

STATISTICAL DESIGN AND DEVELOPMENT OF A LIQUID ORAL FLOATING *IN SITU* GEL OF METFORMIN HYDROCHLORIDE FOR SUSTAINED RELEASE: PHARMACODYNAMIC AND TOXICITY (HISTOPATHOLOGY) STUDIES

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ABSTRACT

Objective: To statistically design, optimize and evaluate a liquid oral, floating *in situ* gel of metformin hydrochloride (MH) to increase the gastric residence time (the absorption window being the upper part of the duodenum), sustain and modulate the release behavior of the drug. No liquid oral SR formulations of MH are yet available in the market.

Methods: A simple mixing based ionic cross-linking method was used for the formulation. A Two-square Factorial Design was employed and the effect of sodium alginate and three categorical levels of HPMC (K4M, K100M, E50) on the response variables were studied.

Results: The optimized formulation gelled instantaneously in simulated gastric fluid and showed >24 h floating. The drug release in 1h was 37.98 %, followed by a moderate sustained release for 12 h. Pharmacodynamic studies showed a significant reduction in blood glucose levels in Wistar rats. Short term preclinical safety studies revealed no toxicity to pancreatic tissues. On the contrary, faster regeneration of the β cells of the islets of Langerhans was observed with the group treated with the optimized formulation. Stability studies revealed a 2-year shelf life.

Conclusion: An elegant, needle-free, *in situ* gelling, SR liquid oral of metformin hydrochloride could be developed with drug release modulated as per official specifications for SR formulations of MH. This would be an interesting alternative for geriatric patients who find it difficult to swallow bulky tablets.

Keywords: Liquid oral floating *in situ* gel, Metformin hydrochloride, Least square fit model, Pharmacodynamic study, Toxicity studies

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INTRODUCTION

The successful design and optimization of effective drug delivery systems have become an integral part in improving various parameters such as the degree and rate at which the administered drug reaches the systemic circulation and research is continuously progressing in this area. Diseases like diabetes need much attention as glucose levels tend to increase after a meal and it should be maintained for the whole day for proper body activity. Metformin hydrochloride is an oral hypoglycemic agent and the first drug of choice to treat patients with Type II diabetes. It has an absolute oral bioavailability of 40% to 60%. It is highly hydrophilic with gastrointestinal absorption occurring mainly in the upper part of the intestine with peak plasma concentrations (C_{max}) at 4.8 h. It has a short half-life of 1.5 to 4.5 h and therefore the development of gastro retentive systems of the drug would help sustain the drug release and improving oral bioavailability. When large doses of MH are needed, the frequency of dosing may be 2 to 3 times a day which may reduce patient compliance and therefore liquid oral sustained-release (SR) formulations that give once a day dosing would be preferable.

The tablet and capsule dosage forms have to be swallowed as a whole unit and in case of dosage adjustment, these cannot be broken into halves as they are designed for controlled release and the floating ability also depends on dimensions of the tablet. Elderly and dysphagic patients find it difficult to swallow bulky tablets and capsules. In such cases, high doses of the drug can be incorporated into liquid oral *in situ* gelling (ISG) formulations [1, 2]. Novel drug delivery systems like *in situ* gelling systems reduce adverse reactions, toxicity and frequency of dosing with improved efficacy, patient compliance and convenience [3-6]. *In situ* gelling occurs due to one or a combination of different stimuli like pH changes, temperature modulation, solvent exchange and can be achieved in a gastric environment [7-9]. Various natural and synthetic polymers

including polysaccharides like carrageenan, gellan gum, pectin and sodium alginate have been used in the development of *in situ* gelling systems which undergoes a phase transition in presence of various di and trivalent ions [10-12]. Sodium alginate is a natural and hydrophilic polymer suitable for the entrapment of water-soluble drugs [13-15], widely used in pharmaceutical formulations [16-18]. HPMC is used as a viscosity modifier. Sorbitol is used as a sweetener in liquid orals and studies have shown that gels containing 10-17 % sorbitol sustained the release of drug in rat stomach and a bioavailability of approximately 90% was achieved from orally administered syrups [19, 20].

The current research work aimed at the development and evaluation of a floating, liquid oral dosage form of Metformin hydrochloride for sustained release, along with providing evidence regarding modulated drug release as per official specifications. No liquid oral sustained-release formulations of Metformin hydrochloride are available in market up to date and therefore an attempt is being to develop the same.

MATERIALS AND METHODS

Materials

Metformin hydrochloride was procured from National Pharmaceuticals, Birgunj. Sodium alginate, pectin, calcium carbonate, HPMC (K4M, K100M, E50), sodium citrate, and sorbitol were obtained from Yarrow Chem Products, Mumbai.

Methods

Pre-formulation studies

Drug-excipient compatibility studies by FT-IR

To investigate any possible interaction between the drug and polymers used, the IR spectrum of the pure drug (MH) and the

binary mixture of drug and polymers was taken using FTIR (UV visible spectrophotometer, Alpha Bruker) [21-24].

