ORIGINAL ARTICLE

Development of a Sustained Release, Liquid Oral *in situ* Gelling System of Montelukast Sodium with Improved Bioavailability: Equivalence Testing using Earth Mover's Distance

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Abstract

Aim: The present study aims at the development of a sustained release liquid oral *in situ* gel of the antiasthmatic drug montelukast sodium with improved bioavailability and patient compliance. The drug has a short biological half-life of 2.5–5.5 h, an oral bioavailability of 64% and is commercially available only as solid dosage forms. **Materials and Methods:** The formulations were statistically designed using central composite design, suitable proportions of thermosensitive polymers such as Pluronic F127, Xyloglucan and other excipients added and a simple mixing (cold method) used for the preparation. The effect of the factors on various responses was evaluated and optimization was done. **Results:** The optimized formulation showed a mean viscosity of 0.039 Pas, gelled at body temperature, gave 94.18 ± 2.15 % drug release in 12 h. *In vivo* studies on New Zealand male rabbits revealed a C_{max} of 192.91 ± 13.363 ng/ml in 1 h and 12 h sustained release. The $AUC_{0-\alpha}$ (4767.942 ± 412.915 ng h/ml) showed 3.8-fold increase in bioavailability. Stability studies indicated a 2-year shelf life at 4°C. Θ_{test} (0.78690) < Θ_{std} (0.7963) obtained using Earth mover's distance revealed that the pharmacokinetic profile of the optimized formulation was better than the reference drug solution. **Conclusion:** This elegant, less bulky, liquid oral *in situ* gelling system with pH-independent release would also be patient compliant and could pave way for a better approach to drug delivery.

Key words: Earth mover's distance, liquid oral *in situ* gel, statistical optimization, sustained release

INTRODUCTION

Liquid oral *in situ* gelling techniques have gained much interest recently.[1-5] Montelukast sodium (MKS) has an oral bioavailability of 64%, a short biological half-life (2.5–5.5 h) and is rapidly absorbed following oral administration.[6-9] The issue with solid unit dosage forms is that they must be swallowed as a whole unit and cannot be broken in halves if designed for controlled release. Emerging delivery strategies are being pursued to circumvent the current limitations to the use of MKS.[10] The polymers and excipients used in this study were Pluronic F127, a thermosensitive polymer (approved by FDA as a food additive), Xyloglucan (from tamarind seeds) as an ice crystal stabilizer, Sorbitol (10-17%) as a sweetener, viscosity enhancer and sodium benzoate (0.1%) as preservative.[11-19] The aim of this work was to develop a liquid oral *in situ* gelling formulation of MKS for sustained delivery, better bioavailability, and patient compliance. Earth mover's

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distance (EMD), a statistical metric, has been used to prove that the developed formulation has better bioavailability than the pure drug solution.

MATERIALS AND METHODS

Materials

MKS was obtained as a gift sample from Apotex Research Private Ltd, Bengaluru, India. PF127 was purchased from Sigma Aldrich Chemicals Private Ltd, USA. Xyloglucan was purchased from Leo Chem, Bengaluru, India and other chemicals used for the study were of analytical grade. Water was distilled and deionized before use.

Preformulation studies

Fourier-transform infrared (FT-IR) spectroscopy

IR studies were performed for the pure drug as well as binary mixtures of the drug and polymers using the FTIR spectrophotometer (Model αE ATR module, BRUKER).[20]

Differential scanning calorimetry (DSC) study

DSC analysis was performed using the differential scanning calorimeter (NETZSCH, DSC 200 PC System, Japan). 3–5 mg samples were weighed and placed in closed, hermetically sealed sample pans with a pinhole. Thermograms were obtained by heating the sample at a constant rate of 100°C/min. A dry purge of nitrogen gas (50 ml/min) was used for all runs to eliminate oxidative and pyrolytic effects. Samples were heated from 0°C to 250°C. The melting point, disappearance of the crystalline sharp peak of the drug, and the appearance of any new peak or peak shape were noted.[21,22]

Experimental design

In the statistical design of experiment (DOE) approach, a series of formulations were created and tested in a planned sequence. The level of the factors was varied and the performance of the formulations was measured. A model was fit into the data and critical factors were identified. Response surface contours were examined graphically and analytically to determine the design space and region of the best values of the response that meet the specifications. Additional confirmatory formulations were then tested to verify model predictions. The design-expert software® (version 9.0.5.1), Stat-Ease Inc., Minneapolis, USA) was used. The effect of the two independent variables (polymer concentrations) on the five dependent variables (responses) was determined. The SAS statistical tool (JMP trial version 14) was also used to obtain the optimized formula with the help of the prediction profiler. A randomized rotatable central composite design (CCD) was employed and the response parameters were statistically analyzed by applying analysis of variance (ANOVA) at 0.05 levels.

