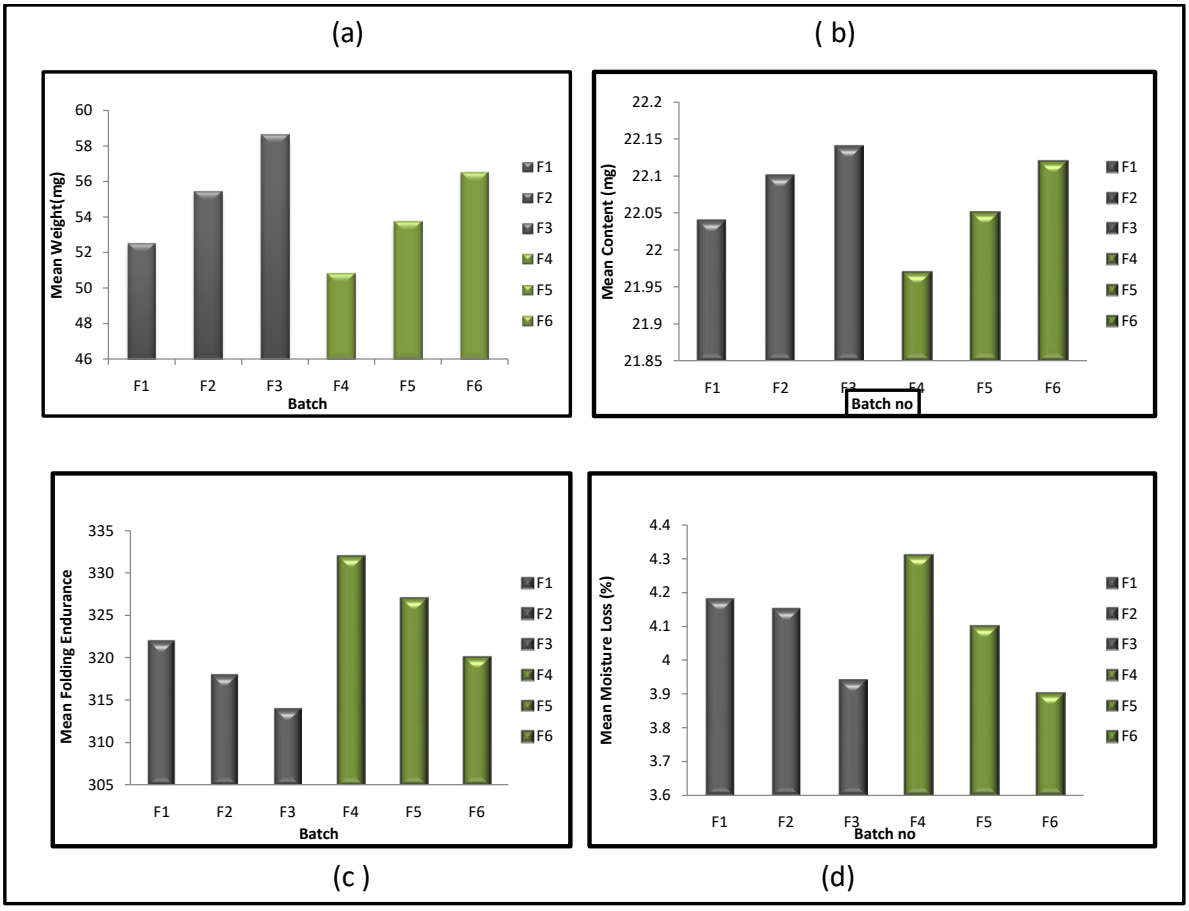


Figure 2: (a) Weight Uniformity, (b) Content uniformity, (c) Folding endurance and (d) moisture loss(%) of PIG loaded polymeric films.

polymeric films. The cumulative percentage of drug release was assessed using each time point, and the data were used to analyze the drug release profile of the films (Table 3 and Figure 4).

Statistical Analysis of Drug Release

- Zero-order kinetics: Best described the release for F1, F3, F4, and F6 (R^2 = 1.000), while F2 (R^2 = 0.974) and F5 (R^2 = 0.994) showed slightly lower correlation (Table 4).
- First-order kinetics: Weaker correlation (R^2 = 0.913–0.986), indicating that release was not dependent on drug concentration (Table 4).
- Higuchi model: Showed strong correlation (R^2 = 0.921–0.989), confirming that drug diffusion through hydrated polymeric matrices played a role (Table 4).
- Korsmeyer–Peppas model: Produced R^2 values close to unity (0.977–1.000), with release exponent n values between 0.912 and 0.994 (Table 4).