Design of experiment and statistical optimization

The conventional methods of experimentation by changing one variable at a time being time-consuming, the Design of Experiment (DOE) was done using the JMP Statistical Software (Trial version 13). The polymers and other quantities were selected based on reported literature on similar studies and initial screening experiments done to identify the optimum conditions and quantities required. Evaluation of preliminary batches was done based on responses such as *in vitro* gelling capacity, drug release and pourability (relative viscosity), to select the working concentration range of polymers (factors). The Two-Square Factorial design was used, taking two levels of the independent variable sodium alginate (Factor 1) coded at high and low levels and three categorical levels of HPMC (Factor 2) i.e., E50, K4M, K100M.

Formulation of metformin HCl oral *in situ* gel by a simple mixing method

Polymeric solutions of sodium alginate (0.7% and 1.25% w/v) with pectin (0.5% w/v) were prepared by adding it to ultrapure water containing sodium citrate (0.25% w/v) and calcium carbonate (1% w/v). This solution was then heated to 60°C with continuous stirring. After cooling to below 40°C, HPMC (0.5% w/v) was added, followed by 500 mg of MH/5 ml, sorbitol (10% w/v) and sodium benzoate (0.1% w/v). Ultra-pure water was used as a solvent [25-27]. A batch minimum of 100 ml was prepared.

Evaluation

pH of the gel

The pH of the formulation was determined using a calibrated pH meter and readings taken in triplicate [28-29].

Determination of *in vitro* gelation time and floating behavior

In vitro gelation time was determined by placing the sols in an open vial tied to the paddle in the USP Type II Dissolution apparatus (Paddle type) containing 900 ml of SGF (0.1 mol L⁻¹HCl, pH 1.2) at 37±0.5°C. The buoyancy lag time and duration of floating was noted. The readings were taken in triplicate [30-33].

Viscosity

The viscosity of the sol and gel in 0.1N HCl was determined using Brookfield viscometer (DV-II+Pro LV model). The selected formulations (250 ml) were poured into the sample adaptor of the viscometer and the viscosity measured at room temperature using SC4-18 spindle for the sols and LV2 spindle for the gels. The angular velocity was gradually increased from 10 to 50 rpm with a 6 sec wait period, the hierarchy reversed and the average taken. The viscosity measurements were made in triplicate [34].

In vitro drug release and kinetics

The drug release study was carried out using USP Type II dissolution apparatus (Paddle type, Electrolab). 5 ml of the formulation was kept in vials and dissolution done. At predetermined time intervals, 5 ml of sample solution was withdrawn, filtered through a 0.45 µm membrane filter, suitably diluted and analyzed by UV spectrophotometrically at 232 nm. The same amount of fresh dissolution medium was replaced immediately after withdrawal of the test sample [35-37]. The mechanism of drug release was studied by fitting the dissolution data in the zero-order, first-order, Higuchi model, Korsmeyer-Peppas equation following model-dependent kinetics. Based on the slope and r² values obtained, the mechanism of drug release was determined [38-41].

Optimization

The values obtained after evaluating the responses experimentally were fed into the JMP software and the data analyzed. The numerical optimization techniques and the least square fit model was employed. The desirability approach (using Prediction profiler) was used to generate the optimum settings for the formulation.

Optimization was done for the responses by keeping *in vitro* gelation and drug release at 1h at maximum while that at 8 h and 12 h were kept in the range [42]. The optimization target and constraints were set based on the official IP specifications.

In vivo pharmacodynamic study

The animal studies were done according to the protocol approved by the Institutional Animal Ethics Committee formed under the CPSCA guidelines (CPSEA Registration No: 1564/PO/a/11/CPSEA-23-11-2012). Wistar rats weighing between 150-250 g were divided into 3 groups, each with six rats. They were acclimatized for a week prior to the experiment, caged in a fully ventilated room at a temperature of 25±2°C, with a 12 h light/dark cycle with free access to standard chow diet and water. These have fasted for 24h and diabetes-induced with Streptozotocin 40 mg/kg (i. v). Treatment was started once the rat became diabetic. Group-1(control) was treated with physiological saline), Group-2 with the pure drug solution (0.116 ml) of MH (58.31 mg/kg dose) and Group-3 was given the optimized liquid oral (0.116 ml) of MH (58.31 mg/kg dose). Comparison was done between the groups to identify any significant differences [43-45].

Statistical analysis

Statistical analysis was carried out using the ANOVA and p-values less than 0.05 were considered statistically significant.

Toxicity (Histopathological) studies

At the end of the animal study, the rats were fasted overnight, anesthetized with Ketamine (80 mg/kg) and Xylazine (8 mg/kg) followed by terminal exsanguination. The pancreas was carefully excised, rinsed in ice-cold saline and stored in 10% formalin for tissue characterization. It was then embedded in paraffin, sectioned at 4 µm using a semi-automated microtome and the tissue sections mounted on glass slides using a hot plate. After deparaffinization with xylene, rehydration with 100%, 90% and 70% dilutions of ethanol, the sections were stained with Hematoxylin and Eosin (H and E) and the changes in pancreatic tissues observed [46].