Preparation of MKS liquid oral

MKS (20 mg/5 ml), xyloglucan (1–1.5% w/v), sorbitol (10 %w/v) and sodium benzoate (0.1%w/v) were dissolved in distilled, deionized water and kept in refrigerator. After cooling the solution, PF127 (18–28%) was added to it, little by little, with continuous agitation and vortexed until the bubbles disappeared. The prepared sol was refrigerated at 4°C until a clear solution was obtained.[23] A batch minimum of 50 ml was prepared [Figure 1].

Evaluation of the MKS liquid oral *in situ* gels

Clarity

The clarity of the formulations before and after gelling was visually observed under light, alternatively against white and black backgrounds.

Measurement of gelation time

The time taken to form a gel that maintained its integrity was noted by transferring 10 ml of the sol into a vial and placing it into 500 ml of 0.1N HCl (pH 1.2) maintained at a temperature of 37 ± 0.5°C in a USP (Type II) dissolution apparatus. Measurements were done in triplicate and the average was

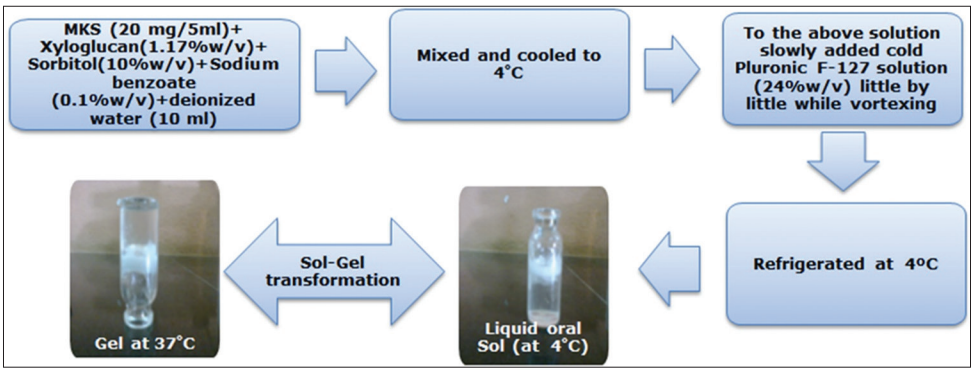

Figure 1: Preparation of the optimized liquid oral *in situ* gelling formulation by simple mixing (cold method)

taken.[24] An alternative method was also adopted where the vials containing the sol were placed in an incubator and the formation of the gel visualized.[25]

Measurement of gel strength

The sol (50 g) was placed in a 100 ml beaker and gelled at 37°C. The gel strength was determined by noting the time taken by the apparatus (weighing 27 g) to sink 5 cm through the gel. Measurements were done in triplicate and the average was considered.[26]

Gelation temperature

About 10 ml of the sol in a 20-ml vial containing a magnetic bar was placed on a low-temperature thermostat water bath and heated at a constant rate along with the constant stirring of the magnetic bar. The temperature at which the gel formed (when the magnetic bar stopped moving) was noted. This was repeated for all the formulations thrice and the average considered.[27]

Rheological studies

Rheological tests on the sols and gels in acid and phosphate buffer saline were performed using a stress-controlled rheometer (MCR 302 Rheometer, Anton Paar) having 25-mm parallel plates. For temperature control, a Peltier system was employed. The samples were poured on the lower plate of the rheometer at 0°C and the viscosity of the sol was measured at 50 rpm. For gel characterization, the same procedure was employed, and the sample was heated to 37°C. Quiescent conditions were maintained for 15 min to reach the thermal stability, and then, isothermal testing was done.[28,29]

In vitro drug release study

The USP Type-II (Paddle type) dissolution test apparatus (Electro lab, ETC-11L) was used with 900 ml of 0.1N HCl as dissolution medium for 2 h followed by phosphate buffer (pH 7.4) maintained at 50 rpm speed and 37 ± 0.5°C temperature. 5 ml of sol equivalent to 20 mg of the drug was taken in a vial and placed in the dissolution vessel where it converted to a gel. At regular intervals of time, an aliquot of 5 ml was withdrawn, filtered through a 45 µm membrane filter and replaced with the same volume of fresh medium. Further dilutions of the sample were done to obtain a concentration of 10µg/ml and the assay was done using the UV-Visible Spectrophotometer (Shimadzu UV-1700, Kyoto, Japan) at 350 nm. The concentrations of the drug were analyzed from the standard calibration curve and the percentage cumulative drug release was calculated.[30,31]

Statistical optimization

For the Statistical optimization (using JMP software), the criteria employed were as follows: Concentrations of PF127 (Factor A) and Xyloglucan (Factor B) were kept in the range , the responses i.e., gelation temperature, gel strength and % CDR at 1h were kept at the maximum while % CDR at 8h and 12h were kept in range. For group comparisons, the one-way layout ANOVA was applied.

