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METFORMIN ADORNED BILAYER TABLETS FOR TYPE II DIABETES MANAGEMENT: FORMULATION DEVELOPMENT AND CHARACTERIZATION

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Abstract

Diabetes mellitus is a chronic metabolic disorder and its management requires uninterrupted drug administration, as failure to show the compliance results in fatal consequences. This study was executed to develop, optimize and characterize metformin HCl adorned bilayer tablets which underwent initial burst release in the stomach followed by a sustained release pattern to meet the once daily requirements for patients acceptability. Metformin HCl is scheduled to be taken three times a day due to having reduced half-life of 2-6 h. Therefore, its development into bilayer tablets having both immediate (IR) and sustained release (SR) layers would be clearly beneficial in reducing the frequency of administration as well as patients compliance. The immediate release layer anticipated the initial burst release in the stomach to quickly produce the antidiabetic effect with subsequent persistent extended action generated by controlled release layer. Direct compression method was used to fabricate bilayer tablets of metformin HCl by incorporating numerous polymers (HPMC K4M, carbopol and chitosan) in varied concentrations. The drug and polymeric admixtures were assessed for compatibility through FTIR and results revealed no interactions among the drug and various polymers. The flow parameters were found to be within the acceptable official range. After compression into bilayer tablets different physico -chemical tests were performed. All the tests were found to be consistent with official limits. The results of *in-vitro* data showed biphasic release pattern with the release from IR layer within 15 minutes and up to 24 h controlled drug release from SR layer. MT3 was chosen as an optimized formulation batch based on the results of *in-vitro* controlled drug release, physico-chemical tests and stability profiles. Kinetic model fitting of the release data of formulations (MT1-MT9) demonstrated Fickian diffusion mechanism with zero order kinetics ($n<0.5$). There was no statistical significant difference found for cumulative drug release of IR layer ($p>0.05$), however, for SR layer a significant difference was observed for the cumulative amount of drug release ($p<0.05$). Thus, the anticipated results revealed the potential of carbopol polymer for assuring the biphasic release of metformin HCl.

Keywords: Diabetes mellitus, bilayer tablet, Carbopol, direct compression, *in-vitro* drug release.

1. Introduction

A universal serious health problem with worldwide economic and social repercussions is diabetes mellitus. It is a chronic condition, characterized by elevated blood glucose levels. It is brought on by either insufficient insulin production, insulin resistance, or a combination of both (1). The oral hypoglycemic drug, metformin HCl, which has excellent water solubility, is used to treat type 2 diabetes mellitus. It lowers blood glucose levels by increasing peripheral and hepatic insulin sensitivity (2). It is a first-line oral biguanide drug with 50-60 % oral bioavailability in fasting condition. The drug exhibits slow absorption profile, with elimination half-life of 6.2 h. Its peak plasma concentrations are attained after 4-8 h of oral drug administration in the form of extended release formulations (3).

The oral route holds a significant platform in drug delivery due to simple administration technique, avoidance of sterility problems, increased patient compliance and adaptability in dosage form design. The appropriate dosage of drug should arrive at the site of action quickly and reside there long enough to have the intended therapeutic effect (4).Controlled release delivery systems, which employ a number of processes to administer and maintain the drug in specific concentrations in the blood stream, provide the perfect dose schedule, which maintains patient compliance and helps prevent overdosing as well as adverse effects (5). The development of innovative and more effective tablet dosage forms is a result of technological advancements and increased awareness of the dire need to modify conventional tablet to improve acceptability and bioavailability. A dual release tablet is an oral dosage form in the shape of a unit-compressed tablet (6).

The successful development of controlled release formulations and numerous characteristics to offer a means of effective drug delivery systems has given rise to a new arena, which is represented by the bilayer tablet. Bilayer tablets were introduced to deliver drugs under controlled conditions with predictable release schedules (7). One of its layers is designed to guarantee the drug's fast extraction and seeks to quickly attain a high serum concentration. A controlled release hydrophilic matrix makes up its second layer, which attempts to sustain an effective plasma level for an extended period of time. The quick release of the drug from the first layer causes an abrupt rise in blood concentration, which is necessary for the pharmacokinetic benefit. However, as the drug is released from the second sustained layer, the blood level stabilizes (8). The goal of using controlled, or sustained drug delivery is to decrease the frequency of doses or increase the drug's efficacy (9). This type of strategy is generally used when attainment of quicker relief is desired. The sustained release phase then controls the drug release behavior to avoid recurrent administration. Recent literature reports reveal the development of metformin HCl loaded bilayer tablets, either alone or in the form of combination therapy. Nguyen *et al*. developed bilayer tablets of metformin HCl, as sustained release layer, and sitagliptin, as immediate release layer for management of type 2 diabetes (10). Similarly, Patil *et al*. prepared metformin HCl and glimepiride loaded bilayer tablets, with metformin HCl as sustained layer (11). Taken into account, the present study is planned for the design, development and evaluation of bilayer metformin HCl tablets, as biphasic drug delivery approach, by employing numerous polymers, such as chitosan, carbopol 940P and HPMC K100M.

2. Materials & Methods

2.1. Materials

Metformin-HCl was received as a generous gift from Wilshire Pharma Private Limited, Lahore. Polymers employed were carbopol, chitosan & HPMC K4M (Sigma Aldrich, USA). Micro-crystalline cellulose (diluent), Kyron T (super-disintegrant),CMC (binder), magnesium stearate (lubricant) & talc (glidant) (Sigma Aldrich, USA). Gomal Center of Pharmaceutical Sciences provided all the requisite instruments for this study. Chemicals of analytical grade without any further purification were employed.