Stability studies

Accelerated stability studies were conducted as per ICH Q1C guidelines on the optimized formulation to assess the stability with respect to physical appearance, drug content and drug release characteristics after storing in a stability chamber (Thermolab) at 40 °C/75% (RH) for 6 mo. The formulation was filled in an HDPE bottle with induction sealed CRC caps [47, 48].

RESULTS AND DISCUSSION

Preformulation study

Drug-excipient compatibility studies by FT-IR

No significant shifts or reduction in the intensity of the FTIR bands of MH were seen. Characteristic shoulders were observed at 1623 cm⁻¹ for the C=N stretching, 1544 cm⁻¹ for the N-H bending, 1164 cm⁻¹ for the of the C-N stretch, 3288 cm⁻¹ for the N-H stretching respectively. These bands were also observed for the physical mixture of polymers along with MH, thus confirming that there was no interaction between MH, polymers and excipients used and that they were compatible with the drug.

Metformin HCl liquid oral *in situ* gel

In the prepared formulation, sodium alginate along with pectin were used as to modulate and prolong the drug release and the combination gave a synergistic effect. HPMC was incorporated as a viscosity enhancer in order to achieve the desired consistency so as to facilitate sustained release and also reduced the amount of sodium alginate used. Calcium carbonate was used as a source of calcium ions, sodium citrate to prevent gelation outside the gastric environment and to complex with the free calcium ions and release them in the highly acidic environment of the stomach. Sorbitol, polyhydroxy alcohol, was used as stabilizing agent cum sweetener (it does not increase the blood glucose levels) and sodium benzoate as preservative. The formulation remained in the liquid form until it reached the stomach where instantaneous gelation occurred, thereby sustaining the drug release. The ion activated gel so

formed floated on the gastric fluids. The basic mechanism of floating was that the calcium carbonate present in the formulation as an insoluble dispersion becomes soluble in the acidic medium, releasing Ca^{2+} and CO_2 gas, causing gelation of the polymer and the gas released in the entrapped gel matrix caused the system to float [49]. The pH of the formulations was 6.8-7.2 which was within the acceptable range for liquid orals. The carbonation also had a synergistic effect in the taste-masking (in the pH range of 4 to 9). The viscosity was near or greater than 1 (the maximum limit a flowing liquid can have).

Design of experiment and ANOVA

The Two-Square Factorial design was used to discover the vital factors in the formulation that influence the various responses, including physicochemical properties and drug release. The factors used in the formulation, their proportions as per the design and the values of the responses are as enlisted in table 1 and the response variables of prepared formulations F1-F6 are seen in table 2. Each of the readings was performed in triplicate and the average taken.

Table 1: Formulation chart of metformin HCl in situ gel as per fit model design (JMP)

Formulation code	F1	F2	F3	F4	F5	F6
Metformin Hydrochloride (g)	1	1	1	1	1	1
Sodium alginate (mg)	125	70	125	70	125	70
Pectin (mg)	50	50	50	50	50	50
HPMC (mg)	E50	50		50	50	
	K4M					
	K100M		50			50
Ultra-Millipore water (ml)	10	10	10	10	10	10

*All other quantities in the formulation are constant i.e., Sodium citrate 25 mg, Sorbitol 10%, Calcium carbonate 100 mg, Sodium benzoate 10 mg

Table 2: Response variables of the prepared formulations F1-F6

Formula code	pH	Gelation time (sec)	Floating lag time (sec)	Viscosity of sol (cP)	Viscosity of gel (cP)	Drug content (%)
F1	7.2	2	75	410	1500	92.35
F2	7.3	10	110	360	1200	94.26
F3	7.1	2	150	1010	4240	95.65
F4	6.9	3	96	380	1620	98.26
F5	6.8	2	130	490	1840	96.73
F6	7.1	4	195	920	4110	93.78

The line of fit for the three grades of HPMC in the plot of sodium alginate versus % Cumulative drug release at 3h and the correlation

between the actual and predicted values for %CDR for 3h is seen in fig. 1a and 1b.

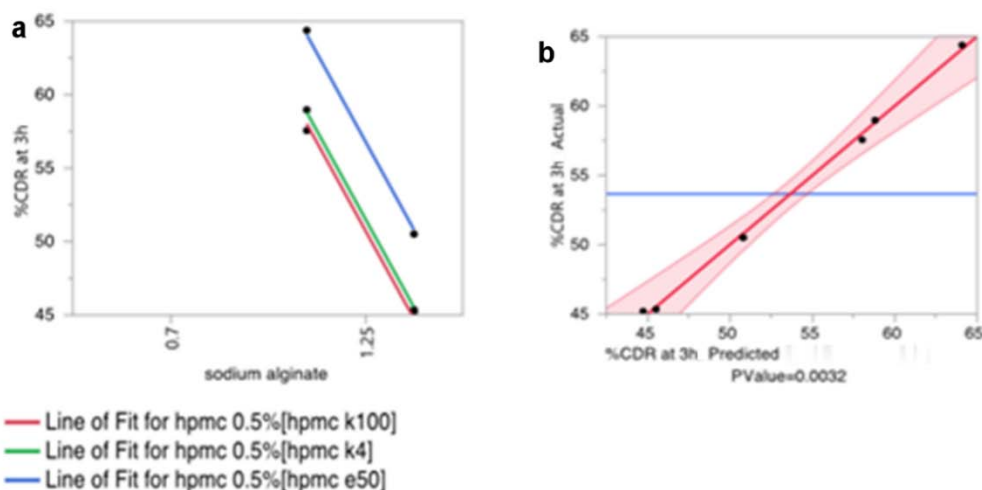


Fig. 1: (a) Line of fit of the different grades of HPMC used for sodium alginate vs %CDR at 3h (b) Predicted vs Actual values