A bioanalytical method high-performance liquid chromatographic (HPLC) for quantitative estimation of MKS in plasma

For the sample preparation, 750 µl of acetonitrile was added to 200 µl plasma sample for the precipitation of proteins and the mixture was kept on a vortex mixer (Spinix, Tarsons, India) for 10 min and then centrifuged (Spinwin, Tarsons, India.) at 13,000 g for 5 min. After centrifugation, the mixture was transferred to glass tubes with caps after filtration through a membrane filter of 0.22 µm (Millipore, Bedford, MA, USA) and 20 µl was injected into the HPLC system. The mobile phase consisting of acetonitrile:potassium dihydrogen phosphate (70:30 % v/v, 0.05 M) adjusted to pH 3.5 ± 0.1 with phosphoric acid was used with a flow rate of 2.0 ml/min. The Agilent HPLC system (LC Compact-1120 model, Germany) with binary pump and EZ Chrome software was used during the study, and the Waters XBridge column (150 mm × 3.9 mm × 5 µm particle size) was used as the stationary phase. The detection was carried out using UV detector at 350 nm.[32,33]

In vivo pharmacokinetic studies on New Zealand male rabbits

The study protocol for the animal experiments was previously approved by the Institutional Animal Ethics Committee (formed under CPCSEA guidelines) as it complied with the Institutional guidelines on Animal Experimentation. Two groups of animals were taken. For each group, six healthy New Zealand white male rabbits, weighing 2.86 + 0.12 kg, were utilized. They were housed individually in stainless steel cages, fed with commercial rabbit diet, given water *ad libitum*, and allowed to fast 18 h before and during the pharmacokinetic study. The animals were conscious throughout the duration of the experiments and were held in rabbit restrainers during the blood sampling. In the crossover study with 1 week apart as washout period, 5 ml of the optimized formulation (test) containing an equivalent of 20 mg of the drug was orally administered to one group using a stomach sonde needle while 5 ml of the pure drug solution (reference) was given to the other group. Blood samples (1.5 ml) were withdrawn through a cannula from the marginal ear vein of rabbits and collected in heparinized tubes at the time intervals of 0, 1, 2, 3, 4, 5, 8, 10, and 12 h. The collected blood samples were centrifuged immediately at 4300 × g at 8°C for 15 min to separate the plasma and analysis performed. The plasma remaining for any pending analysis

was deep-frozen and stored at −20°C. Quantification of MKS in rabbit plasma was done using the HPLC method mentioned in the above section. The software for Pharmacokinetics/Pharmacodynamics analysis, Kinetica 5.0, was utilized for the calculation of the pharmacokinetic parameters.[34,35]

Statistical analysis

The Analysis of Variance was done using the JMP and *p values < 0.05 were considered statistically significant.

Equivalence testing of reference and test plasma profiles based on EMD

The population bioequivalence (PBE) statistical approach has been recommended by the FDA to compare descriptors from the test and reference products to support product equivalence. The PBE approach considered both mean and variance information. Given two sets of profiles (reference vs. test), a reference center was first calculated by taking the grand average of all reference profile data. The EMD, a statistical metric, was then applied to calculate the distance between the reference center and each individual reference profile. Similarly, the distance between the reference center and each individual test profile was also calculated using the EMD. The obtained two groups of EMD distances were then used as input to PBE for conducting a statistical test between the two groups to establish (in)equivalence between the test and reference.[36-38]

Population bioequivalence approach

The population BE approach was done to complement the average. This method considered both the mean and variability of the reference as well as test products for comparison of Bioequivalence. The criterion for PBE was summarized as follows:

$$PBC = \begin{cases} \dfrac{(\mu_T - \mu_R)^2 + (\sigma_T^2 - \sigma_R^2)}{\sigma_R^2} \le \theta, if\ \sigma_R \le \sigma_{T_0} \\ \dfrac{(\mu_T - \mu_R)^2 + (\sigma_T^2 - \sigma_R^2)}{\sigma_{T_0}^2} \le \theta, if\ \sigma_R \le \sigma_{T_0} \end{cases} \quad (1)$$

where μ_T and μ_R were the mean of the reference and test products, σ^2_T and σ^2_R were the total variance of the test and reference drug products, σ^2_{To} a predefined scaling factor, and m represented the BE limit (1.25).