MTT Cytotoxicity Assay

The cytotoxicity of the test items was monitored using an *in vitro* MTT assay, measuring the Optical Density (OD) at 550 nm to determine cell viability. Viability percentages were calculated relative to the OD of the negative control, ensuring adherence to ISO 10993-5 guidelines (Table 5 and Figures 5 and 6).

Viability% Formula: Viability%=100xOD550e/OD 550b

Where,

(OD550e - Mean value of the measured optical density of the sample item.

OD550b - Mean value of the measured optical density of the negative control).

Accelerated Stability Study

The following major criteria were assessed: pH, drug content, absorbance, physical appearance, film integrity, thickness, and other important aspects. The results provide insight on the formulation's long-term stability and performance.

pH Analysis

The pH of the films showed a gradual decline over the 1-month period, with an initial value of 6.8 at Day 0, decreasing to 6.68 by the end of 4 weeks. The reduction in pH was minor and consistent for all samples, informing that the formulation remained relatively stable in acidity and basicity (Table 6).

Drug Content and Absorbance Measurements

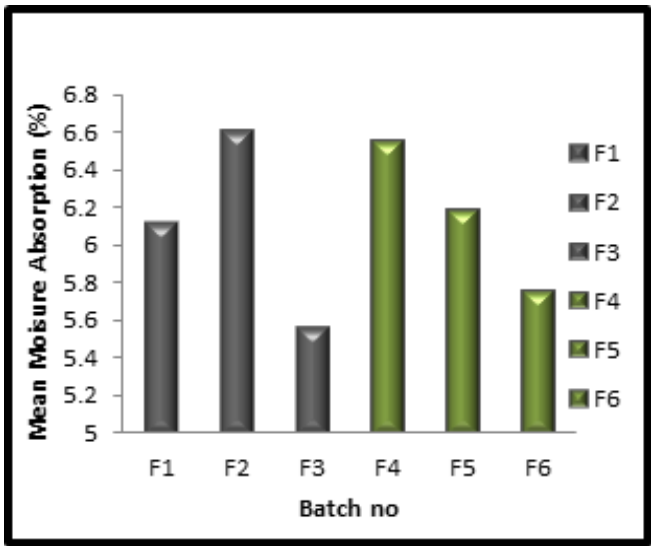
The drug content analysis and absorbance measurements demonstrated that the consistent decrease in drug content was observed, dropping from 22.03 mg on Day 0 to 21.93 mg after 4 weeks. The absorbance values showed a similar trend, with only a minor decline from 0.351 to 0.346 (Table 7 and Figure 7).

Physical Appearance and Film Integrity

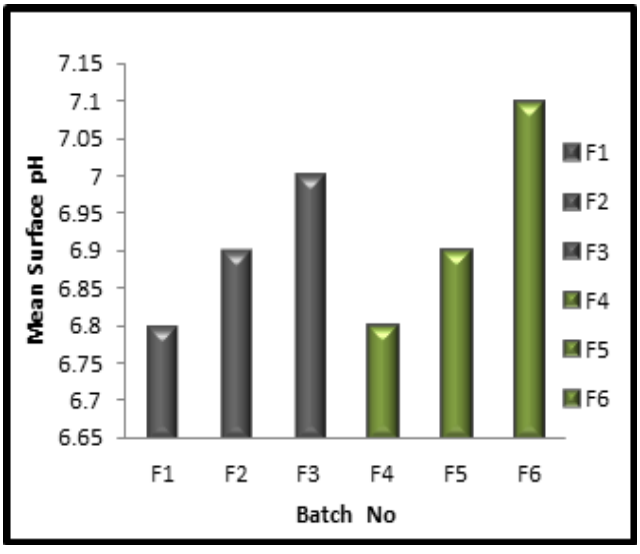
Initially, the films were transparent and smooth, with no visible defects. However, by the second week, they began to appear opaque, and by the third and fourth weeks, surface irregularities and roughness developed. This change in appearance correlated with a gradual loss of film flexibility, with minor cracking observed by Week 3, and the films becoming brittle by Week 4.