The line of fit showed a linear regression line with confidence intervals fixed at 95% confidence for the predicted value (fit). The equations from the linear regression analysis for the responses are as follows:

Gelation time, $R_1 = 3.833 - 1.833 X_1 + 2.166 X_2a - 1.333 X_2b - 0.833 X_2c$ (Eq 1)

Floating lag time, $R_2 = 126 - 7.666 X_1 - 33.5 X_2a - 13 X_2b + 46.52c$ (Eq 2)

Viscosity of sol, $R_3 = 595 + 41.666 X_1 - 210 X_2a - 160 X_2b + 370 X_2c$ (Eq 3)

Viscosity of gel, $R_4 = 2418.333 + 108.333 X_1 - 1068.333 X_2a - 688.333 X_2b + 1756.666 X_2c$ (Eq 4)

%CDR at 1h, $R_5 = 39.258 - 8.401 X_1 + 11.306 X_2a - 2.923 X_2b - 8.383 X_2c$ (Eq 5)

%CDR at 3h, $R_6 = 53.661 - 6.641 X_1 + 3.783 X_2a - 1.501 X_2b - 2.281 X_2c$ (Eq 6)

%CDR at 8h, $R_7 = 90.34 - 3.61 X_1 - 1.48 X_2a + 1.11 X_2b + 0.37 X_2c$ (Eq 7)

%CDR at 12h, $R_8 = 98.66 - 0.826 X_1 - 0.915 X_2a + 0.61 X_2b$ (Eq 8)

Where X1 was the sodium alginate concentration and X2a indicated HPMC E50 (coded level-1), X2b represented HPMC K4M (coded level 0) and X2c represented HPMC K100M (coded level+1) respectively. The 3D surface response curve (fig. 2) also showed the relationship between the polymer concentrations and drug release at 3h. The regression analysis is a statistical tool used to determine the probable change in one variable for a given change in another. The magnitude of the change in response caused by varying the factor level is denoted as an effect. The main effect is the effect of a factor averaged over all the levels of other factors. Such data is suitably modelled to generate mathematical relationships between the independent and dependent variables. The response surface is a graphical depiction of the mathematical relationship [50].

Rheological studies

The formulations were sols at lower pH (indicating ease of pourability and swallowing) while they gelled at gastric pH, thus improving the gastric residence time and sustaining the release. The viscosity of the sols varied from 360 to 1010 cps while that of the gel varied from 1200 to 4240 cps on changing the rpm from 10 to 50 (at 37 °C). The hydrogels exhibited pseudoplastic rheology, the value of the flow index (n) being less than 1. Smaller the flow index values, more marked is the shear thinning. By knowing rheological properties, the composition can be modulated, designing hydrogels with optimal drug release characteristics and adequate consistency [51].

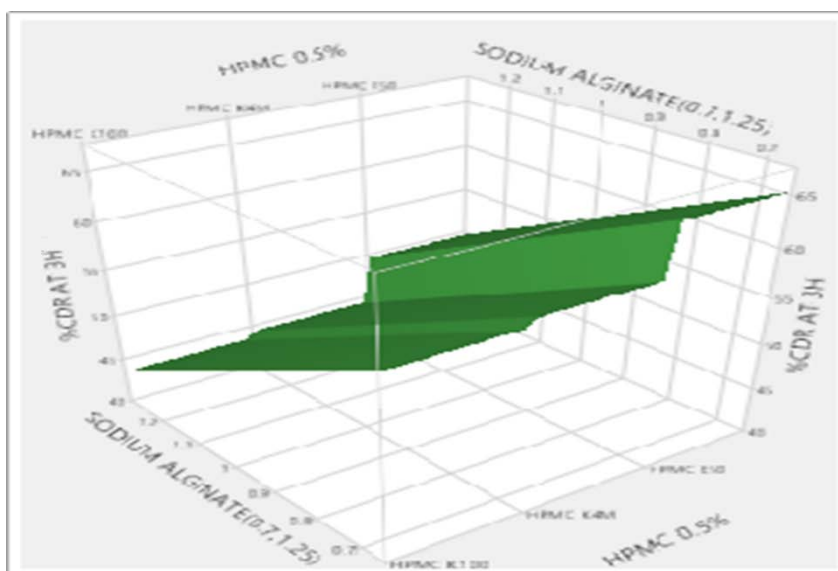


Fig. 2: 3-D surface response graph for 3h drug release

For the above-mentioned responses, the probability value was found to be significant (i.e., $*p < 0.05$) for viscosity of the sol and gel as well as drug release at 12h (table 3).