$$\theta = \frac{(ln(m))^2}{(0.25)^2} \quad (2)$$

Stability studies

To establish the most appropriate storage conditions, stability studies were conducted as per the ICH Q1AR guidelines. Long-term stability studies were done on the optimized formulation at 5°C ± 3°C for 12 months. Accelerated stability studies were done for 6 months at 25°C ± 2°C / 60% RH ± 5% RH. The packaging of the formulation was done in tightly sealed, light shielding glass bottles. The drug content, gel strength, gelation time, and drug release were periodically measured at 3-month intervals for 12 months. The drug content was periodically assessed by taking an aliquot from each sample, mixed with a known volume of acetonitrile, vortexed, centrifuged at 1100 × g, and filtered using 0.45 µm filter, and the resulting solution was analyzed using HPLC.[39]

RESULTS AND DISCUSSION

Preformulation studies

FT-IR spectroscopy

The drug-excipient interaction study done indicated that MKS was compatible with all the excipients used in the formulation as there were no extra peaks or shifting of peaks of the functional groups of the drug (all peaks were within the ± 5 cm^{-1}) in the spectra of binary mixtures of drug and excipients.

DSC study

DSC studies indicated that there was no interaction between the drug and excipients as there was no appearance of new peaks or disappearance of existing peaks. The melting point peak appeared at 120°C for MKS. DSC peaks may vary based on polymorphic transitions in the presence of excipients, differences in inbound solvents, i.e., pseudomorphs, and impurity types and amounts. In general, an amorphous-containing solid form of montelukast exhibits melting within the range from 60°C to 160°C.

Experimental design

The selected factors and their coded levels are summarized in Table 1 while the formulation chart obtained using the CCD is shown in Table 2. The ANOVA for response surface models is summarized in Table 3.

Statistical optimization of the *in situ* gelling formulation

The desirability function was used to find out the optimized formulation. The values of the optimized formulation

Table 1: Factors and corresponding coded levels implemented for the construction of central composite design

Factor	Level				
	−α	−1	0	+1	+α
X1: PF127 (g)	0.917	1.000	1.200	1.400	1.482
X2: Xyloglucan (mg)	58.95	66.00	83.00	100.00	107.04

were obtained from the Prediction profiler [seen in Figure 2] and consisted of 1.2g of Pluronic F127, 83mg of Xyloglucan,17% sorbitol and 0.1% sodium benzoate per 5ml dose.

In vitro evaluation

Clarity

Clear sols were observed at 4°C under light alternatively against white and black backgrounds while a gel was obtained at body temperature (37°C). The gel was transparent when xyloglucan was used in combination with PF127 when compared to a gel containing PF127 alone.

Gelation time

The time taken for the sol to convert to gel at body temperature was determined. The gelation time of the formulations varied from 82 ± 0.84 to 137 ± 0.50 s. Depending on the concentration of polymers used, the time taken for gelation varied. Xyloglucan alone formed gels at much lower concentrations when compared to the combination. Furthermore, gelation time increased with the decreased concentration of poloxamer. The optimized formulation showed a gelation time of 120 s. This optimal gelation time would enable *in situ* gelation in the stomach following oral administration of the formulation.

Measurement of gel strength

The gel strength of the formulations varied from 24 ± 0.87 to 29 ± 0.57 s and it depended on the concentration of the polymers used. The gel strength was found to increase as the concentration of PF127 increased. The final equation in terms of coded factors for gel strength (R1) was as follows:

$$R1=+27.23+1.28A+6.869E-0.17B \quad (3)$$

This kind of linear equation with coded factors was useful to make predictions about the response for the given levels of each factor and was to identify the relative impact of the factors by comparing the factor coefficients. The linear Equation (3) showed that factor A (concentration of PF127) had a positive effect on gel strength while factor B (concentration of Xyloglucan) had a slight negative effect. The effect of factors A and B was further studied with the help of a 3D response surface plot [Figure 3] which showed that the gel strength increased with the increase of PF127 proportion.