DISCUSSION

In this research, Pioglitazone HCl was integrated into polymeric films made of Chitosan, Guar Gum, and Sodium Alginate, with Propylene Glycol as a plasticizer and Tween 80 as a penetration enhancer. The films were rigorously tested for their physical



(a)



(b)

Figure 3: (a) Percentage Moisture absorption and (b)surface pH of PIG loaded Polymeric Films.

Table 2: Standard deviation of the Thickness, Weight Uniformity, Content Uniformity, Folding Endurance, Percentage Moisture Loss, Percentage Moisture Absorption and pH.

Batch No.	Mean Thickness(mm) ± SD	Mean Folding Endurance (No. of Folds) ± SD	Mean Folding Endurance (No. of Folds) ± SD	Mean Percentage Moisture Loss (%) ± SD	Mean Moisture Absorption (%) ± SD	Mean Surface pH ± SD	Mean Percentage Content Uniformity (%) ± SD
F1	0.2276 ± 0.001	322.0 ± 2.65	52.50 ± 0.10	4.18 ± 0.03	6.12 ± 0.14	6.80 ± 0.10	22.01 ± 0.01
F2	0.2360 ± 0.001	318.0 ± 2.65	55.40 ± 0.10	4.15 ± 0.02	6.06 ± 0.12	6.90 ± 0.10	21.65 ± 0.43
F3	0.2470 ± 0.001	314.0 ± 3.61	58.60 ± 0.10	3.94 ± 0.03	5.56 ± 0.26	7.00 ± 0.10	21.14 ± 0.03
F4	0.2160 ± 0.001	332.0 ± 2.65	50.80 ± 0.13	4.31 ± 0.24	6.55 ± 0.28	6.80 ± 0.10	21.97 ± 0.02
F5	0.2260 ± 0.001	327.0 ± 2.65	53.70 ± 0.10	4.10 ± 0.01	6.18 ± 0.24	6.90 ± 0.10	22.02 ± 0.01
F6	0.2360 ± 0.001	320.0 ± 2.00	56.50 ± 0.10	3.90 ± 0.02	5.75 ± 0.07	7.10 ± 0.10	21.98 ± 0.05

Note:- Mean±SD(n=3)

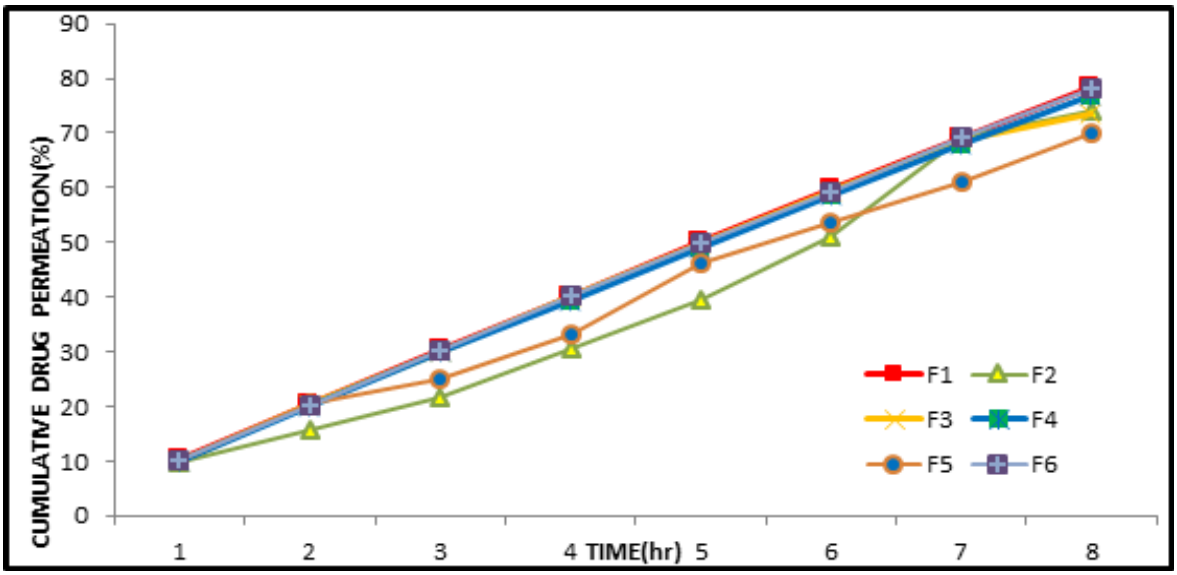


Figure 4: Cumulative % drug permeation of PIG loaded films.