Thus, the ANOVA established the statistical significance of the two factors in formulating a liquid oral sustained release formulation of MH.

Table 3: Summary of ANOVA for significant responses as per least square fit model

Source	Sum of squares	DF	F ratio	p-value Prob>F
Viscosity of sol				
Model	423616.67	3	302.5833	0.0420*
Sodium alginate	10416.67	1	22.3214	0.0023*
HPMC	413200.00	2	442.7143	0.0033*
Corr Total	424550.00	5	-	-
Viscosity of gel				
Model	9472450.0	3	3157483	0.0011*
Sodium alginate	70416.7	1	19.4700	0.0477*
HPMC	9402033.3	2	1299.820	0.0008*
Corr Total	9479683.3	5	-	-
%CDR at 12h				
Model	6.7049667	3	25.3065	0.0383*
Sodium alginate	2.6047000	1	46.4269	0.0209*
HPMC	2.6047000	2	14.7464	0.0635
Corr Total	6.8816000	5	-	-

(*p value significant i.e., < 0.05)

Table 4: Effective summary as per least-square fit model (JMP)

Source	Log worth	Value
Sodium alginate (0.7,1.25)	2.908	0.00124 (significant)
HPMC 0.5%	2.647	0.00225 (significant)

The logworth values as seen in table 4 are also a good statistic for plotting and assessing significance. A log worth value exceeding 2 indicates that the effects are significant at 0.01 level.

In vitro gelation, floating and drug release studies

The nature of floating was as seen in fig. 3. The formulation

instantaneously gelled and floated for more than 24h, on contact with SGF.

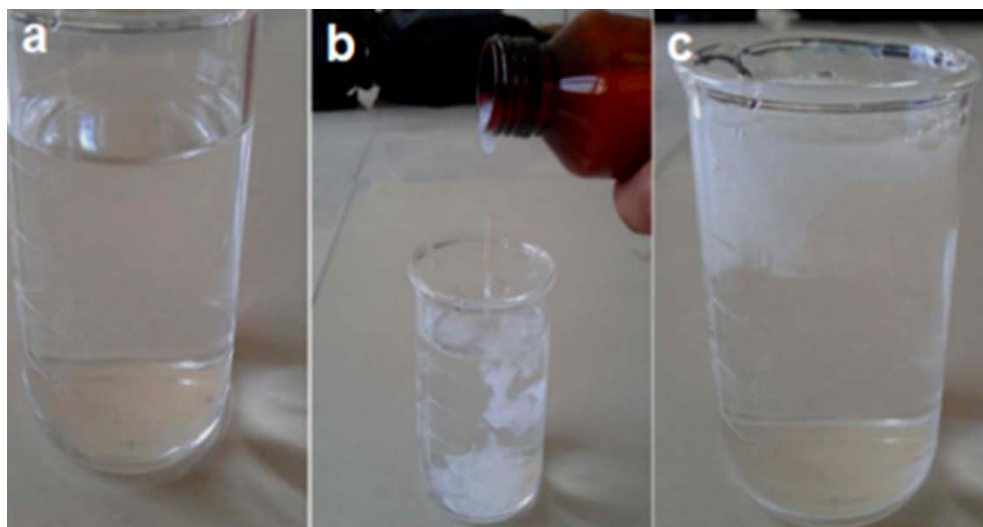


Fig. 3: (a) Liquid oral (b) Ionic gelation of the liquid oral on contact with SGF, (c) Floating *in situ* gel

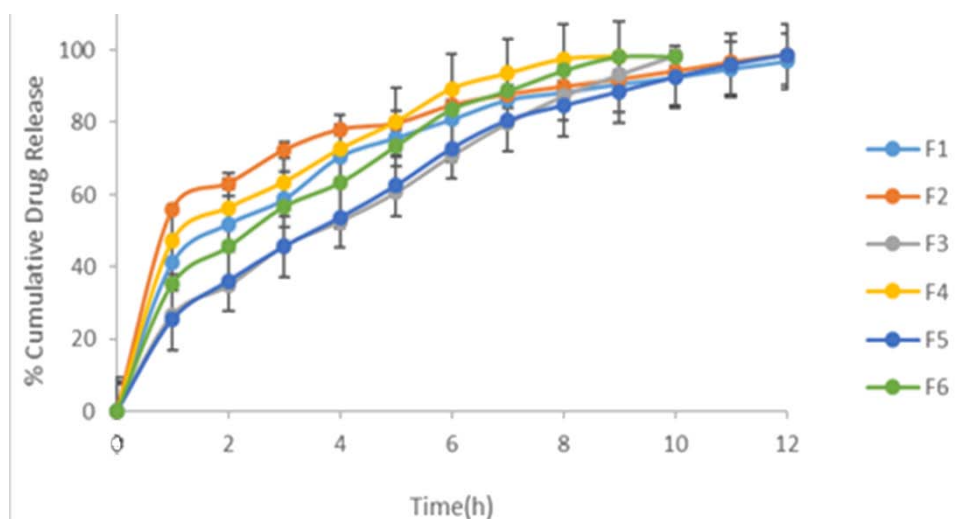


Fig. 4: *In vitro* dissolution profiles of the drug from the prepared formulations F1-F6 *Each reading was determined in triplicate (n=3), average taken, data given as mean \pm SEM)

The *in vitro* drug release studies for the formulations were done and the results compared (fig. 4). The formulation F5 showed maximum sustained drug release. The drug release data fitted into the various models showed very close r^2 values (above 0.8). The r^2 values of the Higuchi model of all the formulations showed higher values indicating that the drug release was directly proportional to square root of time. But n values ranging from 0.2033 to 0.5239 indicated Fickian diffusion mechanism. It may be coincident. However, n values of Korsmeyer-Peppas strongly indicated Fickian diffusion mechanism.