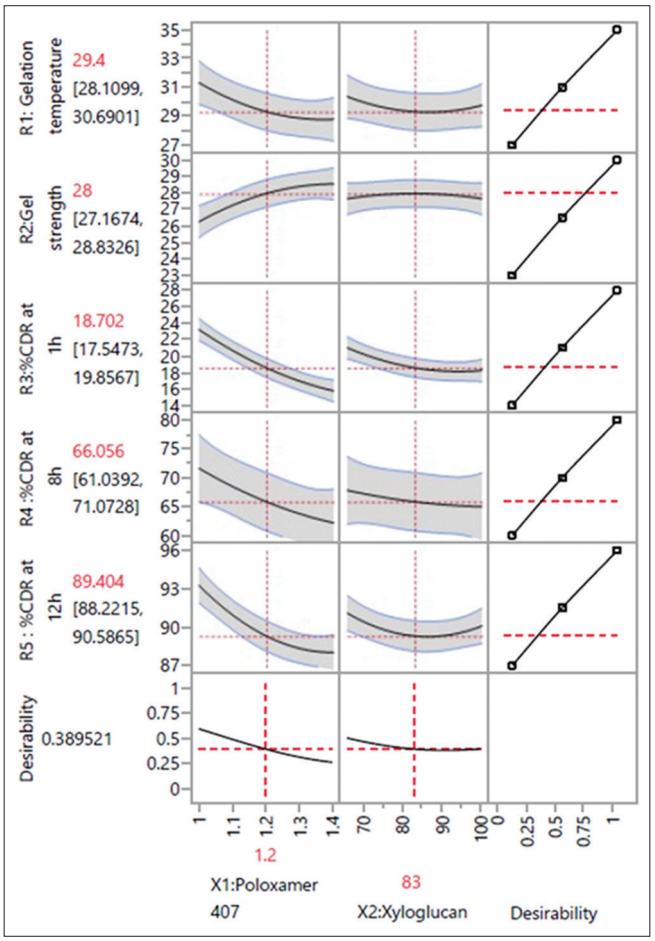

Figure 2: Prediction profiler showing the optimized formula

Measurement of gelation temperature

The gelation temperature of the formulations, i.e., the temperature at which gel formation took place varied from 29 ± 0.16 to 32 ± 0.34°C, and the variation depended on the concentration of polymers used. There was an increase in the gelation temperature with a decrease in PF127. The three-dimensional response surface plot also showed that the gelation temperature increased with a decrease in PF127 concentration and there was a significant correlation between actual and predicted values. The final equation obtained for the gelation temperature (R2) in terms of coded factors is given as follows.

Table 2: Formulation chart for MKS *in situ* gelling system as per central composite design along with independent variables and the responses

Formulation code	Factors		Responses				
	FactorA	Factor B	R1	R2	R3	R4	R5
	PF 127 (g)	Xyloglucan (mg)	Gel strength (sec)	Gelation temperature (°C)	% CDR 1 h	% CDR 8 h	% CDR 12 h
F1	1.200	83.00	28±0.43	29±0.86	18.45±0.97	66.67±1.44	89.53±1.20
F2	1.400	66.00	28±0.78	30±0.24	17.69±1.97	60.85±1.36	89.98±1.24
F3	1.400	100.00	28±0.29	29±0.16	15.43±2.02	63.57±1.67	88.78±1.28
F4	1.200	107.04	27±0.12	31±0.67	18.79±0.90	62.57±0.98	90.12±2.03
F5	0.917	83.00	24±0.87	34±0.36	26.82±0.42	79.71±1.06	95.47±0.39
F6	1.200	58.95	27±0.54	32±0.08	24.23±1.22	76.13±2.09	92.54±0.77
F7	1.200	83.00	28±0.25	31±0.34	19.12±0.78	65.51±1.48	91.18±2.15
F8	1.482	83.00	29±0.57	29±0.56	15.92±0.88	61.71±1.53	89.67±1.37
F9	1.000	66.00	27±0.62	31±0.07	23.84±1.02	65.00±1.02	94.74±0.78
F10	1.000	100.00	27±0.34	31±0.28	22.89±0.86	70.28±0.67	93.98±0.96