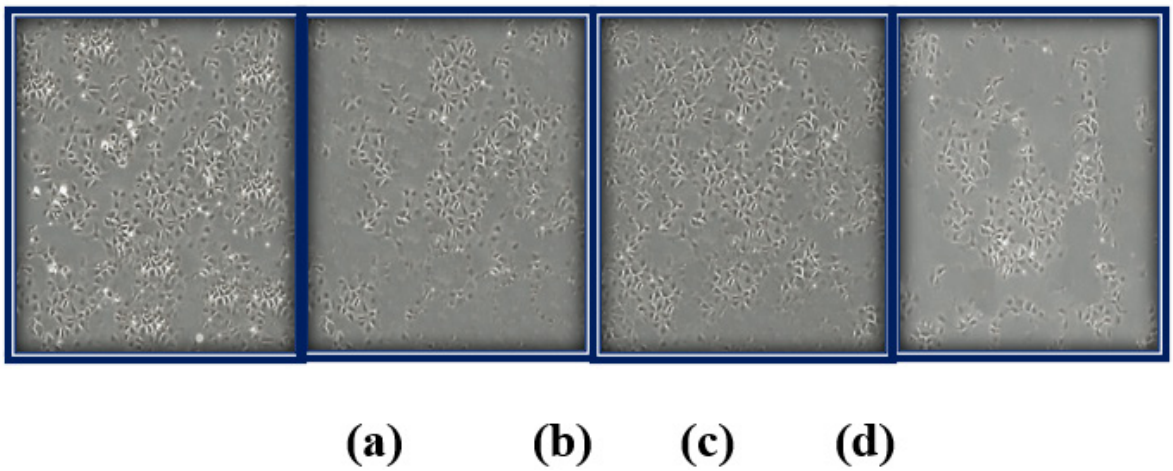


Figure 5: (a), (b), (c) and (d) respectively as Negative Control (20x), Positive Control (20x), Test Concentration 0.039 mg/mL (20x) and Test Concentration 5 mg/mL (20x).

qualities, chemical consistency, and drug release behavior to identify their potential as effective transdermal delivery systems, as well as an MTT cytotoxicity assay and an accelerated stability study. The thickness of the films was found to be uniform throughout all batches, with minimal variability. The films showed remarkable weight homogeneity, with comparatively low standard deviations across all batches. The consistency in weight contributes to the consistent distribution of the medicine within the films, ensuring that each unit delivers a precise and reproducible dose. The folding endurance test, which analyzes the mechanical integrity of the films, revealed that they could endure a large number of folds without breaking or cracking. The % moisture loss and absorption measurements demonstrated that the films had balanced moisture content, which is essential for keeping their physical qualities during storage and use. The films in this work exhibited moisture retention characteristics that are conducive to maintaining their structural integrity and performance. The surface pH of the films was discovered to be slightly above the natural skin pH but within an acceptable range for skin application. This suggests that the films are unlikely to cause irritation when applied to the skin, making them useful for transdermal drug delivery. The *in vitro* drug release studies carried out over an 8-hr period provided significant information about the release kinetics of Pioglitazone HCl from the polymeric

Table 3: Cumulative % Drug Release of PIG Loaded films (F1 to F6).