2.2. Methods

2.2.1. Pre-formulation Studies

Pre-formulation analysis is mandatory to get an insight into the optimum selection of both active and excipients for final formulation that gave necessary physico-chemical properties, ultimately providing safe, effective, stable and consistent dosage forms. Among the formulation ingredients, there is a direct relation between active moiety and excipients to facilitate not only administration but also control the drug release as well as provide protection from extraneous environment (12). Moreover, despite inactive the excipients play their role in interacting with active ingredient and alter physico-chemical attributes like drug stability, dissolution or can potentiate the drug deterioration. Thus a careful selection of excipients is compulsory to yield a robust and efficacious formulation highlighting patient compliance via ease in administration leading to improved control over drug release and improved bioavailability and shelf life. Thus, pre-formulation is regarded as an important step in drug delivery research and vital criteria for initiating the research work (13).

2.2.1.1. Metformin-HCl Standard Calibration Curve

Originally, a stock solution of the drug was made in phosphate buffer solution of pH 7.4 by dissolving 100 mg of active drug in 100 ml of the solvent to produce 100 ml (1000 µg/ml) in volumetric flask. From the stock solution various dilutions were made by using phosphate buffer solution of pH 7.4. The dilutions were run on UV spectrophotometer (UV-1601, Shimadzu, Japan) at 232 nm λmax compared to blank solution to get the absorbance. The experiment was triplicated and results averaged (14).

2.2.1.2. FTIR Analysis

Any pharmaceutical dosage form fabrication demands drug excipient compatibility. It must be ensured that there is complete absence of any sort of interaction among active and inactive formulation ingredients such that they do not alter the shelf life profile of the drug product. So. it is dire need to carefully manipulate excipient choosing for solid dosage forms, contributing towards its efficacy and stability. Compatibility study is performed to investigate the potential of various excipients for stable formulation lacking any interaction. Also there is ruling out of any toxic potential of the excipients (15).

KBr pellet technique was used for active drug, polymers and their admixtures to perform FTIR spectra (Spectrum 100, Perkin Elmer, USA). FTIR scans were documented from 4000 – 625 cm^{-1} . The spectra were compared to note any discernible changes (16).

2.2.1.3. Micromeritical Characteristics

2.2.1.3.1. Angle of Repose

The greatest angle that may be produced between a pile of powder's surface and horizontal surface known as angle of repose was determined by noting height (h) and radius (r) of powder bulk and fitted in the equation below to calculate the angle of repose (17):

Angle of repose (θ) = $\tan^{-1}$ h / r

2.2.1.3.2. Bulk & Tapped Density

A specific powder weight (W) was introduced in a graduated cylinder and bulk volume was noted. Bulk density was calculated by the following equation:

Bulk Density (ρ_{bulk}) = W / V_{bulk}

Similarly, tapped density was determined by the same graduated cylinder method, except tapping of the cylinder was performed 500, 750, and 1250 times, until the volume difference between successive taps was < 2 %. The last reading was shown by (V_{tapped}) (18).

Tapped Density (ρ_{tapped}) = W / V_{tapped}

2.2.1.3.3. Compressibility / Carr's Index

It is simple, rapid and popular technique for predicting powder flow characteristics (19).
Carr's Index = $(\rho_{tapped} - \rho_{bulk} / \rho_{tapped}) * 100$

2.2.1.3.4. Hausner's Ratio

Comparison of ratio of taped versus bulk density provided Hausner's ratio value and discloses powder flow properties (20).
Hausner's Ratio = $\rho_{tapped} / \rho_{bulk}$

2.3. Preparation of Metformin-HCl Bi-layer Tablets

The preparation of drug loaded bilayer tablets involved loading of 250 mg of metformin-HCl in each of immediate release as well as controlled release layers. An immediate release layer was constituted after accurate weighing of drug as well as super disintegrant (Kyron T) and diluent (MCC). This mixture was passed through sieve number 40, followed by thorough mixing in the plastic bag for 5 min. Magnesium stearate was dusted onto the immediate release fraction to facilitate lubrication step. The whole contents were thoroughly mixed for additional 2 min, followed by passing through sieve number 60.
Similarly, controlled release layer was constituted by adequate mixing of drug portion with already established proportions of release retardants such as Carbopol, chitosan and HPMC K4M. There was an addition of dry binder (CMC) and diluent (MCC) to make the resultant bulk. It was passed through sieve number 40 and through mixing in the plastic bag. Finally, lubricant and glidant (Mg. stearate and talc) were added. The whole mixture was passed through sieve number 60 with adequate admixture (21). The composition of IR and CR layers of metformin bilayer tablets is summarized in Tables 1 and 2, respectively.

Table 1. Composition of immediate release layer of metformin HCl bilayer tablets

Ingredients	Amount (mg)
Metformin HCl	250
Kyron T	48
CMC	1.5
Magnesium stearate	0.5
Total	300

Table 2. Composition of controlled release layer of metformin HCl bilayer tablets

Ingredients (mg)	MT1	MT2	MT3	MT4	MT5	MT6	MT7	MT8	MT9
Metformin HCl	250	250	250	250	250	250	250	250	250
Carbopol	75	100	125	-	-	-	-	-	-
HPMC K4M	-	-	-	75	100	125	-	-	-
Chitosan	-	-	-	-	-	-	75	100	125
MCC	70	45	20	70	45	20	70	45	20
CMC	3	3	3	3	3	3	3	3	3
Magnesium Stearate	1	1	1	1	1	1	1	1	1
Talc	1	1	1	1	1	1	1	1	1
Total	400	400	400	400	400	400	400	400	400

2.3.1. Compression Process

Single punch tablet machine (AR 400, Erweka, GMBH, Germany) was used to pre-compress CR layer in the die cavity, followed by placement of requisite amount of IR layer fraction to directly compress both layers with adequate hardness value of 5-10 $kgcm^{-2}$ (22).