Statistical optimization

For each of the responses (Y_i), the desirability function $D(Y_i)$ assigned values ranging from a completely undesirable value of 0 to

a completely desirable response of 1. The optimized formulation was prepared according to Prediction profiler (fig. 5) and evaluation is done. Profiling is an approach to visualize response surfaces (cross-sectional view) by seeing what could happen if one or two factors are changed at a time. Interactive profilers help in exploring opportunity spaces. There was a good relationship between the experimented and predicted values, which confirmed the practicability and validity of the model.

The *in vitro* drug release of the optimized formulation and marketed product were compared (table 5) and the similarity factor was found to be 75.404. The marketed product released the drug at the minimum limit (25%) while the optimized product released the drug towards the average value of the official limit (37.98%) and has been graphically represented as seen in fig. 6.

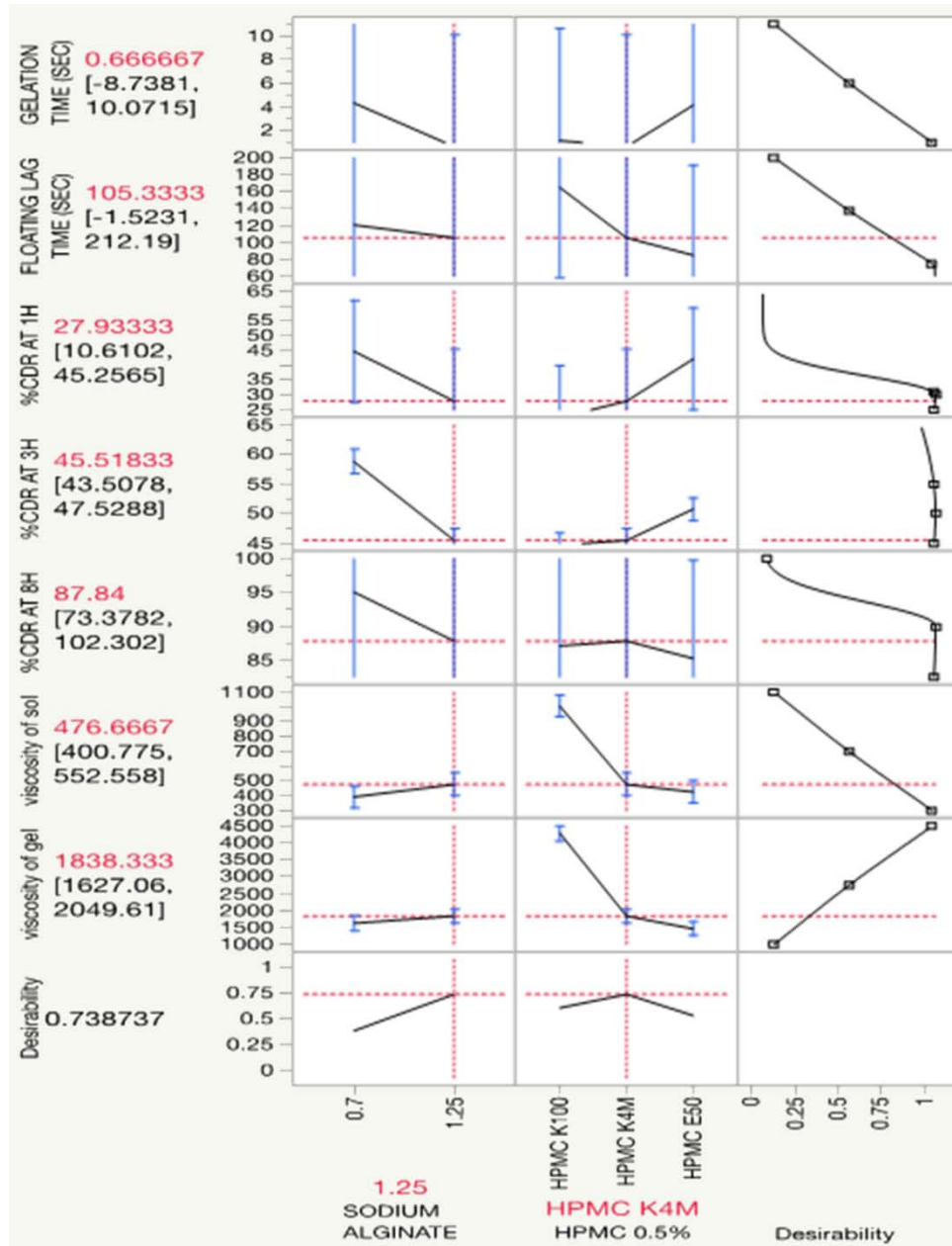


Fig. 5: Statistical optimization done using prediction profiler

Table 5: Drug release profile of optimized formula and comparison with marketed SR product of metformin hydrochloride