MKS: Montelukast sodium

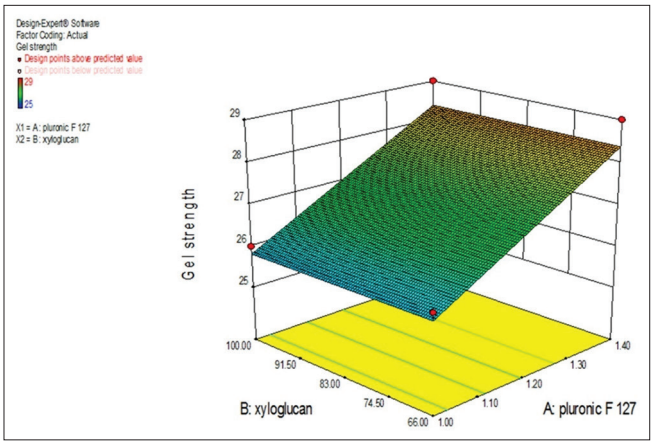

Figure 3: Three-dimensional response surface graph for gel strength

$$R2=+29.10-1.16A+0.052B \quad (4)$$

Rheological properties of the prepared sols and gels

The sols were liquid at <10°C while they gelled when warmed to the body temperature. The temperature at which the gel flowed as a liquid was regarded as the transition point. Below the sol-gel transition temperature, the formulations showed Newtonian properties (sol-like) while above the sol-gel transition temperature, they showed non-Newtonian (gel-like) flow. The optimized formulation showed a remarkable change in viscosity with a change in the stress. Higher concentrations of PF127 showed an abrupt increase in viscosity at lower temperatures. 1.5% (w/w) xyloglucan solution (without PF127) formed a soft gel after several minutes, which had a poorly defined shape, while, with PF127 solution, an elegant gel formed immediately. The addition of 17% (w/v) sorbitol moderated the effect of viscosity of the sol. These changes in viscosity have implications for the administration of sols of the formulation. Lower the viscosity, greater the ease of swallowing the sol. The mean viscosity of the optimized formulation was 0.039 Pa. s for the sols while gels showed a mean viscosity of 205 ± 0.324 Pa. s in pH 7.4 phosphate buffer at $1s^{-1}$ at 37°C. The values were statistically significant (*$P < 0.05$).

In vitro drug release studies

The comparative *in vitro* drug release profiles of formulations F1–F10 (Figure 4a and b) indicated that, as the concentration of polymers increased, the drug release decreased. The initial burst in the drug release during the initial hours of administration could be due to various factors such as unequal distribution of drug inside the polymeric matrix network. The amount of MKS released from each formulation was found to vary depending on the polymer concentrations used. The linear equations for *in vitro* drug release at 1, 8, and 12 h, i.e., R3, R4, and R5 in terms of coded factors were as follows:

Table 3: ANOVA for response surface models

Source	Sum of squares	DF	Mean square	F value	Probability
Gel strength					
Model (linear)	13.11	2	6.56	20.53	0.0003 (significant)
A	13.11	1	13.11	41.06	<0.0001
B	1.776E–015	1	1.776E–015	5.562E–015	1.0000
Gelation temperature					
Model (linear)	10.70	2	5.35	17.88	0.0005(significant)
A	10.68	1	10.68	35.68	0.0001
B	0.021	1	0.021	0.072	0.7944
% CDR at 1h					
Model (linear)	89.35	2	44.68	63.90	<0.0001(significant)
A	85.78	1	85.78	122.70	<0.0001
B	3.57	1	3.57	5.11	0.0473
% CDR at 8 h					
Model (linear)	172.86	2	86.43	7.90	0.0088 (significant)
A	164.86	1	164.86	15.06	0.0031
B	8.00	1	8.00	0.73	0.4126
% CDR at 12 h					
Model (quadratic)	52.02	5	10.40	35.15	<0.0001(significant)
A	40.87	1	40.87	138.10	<0.0001
B	3.65	1	3.65	12.34	0.0098
AB	0.032	1	0.032	0.11	0.7504
A2	6.93	1	6.93	23.42	0.0019
B2	1.13	1	1.13	3.82	0.0915

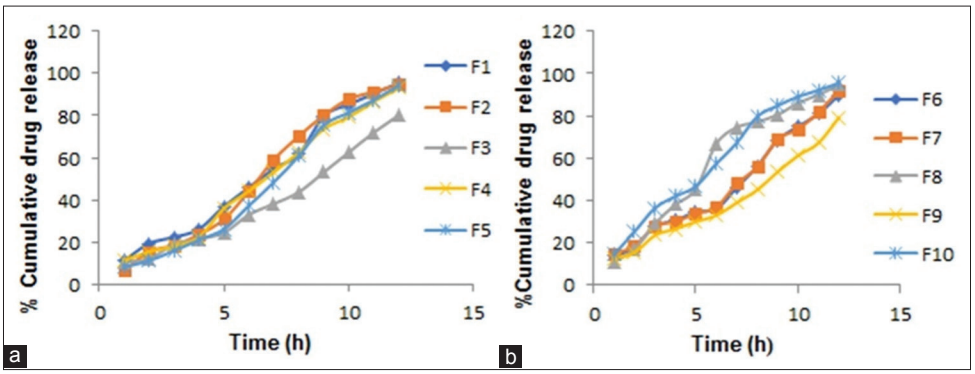

Figure 4: Comparative *in vitro* dissolution profiles of the prepared formulations (mean ± standard deviation, *n* = 3) for the formulations (a) F1–F5 and (b) F6–F10