2.4. Characterization of Bi-layer Tablets

2.4.1. Diameter & Thickness

Bilayer tablets dimensional specifications were calculated by using vernier-caliper (Erweka, Germany). Mean ± SD was used to indicate the average values.

2.4.2. Hardness

A hardness attribute of bilayer tablet physical test is crucial to withstand the necessary force during packaging and transportation. It was measured by Erweka hardness tester (Germany). This test was performed on randomly taken sample of 10 tablets from each formulation batch, the results were taken thrice and averaged to get mean ± SD (23).

2.4.3. Friability

Roche friabilator was used to determine the friability by randomly selected tablets. Initial weighed (W_0) was noted before treatment in the friabilator. After 4 minutes treatment the tablets were again weighed (W) (24). The following formula is utilized to calculate friability:

Friability (%) = $(W_0 – W / W_0) * 100$

2.4.4. Weight Variation

The weight variation analysis utilized initially weighing randomly taken 20 tablets to calculate an average weight followed by taking the individual tablet weight and compared with the average. The percent allowable limit for our bi-layer tablets was 7.5 % (25).

2.4.5. Content Uniformity

Content uniformity test was performed on randomly selected 10 tablets, crushed and an aliquot equivalent to 500 mg of Metformin-HCl was precisely weighed dissolved in PBS of pH 7.4 followed by its sonication and vortexing in order to completely dissolve the drug. The quantification of Metformin-HCl was done at 232 nm by means of a UV-spectrophotometer (UV-1601, Shimadzu, Japan). Triplicate readings were recorded (17). The following formula was employed for calculating % drug content:

$$\text{Drug Content percentage} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100$$

2.4.6. *In-vitro* Drug Release & Effect of Polymer Concentration

The compressed bi-layer tablets were assessed for *in-vitro* drug release by using USP type 1 (rotating basket) method. Dissolution medium (PBS pH 7.4, 900 ml) kept at 37 ± 0.5 ºC was used for this experiment. The tablets were placed in the baskets that were subjected to rotation at 50 rpm. After pre-selected time intervals, about 5 ml sample was withdrawn with the help of syringe having pore size 0.45 µm and quickly replenished with the same volume of fresh dissolution media to maintain the sink conditions. The dissolution medium should be placed at the same temperature. UV-spectrophotometer (UV-1601, Shimadzu, Japan) was used to determine Metformin-HCl contents at 232 nm λmax. From in-vitro data, cumulative percent drug release was determined with the help of equation generated through standard calibration curve. Triplicate reading were taken and averaged (26).

2.4.7. Kinetic Modeling

Model dependent approach was employed to note the kinetic profiling by fitting the *in-vitro* drug release data into various kinetic models such as Higuchi, Peppas, Hixon-Crowell, first and zero order kinetics, to investigate the mechanism of drug release from bi-layer tablets (27).

Zero order model shows the relationship between time versus fraction drug release, represented by the following equation:

$$Q = k_0 t \quad (8)$$

Where, Q shows drug release fraction; 't' represents time; k_0 shows zero order release rate constant.
First order model assumes lowering of matrix surface area over time upon its dissolution. This model chiefly fits the in-vitro release of controlled release matrix tablets. Following equation describes first-order kinetics:

$$\ln (1-Q) = -k_1 t \quad (9)$$

Where, Q represents drug release fraction; 't' represents time; k_1 denotes zero-order release rate constant.
Higuchi model highlights the linear relationship between time (square root) versus drug release fraction (Q), favoring Fickian diffusion.

$$Q = k_2 t^{1/2} \quad (10)$$

Where, k_2 signifies drug release rate constant.
Matrix erosion could be studied by utilizing erosion equation.

$$Q = 1- (1-k_3 t)^3 \quad (11)$$

Where, Q shows drug release fraction; 't' represents time; k_3 signifies drug release rate constant.
Peppas equation was utilized for predicting the drug release mechanism after putting drug release data in the following equation:

$$M_t / M_\infty = k_4 t^n \quad (12)$$

Where, M_t / M_∞ indicates drug release fraction; 't' represents time; k_4 shows release rate constant. The values of "n" highlight various mechanisms for drug release in case of cylindrical tablets (28).

2.4.8. Short-Term Stability Determination

The optimized formulation was chosen and subjected to short-term stability study at different values of temperatures and relative humidity. The room temperature (25 ± 2 °C, 65 % RH) and accelerated temperature (40 ± 5 °C, 75 % RH) were provided by utilizing stability chamber (Ti-Sc-THH-07-0400 Faisalabad, Pakistan). After specific time intervals, suitable aliquots were taken and necessary searching checks (official physico-chemical tablet tests) for the bilayer tablets were performed to note any significant variation upon storage at ambient and accelerated conditions (29).