Time (h)	%CDR (Optimized formula)	%CDR (Marketed product)	IP specifications
1	37.986	25.593	Not less than 25% and not more than 50% in 1h
2	48.076	31.746	
3	61.669	45.12	Not less than 45% and not more than 75% in 3h
4	69.463	53.090	
5	73.496	62.526	Not less than 80% in 8h
6	75.983	72.506	
7	79.173	80.780	
8	83.280	84.27	
10	87.243	89.293	
12	93.263	93.429	

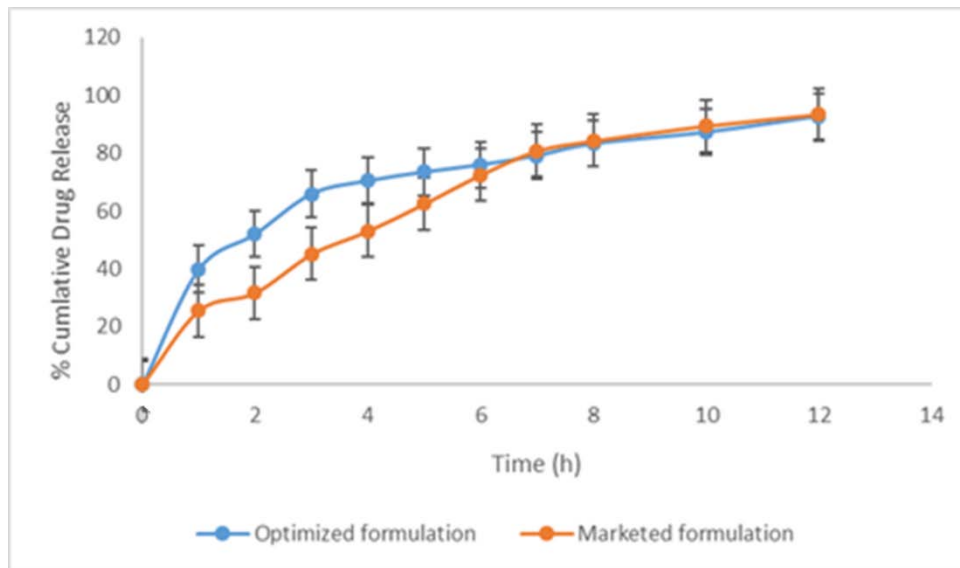


Fig. 6: Comparison of dissolution profiles for marketed product and optimized formulation. (Readings were taken in triplicate (n=3) and averaged (data given as mean \pm SD))

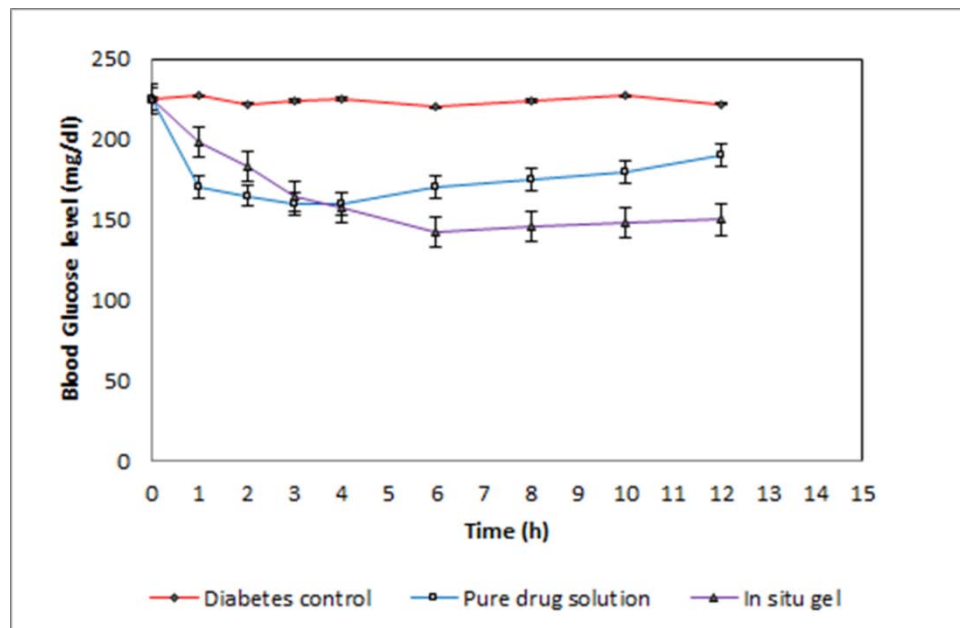


Fig. 7: *In vivo* pharmacodynamic study showing reduction in blood glucose level following oral administration of MH solution and the in-situ gel in streptozotocin induced diabetic rabbits (n=6, data given as mean \pm SEM)

Pharmacodynamic study

The results of the pharmacodynamic study indicated that the optimized formulation showed a reduction in blood glucose levels in the Wistar rats (fig. 7).