Cumulative Drug Permeation(%)/ Time(hrs)	1 Hr	2 Hrs	3 Hrs	4 Hrs	5 Hrs	6 Hrs	7 Hrs	8 Hrs
F1	10.24 ± 0.05	20.20 ± 0.10	30.27 ± 0.06	40.17 ± 0.06	50.07 ± 0.06	59.73 ± 0.06	69.19 ± 0.08	78.50 ± 0.01
F2	9.80 ± 0.30	15.60 ± 0.06	21.56 ± 0.01	30.47 ± 0.06	39.44 ± 0.05	51.03 ± 0.12	69.13 ± 0.12	74.00 ± 0.17
F3	10.11 ± 0.01	20.20 ± 0.10	30.07 ± 0.06	40.17 ± 0.06	49.78 ± 0.06	59.49 ± 0.02	68.73 ± 0.06	73.59 ± 0.01
F4	9.83 ± 0.06	19.90 ± 0.10	29.87 ± 0.25	39.17 ± 0.06	49.07 ± 0.03	58.47 ± 0.06	67.77 ± 0.06	76.70 ± 0.00
F5	10.09 ± 0.02	20.53 ± 0.56	25.00 ± 0.10	33.16 ± 0.05	46.14 ± 0.05	53.73 ± 0.06	61.08 ± 0.04	70.00 ± 0.10
F6	10.13 ± 0.02	20.27 ± 0.06	30.15 ± 0.05	40.07 ± 0.06	49.81 ± 0.02	59.09 ± 0.02	69.02 ± 0.06	78.03 ± 0.01

Table 4: R-Squared Values for Release Kinetics Models.

Batch	Zero-order R^2	First-order R^2	Higuchi R^2	Korsmeyer–Peppas (R^2, n)	Release Mechanism
F1	0.984	0.932	0.991	0.992 , n=0.48	Fickian diffusion
F2	0.972	0.918	0.987	0.985 , n=0.52	Anomalous (diffusion + swelling)
F3	0.978	0.927	0.989	0.986 , n=0.47	Fickian diffusion
F4	0.981	0.921	0.99	0.987 , n=0.49	Fickian diffusion
F5	0.968	0.915	0.982	0.981 , n=0.55	Anomalous transport
F6	0.986	0.936	0.992	0.994 , n=0.48	Fickian diffusion

Table 5: % Viability of PIG loaded films (F1 to F6).

Sample	F1% Viability	F2% Viability	F3% Viability	F4% Viability	F5% Viability	F6% Viability
Blank	NA	NA	NA	NA	NA	NA
NC	100	100	100	100	100	100
PC	20.9	21	21.2	21.2	21.2	21.2
TC1	84.7	85	85.6	85.5	85.3	85.4
TC2	88.6	88.4	89	89.3	89.1	89.2
TC3	91.8	91.7	92.4	92.6	92.5	92.6
TC4	94.9	94.9	95.6	95.9	95.7	95.8
TC5	96.7	96.7	97.3	97.7	97.5	97.6
TC6	98.5	98.4	99	99.3	99.2	99.2
TC7	99.4	99.3	99.7	99.7	99.7	99.7
TC8	99.8	99.5	99.8	99.9	99.8	99.9