2.5. Statistical Analysis

Statistical analysis was performed using GraphPad prism, version 8.0.2. Triplicate readings of all the tests were computed to generate mean ± SD. Statistical significance was checked by using one-way ANOVA and Student t-test. A value of 0.05 was taken as statistically significant p value.

3. Results & Discussion

3.1. Metformin HCl Standard Calibration Curve

The standard calibration curve was determined by plotting concentration vs absorbance values. Thevalue of R^2 (0.9948) showed good linearity (Figure 1). The generated equation Y = 0.03632*X-0.04333 further utilized to estimate drug contents and *in-vitro* drug release.

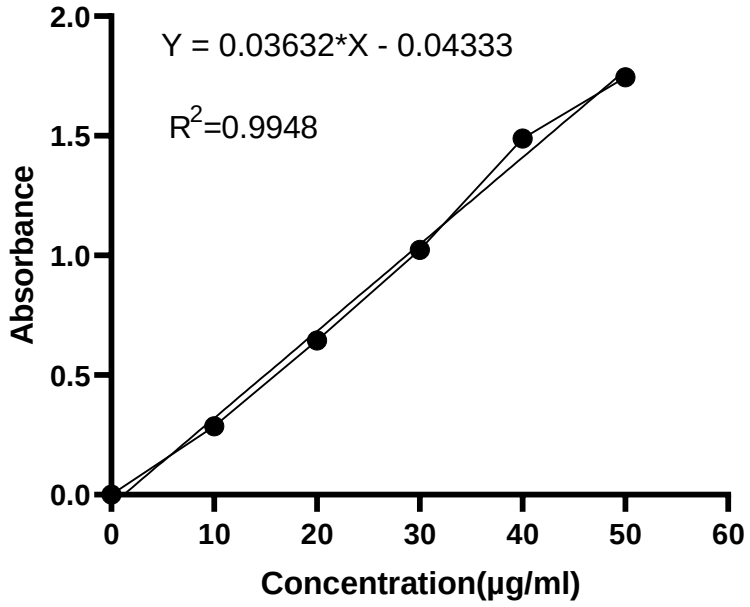


Figure 1. Standard calibration curve of metformin HCl in PBS; pH 7.4.

3.2. Granules Evaluation

Powder flow characteristics are regarded as crucial in various operations like mixing and mixture flow from hopper towards turret for compressional process. An important issue with poorly flowing powders in the pharmaceutical sector is the uneven and non-uniform mixture flow from the hoppers (30). Excellent flowability and compressibility attributes have been demonstrated by all formulations, regardless of the type and concentration of hydrophilic polymers. The values have shown that angle of repose was in the range of 23.18 ± 1.69 º to 27.44 ± 1.23 º, compressibility index between 10.23 ± 0.92 % to 14.89 ± 0.80 % and Hausner's ratio between 1.08 ± 0.10 to 1.19 ± 0.09, as evident in Table 3. Thus these pre-compressional characteristics of various formulation batches were shown to be within acceptable standard limits and highlight good flow behavior because the excipients utilized in the formulation of bilayer tablets were directly compressible. Our results agreed those of previous research conducted by Djebbar and his colleagues, who prepared metformin HCl effervescent floating tablets by melt granulation technique utilizing HPMC K4M and acacia gum as hydrophilic polymers (31).

Table 3. Evaluation of pre-compressional characteristics of metformin HCl trial formulations (MT1-MT9) (mean ± SD, n=3)

F. Codes	Angle of Repose (º)	Compressibility Index (%)	Hausner's Ratio
MT1	24.50 ± 1.22	11.56 ± 0.02	1.17 ± 0.03
MT2	26.33 ± 1.34	10.36 ± 0.11	1.09 ± 0.02
MT3	23.32 ± 0.56	11.47 ± 0.23	1.11 ± 0.11
MT4	25.45 ± 0.47	12.69 ± 0.56	1.08 ± 0.10
MT5	27.44 ± 1.23	13.56 ± 0.47	1.13 ± 0.06
MT6	25.27 ± 1.58	14.89 ± 0.80	1.15 ± 0.21
MT7	23.18 ± 1.69	14.77 ± 0.98	1.14 ± 0.03
MT8	26.89 ± 2.03	12.60 ± 0.59	1.16 ± 0.08
MT9	24.72 ± 0.99	10.23 ± 0.92	1.19 ± 0.09

3.3. FTIR Spectroscopy

The interactions between the drug and polymers employed in bilayer tablet formulation were explored using FTIR analysis, the results of which are illustrated in Figure 2. The FTIR spectrum of pure drug showed characteristic peaks as follows:

- N-H stretching represented by 3 absorption peaks of high intensity at 3365.19 cm^{-1}, 3285.46 cm^{-1} and 3138.54 cm^{-1}.
- C=N stretching represented by 2 absorption peaks of high intensity at 1630.09 cm^{-1} and 1538.74 cm^{-1}.
- Aliphatic CH_3 bending represented by 3 absorption peaks around 1469.89 cm^{-1}, 1440.75 cm^{-1} and 1420.23 cm^{-1}.
- C-N stretching of aliphatic diamines represented by 2 low intensity peaks at 1169.58 cm^{-1} and 1060.23 cm^{-1} (32).

The outcomes of FTIR analysis confirmed identification of metformin HCl in prepared drug admixture with polymers. Moreover, no new peak was observed in FTIR spectrum of drug physical mixture with polymers. The absence of any type of interactions between drug and excipients further corroborated the findings of analysis (22).