$$R3=20.11-3.27A-0.67B \quad (5)$$

$$R4=65.81-4.54A+1.00B \quad (6)$$

$$R5=90.48-2.26A-0.68B-0.90AB+0.90A2+0.30B2 \quad (7)$$

In almost all the cases, both the factors A and B decreased the drug release from the gels. The effect of A and B can be further elucidated with the help of response surface plot [Figure 5]. There was a significant correlation between actual and predicted values. The amount of MKS released in 1, 8, and 12 h from the optimized formulations was found to be 21.12 ± 0.78%, 65.51 ± 1.48 %, and 94.18 ± 2.15%, respectively.

In vivo pharmacokinetic studies

The HPLC method used for the quantification of MKS in plasma showed that the drug was well separated. The HPLC

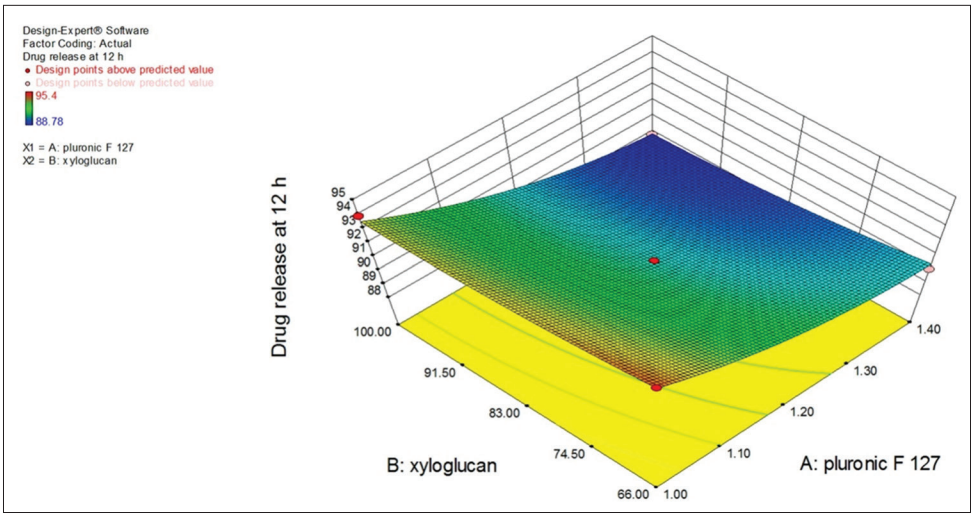

Figure 5: Three-dimensional response surface graph for % cumulative drug release at 12 h

chromatogram of the rabbit plasma with drug showing the retention time of MKS is given in Figure 6.

Pharmacokinetic studies on New Zealand rabbits revealed that the optimized formulation of MKS was rapidly absorbed after oral administration. The peak plasma concentration was achieved in 1 h and found to be 192.91 ± 13.363 ng/ml. The AUC0–α, which reflects the total amount of active drug which reached the systemic circulation, was found to be 4767.942 ± 412.915 ng h/ml which was higher than that of the control (1267.47 ± 126.31 ng h/ml and also statistically significant ($P < 0.05$) indicating a 3.8-fold improvement in bioavailability. The optimized formulation maintained relatively constant plasma drug levels within the therapeutic window (above the minimum effective concentration of about 120 ng/ml) for a period of 12 h, while the control (reference) showed fluctuations in the plasma drug concentration due to the peak and valley profile of the conventional dosage form. The plasma drug concentration of the reference was found to be below the therapeutic levels after 4h [as seen in Figure 7] thus indicating that the pure drug solution works only for a 4h duration.

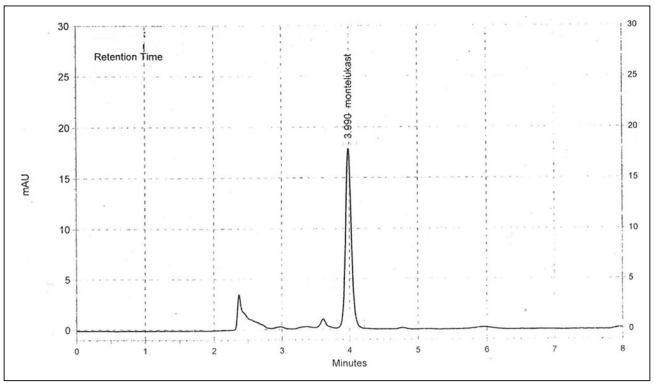

Figure 6: Chromatogram of an extract of rabbit plasma with formulation showing retention time of montelukast sodium