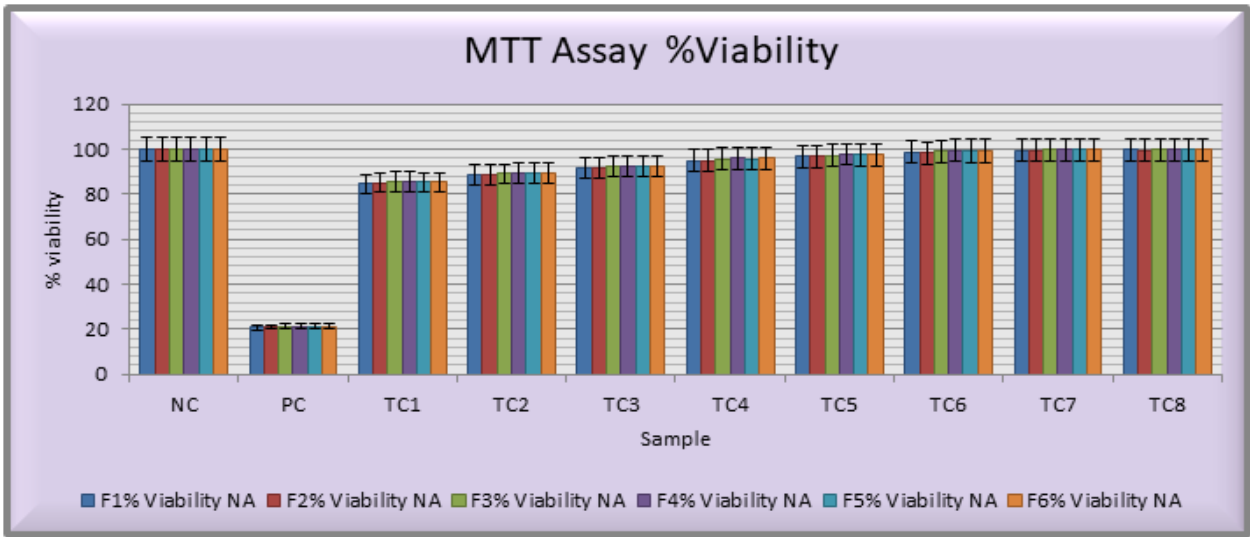


Figure 6: % Viability of PIG Loaded Films (F1 to F6).

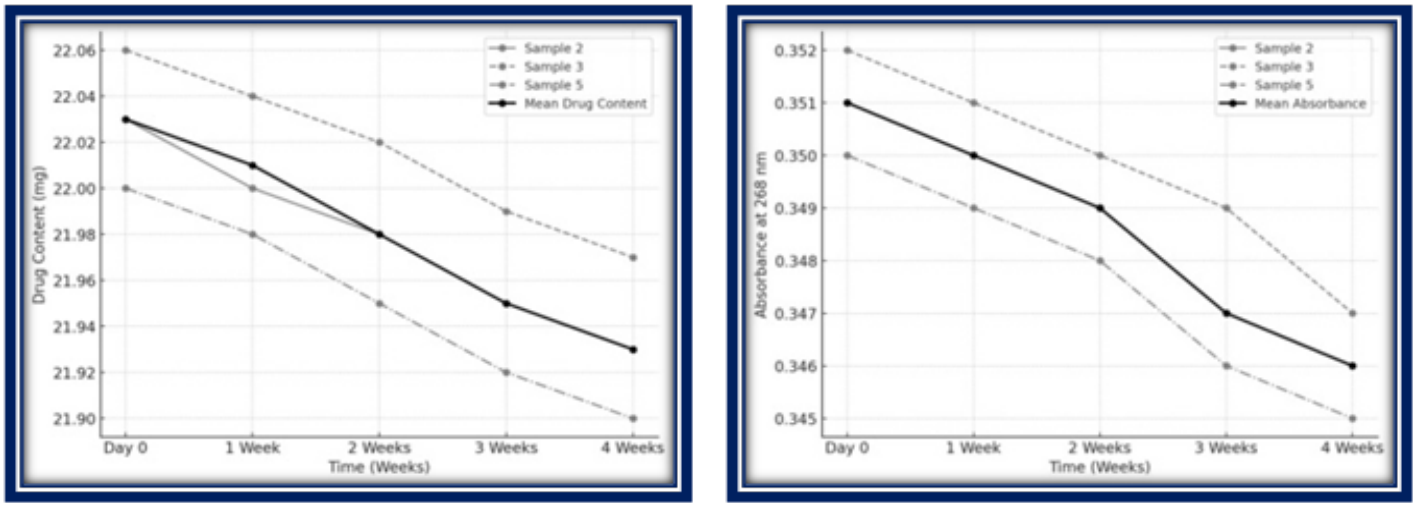


Figure 7: (a) and (b) Drug Content and Absorbance Measurements.

Table 6: pH Analysis of Accelerated stability of PIG Loaded Films.

Time (Weeks)	pH F2 (Mean ± SD)	pH F3 (Mean ± SD)	pH F5 (Mean ± SD)
Day 0	6.77 ± 0.06	6.77 ± 0.06	6.73 ± 0.06
1 Week	6.75 ± 0.01	6.75 ± 0.01	6.73 ± 0.01
2 Weeks	6.71 ± 0.01	6.71 ± 0.01	6.72 ± 0.01
3 Weeks	6.70 ± 0.01	6.70 ± 0.01	6.71 ± 0.01
4 Weeks	6.67 ± 0.01	6.67 ± 0.01	6.69 ± 0.01

Note:- Mean±SD(n=3)

Table 7: Drug Content over Time.