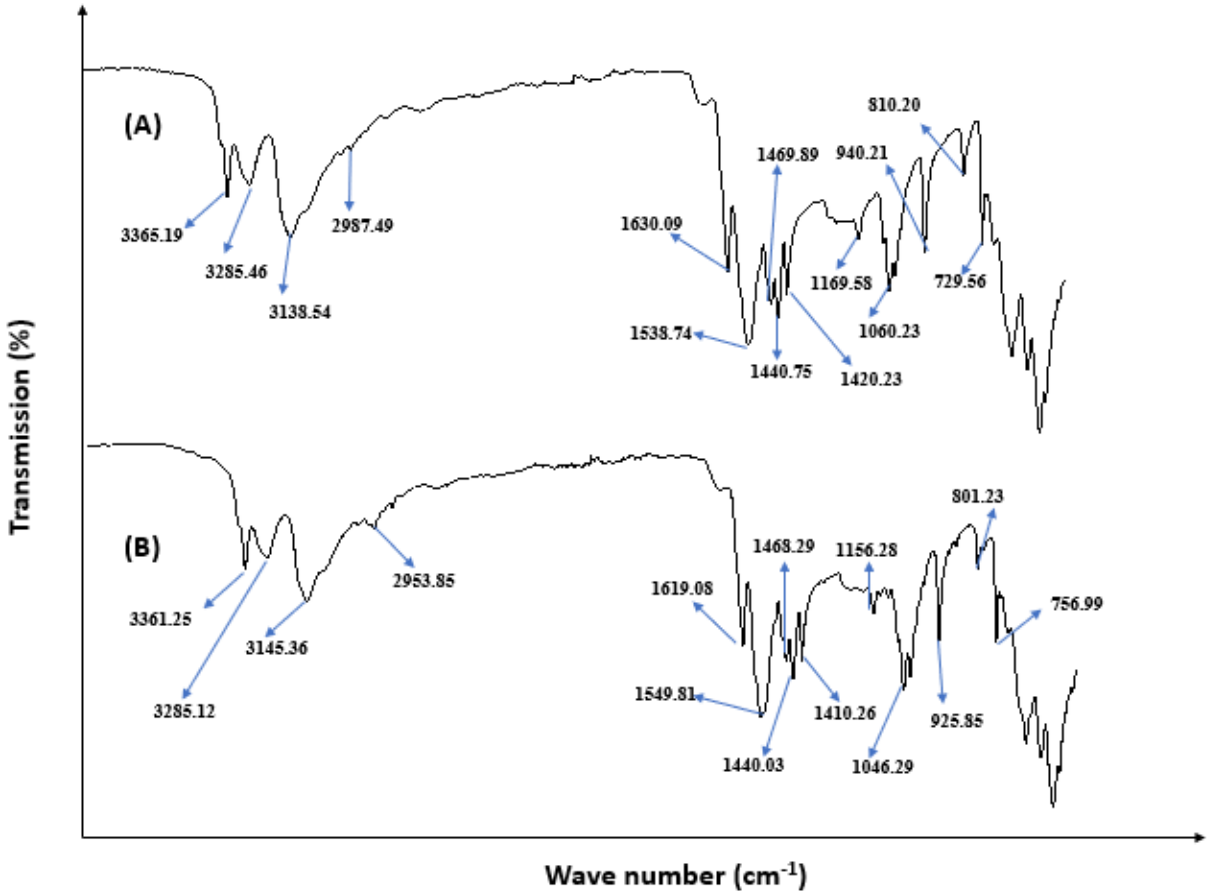


Figure 2. FTIR spectra of (A) metformin HCl and (B) physical drug mixture with polymers

3.4. Preparation of Metformin HCl Bilayer Tablets

Metformin HCl loaded bilayer tablets were successfully prepared by direct compression approach using various polymers in different concentrations. For developmental purpose, various tests were carried out, and nine bilayer tablet formulations were ultimately synthesized. The drug concentration remained constant in all formulation batches i.e. 250 mg. However, several polymers, including carbopol (MT1-MT3), HPMC K4M (MT4-MT6) and chitosan (MT7-MT9) were employed in the

formulations. The choice of direct compression technique was based on simplicity, cost-efficiency and less time consuming (10).

3.5. Evaluation of Metformin HCl Bilayer Tablets

The bilayer tablets produced by direct compression technique were subjected to post-compressional tests, as shown in Table 4. All tablet formulations demonstrated reproducible and satisfactory physical parameters. There was complete absence of any type of tablet flaws like picking, chipping or capping phenomena. The formulated bilayer tablets exhibited hardness in the range of 5.99 ± 0.55 to 7.25 ± 0.22 kg/cm^2. It was also observed that as the concentration of polymers in the formulations increased, there was proportional increase in hardness values. It could be attributed to the high entanglement and binding potential between drug and other constituents (33). Similarly, percent friability values were reported in the range of 0.24 ± 0.08 to 0.93 ± 0.06 %, which were in accordance with official compendial limit of < 1 %. This indicated robustness of tablets surfaces to withstand mechanical attrition or stress upon storage, transportation and handling (34). The results of dimensional specifications i.e. diameter and thickness, were also shown to be within acceptable limits. The findings of weight variation test ranged from 675±10.01 to 715±5.87 mg which complied with acceptable limit of ± 5 %. It was because of good flowability and adequate mixing of drug with other formulation excipients (27). The percent drug content of all formulated batches was also shown to be within acceptable range of 90 – 110 %. Low values of SD for drug content highlighted uniform distribution of drug within all formulations (35). Similar values of post-compressional parameters were also reported in earlier studies (6,36). In summary, the physical parameters of metformin HCl bilayer tablets showed no significant variations and complied with official limitations.