Also, prolonged release of the MH in situ gel has been observed due to the persistence of drug in the body [52].

Histological safety evaluation

The light microphotographs of pancreatic tissue samples (Hematoxylin and Eosin stained, 40 \times) of the normal control as seen in fig. 8 showed (a) normal architecture of the islets of Langerhans and these were interspersed in between the acinar cells. The blood vessels and pancreatic tissue were also intact without any red swelling or inflammatory infiltration in the control group. However, the diabetic control group (b) showed pathological changes in both

the exocrine and endocrine components. The acinar cells were swollen and small vacuoles were seen in almost all the cells. The β cells of the islet of Langerhans were almost entirely destroyed in the STZ treated group as seen in the fig. 8b where no islets are visible. In the group treated with MH solution (c) there was slight distortion in the architecture while in the optimized formulation i.e. MH in situ gel treated group (d), the endocrine portion of pancreas indicated less severe atrophic changes in the acinar cells and the border between the endocrine and exocrine portions were more distinct. Normal architecture of exocrine glands with columnar epithelial cells with a basal nucleus and pink staining secretions was observed with no degeneration islets of Langerhans cells. In fact, the replenishment of insulin-producing pancreatic β cells was observed due to this optimized, controlled release formulation of MH in situ gel which is crucial in treating diabetes and its complications thus overcoming inadequacies of the current treatment strategies.

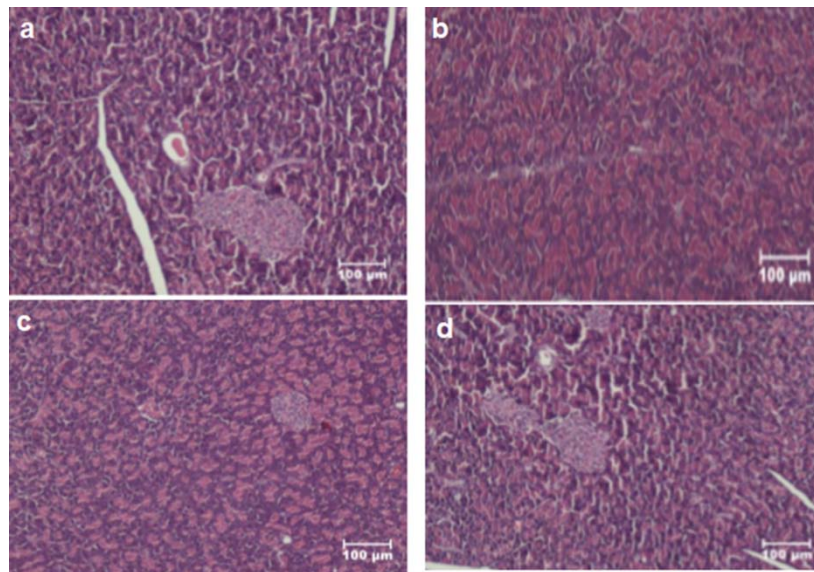


Fig. 8: Light microphotographs of (a) control mice showing normal pancreas b) diabetic mice with streptozotocin-induced diabetic pancreas showing degenerative, necrotic changes and disappearance of the β -cells c) diabetic mice treated with Metformin solution showing reappearance of the β -cells d) diabetic mice treated with optimized MH in situ gel showing marked improvement with regeneration of β cells of the islets of Langerhans) (*n=6, all values expressed as mean \pm SD)

These results are in agreement with previous studies done [53] which have confirmed that there is a progressive deterioration in β -cell function and mass in Type 2 diabetes. The islet function declined to 50%, at the time of diagnosis and a reduction in β -cell mass of about 60% was shown at necropsy, the latter being attributable to accelerated apoptosis. Impaired β -cell function and possibly β -cell mass appear to be reversible, particularly at early stages of the disease. Interventions to preserve or rejuvenate β -cells can thus help improve the function of these cells [54]. Thus, histopathological studies have confirmed that the administration of the optimized SR formulation of MH in situ gel could again increase the number and size of the islet cells.

Stability studies

Stability studies carried out on the optimized formulation as per ICH guidelines (Q1C) revealed no significant changes in the physicochemical properties, gelling capacity or drug release of the formulation even on the aging of the in-situ gel under the different storage conditions. Also, shelf life of up to 2 y was observed.

CONCLUSION

It can be concluded that a promising, stable sustained release, liquid oral floating *in situ* gelling systems of Metformin Hydrochloride that met the official specifications of sustained drug release could be successfully developed using Statistical design and optimization techniques. Therapeutic concentrations of the drug could be achieved in plasma in a controlled manner and the drug release was sustained for 12h when compared to the conventional dosage forms (tablets). The formulation also helped in the regeneration of the β cells of the islets of Langerhans. Incorporation of large doses of drug would be possible with such liquid oral formulations and the simple, economic method of formulation indicate the feasibility and ease of production on a larger scale.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest

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