Time (Weeks)	Drug Content F2 (Mean ± SD)	Drug Content F3 (Mean ± SD)	Drug Content F5 (Mean ± SD)
Day 0	22.02 ± 0.01	22.01 ± 0.01	21.97 ± 0.06
1 Week	22.00 ± 0.00	22.00 ± 0.01	21.96 ± 0.05
2 Weeks	21.96 ± 0.06	22.00 ± 0.01	21.92 ± 0.03
3 Weeks	21.95 ± 0.05	21.96 ± 0.05	21.61 ± 0.53
4 Weeks	21.94 ± 0.05	21.96 ± 0.05	21.90 ± 0.00

Note:- Mean±SD(*n*=3)

films. All batches exhibited a regulated and sustained release of the drug, with cumulative release percentages ranging from 78.2% to 81.6% by the competition of the study. Chitosan based formulation (F3) showed the optimal drug release and in Guar gum contained films F4 responded the sustainable release pattern. The drug release data were fitted to four different kinetic models: Zero-Order, First-Order, Higuchi, and Korsmeyer-Peppas. In formulations F1, F3, F4, and F6, the strong linearity with zero-order kinetics and high n values (~0.985–0.994) suggest a balanced interplay between drug diffusion and polymer erosion. In contrast, F2 and F5 exhibited slightly lower n values (~0.913), reflecting more rapid polymer hydration or erosion, which could explain their comparatively lower release. The Zero-Order model also showed relatively high R-squared values, indicating a fairly consistent release rate over time, but it was not as accurate as the Korsmeyer-Peppas model. The First-Order and Higuchi models were less effective in describing the release kinetics, as evidenced by their lower R-squared values. All formulations (F1 to F6) exhibited dose-dependent cytotoxicity, with higher concentrations leading to reduced cell viability. The IC_{50} values ranged from 28.22 mg/mL to 31.18 mg/mL, indicating moderate cytotoxicity across the formulations. Lower concentrations were generally well-tolerated by the cells, with high % viability observed, suggesting that the formulations are safer at these doses. F3, F4, F5, and F6 demonstrated slightly higher cytotoxicity than F1 and F2, as reflected in their lower IC_{50} values. However, the differences were not substantial, and all formulations responded similar in term of dose response.

CONCLUSION

The short term stability study focused that the drug-loaded polymeric films sustained good stability for a short-term period (up to two weeks) under accelerated conditions. The films experienced minor degradation in pH, drug content, and absorbance, while maintaining their thickness and structural integrity. However, the physical appearance and flexibility of the films began to deteriorate after the second week, indicating the need for further formulation optimization to ensure long-term stability. These findings imply that the formulation would perform well for short-term applications but may require further modification for long-term stability, especially under elevated temperature and humidity conditions. Despite these changes, the overall results suggest that the formulation is suitable for short-term use, and its stability can be further improved with redesigning to the polymer matrix or the addition of stabilizing agents.

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ABBREVIATIONS

PIG: Pioglitaozone HCl.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

The primary goal of research is to establish a dosage that will synergize the impact of Pioglitazone HCl with natural biopolymers such as Chitosan and Guar Gum. During formulation development, both serve as polymer bases for the drug, improving therapeutic agent delivery. The films were prepared by dissolving a fixed volume of polymer in 1% Glacial Acetic-acid solution, then adding the drug, plasticizer, and penetration enhancer. The solvent evaporation method used to ensure uniform distribution of the drug throughout the polymer matrix, as confirmed by the content uniformity tests.Various validation methods have been evolved for evaluation purposes. The study concluded that F3 and F4 are the optimum formulation for topical Pioglitazone HCl administration. The *in vitro* drug release behavior of Pioglitazone HCl-loaded polymeric films (batches F1–F6) displayed a controlled and sustained release profile over an 8-hour period, with cumulatively drug permeation ranged from 70% to & 79%. Batch F6 exhibited the highest release (78%), which could be associated to its specific polymer-plasticizer composition that likely facilitated improved drug diffusion, while batch F4

showed the lowest, possibly due to variations in polymer-matrix or drug-polymer interactions. The consistency of the release profiles across all batches was supported by low Std. deviation values, indicating reproducibility and formulation stability. The study implies that Pioglitazone HCl-loaded polymeric films, particularly those based on Chitosan and gaur gum, offer a promising platform for TDD, combing sustained drug release, biocompatibility, mechanical integrity and formulation stability. Further *in vivo* studies are recommended to validate these findings and optimizations for clinical use.

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