Table 4. Post-compression characteristics of metformin HCl bilayer tablets (mean ± SD)

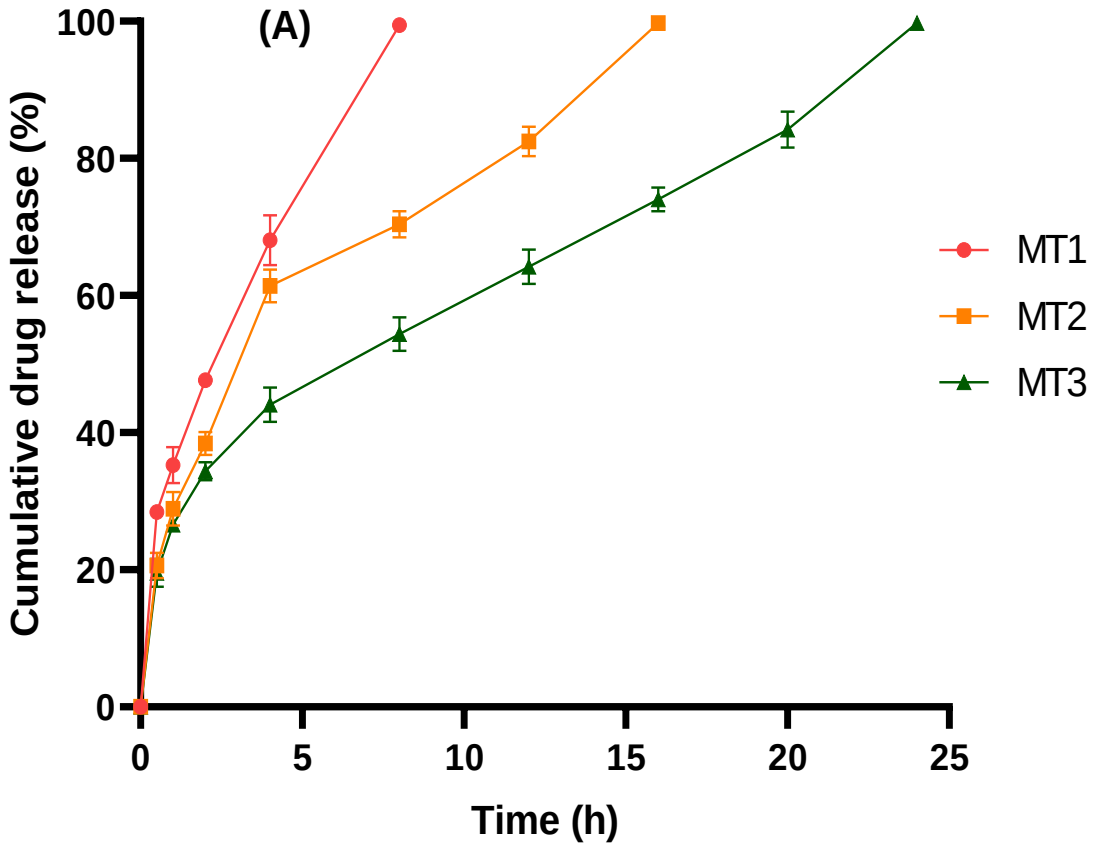
F/ Codes	Hardness (kg/cm^2)[a]	Friability[a] (%)	Thickness (mm)[a]	Diameter (mm)[a]	Weight Variation (mg)[b]	Percent Drug Content[a]
MT1	6.23±1.23	0.65±0.05	3.43±0.09	5.99±0.41	689±9.28	92.31±5.20
MT2	6.45±0.99	0.55±0.11	4.08±0.58	6.02±0.22	701±6.23	94.15±4.17
MT3	6.99±0.87	0.87±0.23	4.22±0.69	6.07±0.37	715±5.87	99.69±7.85
MT4	5.99±0.55	0.93±0.06	3.89±0.74	6.15±0.08	696±8.85	98.52±9.63
MT5	6.41±0.63	0.87±0.09	3.67±0.28	6.26±0.07	680±10.23	95.14±5.28
MT6	6.70±1.01	0.24±0.08	4.01±0.91	6.34±0.47	683±9.61	99.14±6.61
MT7	6.26±0.33	0.34±0.14	4.10±0.08	6.85±1.23	714±9.63	97.49±9.59
MT8	6.89±0.83	0.66±0.21	3.85±0.41	6.19±0.89	709±7.81	96.08±8.48
MT9	7.25±0.22	0.79±0.06	3.42±0.61	6.06±0.90	675±10.01	99.67±9.75

[a] = (n=10) and [b] = (n=20)

3.6. *In-vitro* Drug Dissolution & Relationship with Polymer Concentration

In this investigation, a variety of retarding polymers like carbopol, HPMC K4M and chitosan were employed in varied concentrations for the development of bilayer tablets. All tablet formulations (MT1-MT9) caused > 90 % of drug release (Figure 3). Results of *in-vitro* drug release showed that bilayer tablets prepared with carbopol i.e. MT1, MT2 and MT3 caused > 99 % drug release in 8, 16 and 24 h, respectively. This could be explained by the fact that formulations prepared with carbopol form a strong gel layer around tablets with less micro-viscosity in the swelled tablets due to the polymer anionic nature. The quick formation of gel layer formed barrier for the diffusion of drug, resulting in prolonged drug release (37). Moreover, the tablets swelling could also occur due to polymer hydration, which causes a rapid reduction in glass transition temperature of polymer to the dissolution medium temperature (38). It was also observed that as the concentration of carbopol in formulations increased, the drug release was further sustained. This was because of reduced regions of lesser micro-viscosity and closure of micropores inside the swelled tablets (39). Likewise, for MT4,