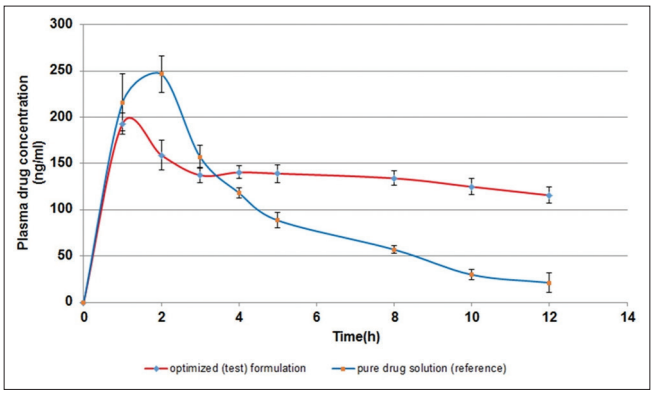

Figure 7: Plasma drug concentration versus time profile of pure drug solution and *in situ* gelling optimized formulation (mean ± standard deviation, $n = 6$) following oral administration

Equivalence testing of reference and test plasma profiles based on EMD

In this study, the EMD was used as a metric for comparing the plasma profiles of the reference (drug solution) and optimized (test) formulation. It was used to assess the (dis)similarity between two multidimensional distributions in some feature spaces where a distance measure between single features (i.e., ground distance) was given. This approach (Figure 8) was sensitive and useful in comparing test and reference profiles for BE testing and was superior to the commonly used distance measures such as Euclidean and Kolmogorov–Smirnov distances. The value of the population BE criteria (Θ) was found to be 0.7869 which is less than the standard Θ (0.7963) and hence, it can be concluded that the optimized formulation prepared (test) was better when compared with the drug solution (reference).

Stability studies

Stability studies at the end of 12 months revealed that there was no significant change in the properties of the formulations such as gelation time, gelation temperature, and drug release, and the drug content was found to be 98.23 ± 2.3 %. The liquid oral *in situ* gelling system of MKS was found to be stable for

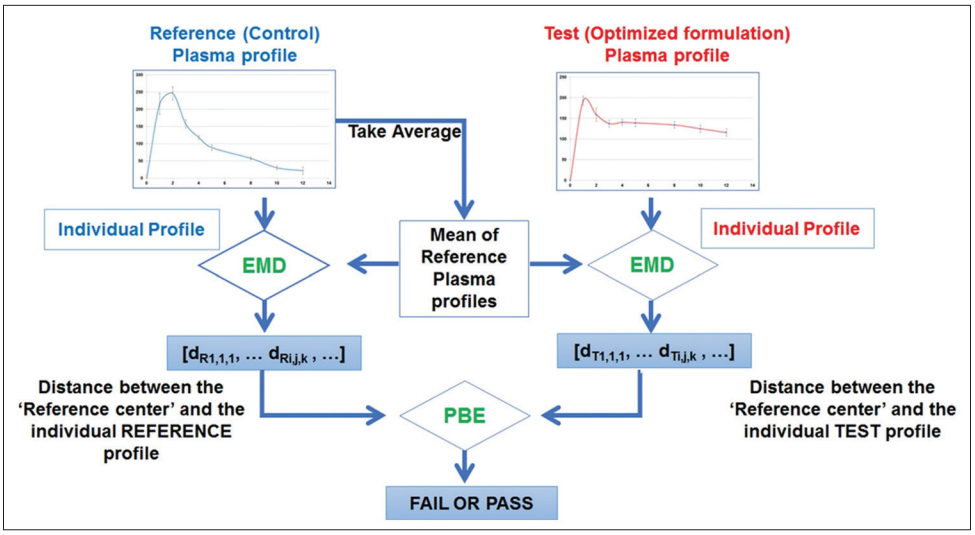

Figure 8: The flow chart for equivalence testing using the population bioequivalence approach and earth mover's distance

2 years when stored in light shielding closed glass containers and remained as a sol under refrigerated conditions.

CONCLUSION

A liquid oral *in situ* gel of MKS formulated employing statistical design, optimization techniques, using thermosensitive polymers, could sustain the release of the drug up to 12 h, give a better oral bioavailability and patient compliance. Therefore, it can be used as a better approach for the drug delivery of MKS. The simple and economical method of preparation of the formulation indicates the possibility of scale-up and commercial production of the same after conducting clinical trials so as to reach the patient at the earliest.

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