MT5 and MT6 formulations utilizing HPMC K4M as rate retarding polymer, > 90 % drug release was observed in 4, 12 and 16 h, respectively. The results also suggested that increased polymer concentration at the surface resulted in rapid gel layer formation after coming in contact with the medium, avoiding initial burst drug release (40). Based on these findings, it was demonstrated that carbopol is more effective to sustain drug release for greater time period as compared to cellulosic substances like HPMC when employed at similar concentrations. This could be explained by slightly cross-linked nature of carbopol which causes drug entrapment in hydrogel domains, resulting in slower erosion. On the contrary, the linear nature of HPMC and the absence of covalent cross-linking causes formation of gelatinous layer on tablets surface upon hydration which erode quickly (41). Complete drug release was observed in 4, 8 and 12 h when chitosan was employed in formulations MT7, MT8 and MT9, respectively. Chitosan was ranked lesser as compared to carbopol and HPMC K4M on the basis of retardant ability. It could be due to greater erosion rate of polymer which caused greater drug release in short time period, thus indicating greater disentanglement of polymeric chains. Thus, based on these findings, MT3 (carbopol containing formulation) was shown as optimized formulation due to its greater sustaining behavior.



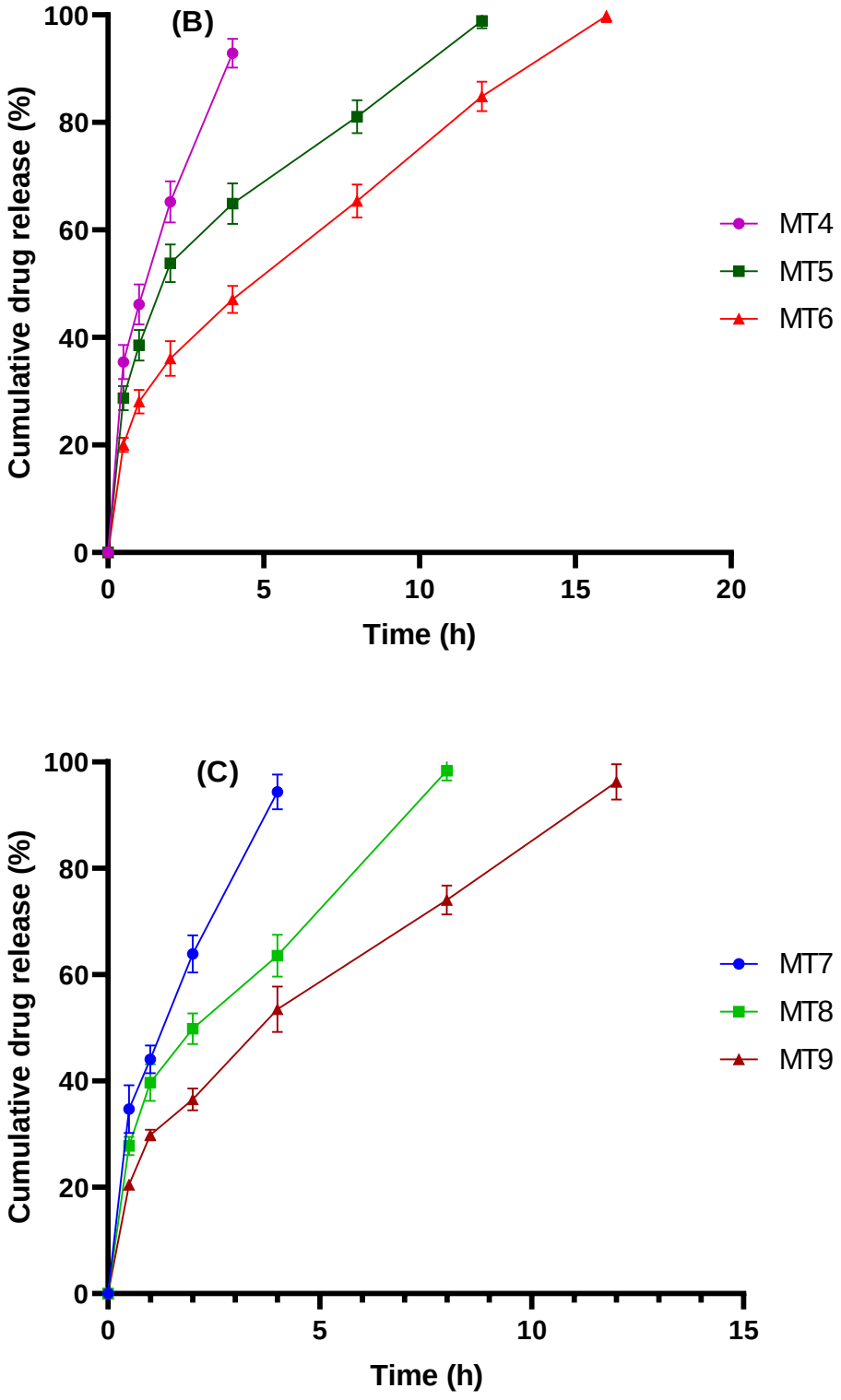


Figure 3. *In-vitro* drug release from (A) MT1-MT3 formulations containing carbopol, (B) MT4-MT6 formulations containing HPMC K4M and (C) MT7-MT9 formulations containing chitosan (mean ± SD, n=3)

3.7. Drug Release Kinetics

All formulations of metformin HCl loaded bilayer tablets (MT1-MT9) followed zero order kinetics, as shown by greater values of "r" as compared to other kinetic models (Table 5). The findings suggested "r" values in the range of 0.9623 to 0.9971. The outcomes of Higuchi and Hixon-Crowell

models to release data corroborated the contribution of diffusion and erosion phenomena in drug release. Generally the process of diffusion is responsible for drug release from matrix tablets composed of hydrophilic polymers (42). The greater "r" values for Higuchi model i.e. 0.9120 to 0.9702, indicated that all tablet formulations followed diffusion process. Fickian diffusion was the predominant mechanism of drug release as obvious from "n" values which were < 0.5 (43). ANOVA showed that there was insignificant difference in drug release from any tablet formulation after 15 min, but there was significant difference between MT3 and other formulations after 24 h ($p<0.05$).

Table 5. Kinetic model fitting of formulations MT1-MT9 of metformin HCl

F. Codes	Zero order		First order		Higuchi model	Hixon Crowell model	Korsemeyer-Peppas model	
	r	K_0	r	K_1	R	r	n	r
MT1	0.9623	10.01	0.9213	0.55	0.9543	0.9147	0.3412	0.9802
MT2	0.9745	6.52	0.8456	1.31	0.9623	0.8887	0.3859	0.8741
MT3	0.9862	6.89	0.8789	0.96	0.9471	0.8420	0.4047	0.8526
MT4	0.9899	10.36	0.8512	0.99	0.9610	0.9542	0.3983	0.9312
MT5	0.9852	11.47	0.8469	1.04	0.9702	0.9013	0.4012	0.9456
MT6	0.9963	8.59	0.9014	1.25	0.9459	0.9417	0.3546	0.9017
MT7	0.9971	12.30	0.9328	0.87	0.9623	0.9568	0.4087	0.9743
MT8	0.9850	8.09	0.8712	0.69	0.9120	0.8974	0.4247	0.9845
MT9	0.9829	5.24	0.8603	0.50	0.9204	0.9099	0.3960	0.9624

3.8. Stability Studies

For the determination of stability, the optimized formulation (MT3) composed of carbopol was subjected to short-term stability studies. For this purpose, MT3 was kept at ambient and accelerated conditions i.e. 25 °C with RH 65 % and 40 °C with RH 75 %, respectively, for 28 days. After specified time period, the formulation was assessed on the basis of various physical and physico-chemical characterization tests like friability, hardness, drug content, weight variation and *in-vitro* drug dissolution. The examination was conducted on various pre-determined time periods of 0, 7, 14, 21 and 28 days (44). The results of stability study indicated no significant variations in above-mentioned attributes after pre-selected time intervals, as shown by Tables 6 and 7. It could be inferred that metformin HCl loaded bilayer tablets exhibited stability, reliability and reproducibility as there was statistically insignificant difference in percent drug dissolution before and after stability testing ($p>0.05$).

Table 6. Stability studies of metformin HCl bilayer tablets of optimized formulation (MT3) under ambient environment (mean ± SD, n=3)

Time intervals (days)	Hardness (kg/cm^2)	Friability (%)	Weight variation (mg)	Drug content (%)	*In-vitro* drug release (%)
0	6.99±0.87	0.87±0.23	715±5.87	99.69±7.85	99.74±5.63
7	6.82±0.32	0.74±0.11	701±3.25	98.56±4.10	99.63±3.08
14	6.86±0.99	0.55±0.09	703±10.23	96.23±5.26	95.42±3.73
21	6.64±1.24	0.48±0.32	692±11.56	97.45±5.58	97.14±5.19
28	6.10±2.56	0.26±0.05	690±9.78	93.59±4.79	93.19±2.58

Table 7. Stability studies of metformin HCl bilayer tablets of optimized formulation (MT3) under accelerated environment (mean ± SD, n=3)

Time intervals (days)	Hardness (kg/cm^2)	Friability (%)	Weight variation (mg)	Drug content (%)	*In-vitro* drug release (%)
0	6.99±0.87	0.87±0.23	715±5.87	99.69±7.85	99.74±5.63
7	6.52±1.23	0.74±0.01	695±10.23	97.46±5.26	96.53±4.25
14	6.01±1.58	0.52±0.20	687±9.96	95.24±5.86	90.23±3.80
21	6.09±2.91	0.49±0.10	651±11.80	91.19±4.49	94.28±6.09
28	5.99±3.43	0.22±0.11	645±11.52	92.58±6.14	89.67±4.72

4. Conclusion

Diabetes mellitus management requires uninterrupted drug administration. MT3 was chosen as an optimized formulation based on the results of *in-vitro* controlled drug release, physico-chemical tests and stability profiles. It was composed of carbopol polymer that delayed the drug release appropriately up to 24 h. The drug release is markedly affected by nature as well as amount of polymers. This biphasic release circumvents the major demerits of controlled release tablets due to offering an initial loading dose (burst release) from IR layer (within 15 minutes) followed by a sustained release over an extended period of time. Taking altogether, it could be concluded that bilayer tablets are convincingly effective for the management of a chronic disease like diabetes mellitus.

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