Formulation Development and Evaluation of Gastro Retentive Matrix Tablet of Levamisole Hydrochloride

Shoaeb Mohammad Syed^{1*}, Kiran Gawale¹, Z S Farooqui²

¹Assistant Professor, Dr. Vedprakash Patil Pharmacy College, Aurangabad MH, India

²Lecturer Kamala Nehru Polytechnic Pharmacy, Aurangabad MH, India

ABSTRACT

The aim of current study was to develop suitable gastroretentive tablet of Levamisole HCl for prolonging the retention of Levamisole in stomach. Floating tablets were prepared by using HPMC K4M and Carbopol 934p in combination and their effect on floating, swelling and release of Levamisole HCl was studied. Optimization of drug release were carried out by taking different concentration of HPMC K4M and Carbopol 934p. It was found that HPMC and Carbopol with concentration 35% and 22% in tablet give satisfactory release and batch was selected for 3² factorial design. In this study all the formulation were subjected to physical evaluation parameter such as Hardness, Friability, content uniformity all were found to be within limits. *In-vivo* study F₅ formulation was carried out to check the buoyancy of tablet. Tablet float in stomach of rabbit for 8 hours without adhering to gastric mucous. Stability studies of F₅ formulation indicated that formulation is stable for 3 months at 40°C and 75% RH. The formulation retained its integrity and *In-vitro* performance.

Keywords: Gastroretentive, Levamisole HCl, Factorial design, HPMC, Carbopol, *In vivo* buoyancy

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Corresponding Author:

Shoaeb Mohammad Syed, Assistant Professor, Dr. Vedprakash Patil Pharmacy College, Aurangabad MH, India

Email: ybccpsh@gmail.com

Mobile: +91 9175599468

INTRODUCTION

Sustained release technology is relatively new field and as a consequence, research in the field has been extremely fertile and has produced many discoveries. With many drugs, the basic goal is to achieve a steady state blood level that is therapeutically effective and non-toxic for an extended period of time.

Sustained release, sustained action, prolonged action, controlled release, extended action, timed release and depot dosage form are term used to identify drug delivery system that are designed to achieve prolonged therapeutic effect by continuously releasing medication over an extended

period of time after administration of a single dose. In the case of oral sustained released dosage form, an effect is for several hours depending upon residence time of formulation in the GIT¹⁻⁴.

Physician can achieve several desirable therapeutics advantages by prescribing sustained release dosage form. Since, the frequency of drug administration is reduced, patient's compliances can be improved and the drug administration can be made more convenient as well, the blood level oscillation characteristics of multiple dosing form of conventional dosage form is reduced, the safety margin of high potency drug can be increase and the

incidence of both local and systemic adverse effects can be reduced in sensitive patients⁵⁻⁷.

Gastro retentive drug delivery system belongs to oral controlled drug delivery system group that are capable to retain in the stomach by bypassing the gastric transit. These dosage forms are also defined as floating drug delivery system, which can float in the contents of the stomach and release the drug in a controlled manner for prolonged periods of time. The release rate will be controlled depending upon the type and concentration of the polymer that swells, leads to diffusion and erosion of the drug⁶⁻⁹.

GRDDS is retained for longer periods of time in the stomach e.g. Hydrophilic matrix tablets, floating capsules and bioadhesive tablet. Thus the longer period of gastric retention as compared to other oral controlled drug delivery system can be attributed. The floating results in release of the drug in to the stomach and the small intestine rather than into the large intestine where drug absorption is poor or erratic. This is achieved by adjusting the time period of release for the drugs by changing the concentrations of polymers⁷⁻¹⁰.

Levamisole HCl is immunomodulator and for achieving long term effect there is need to give Levamisole as sustained drug delivery. Levamisole HCl have short half life about 3-4 hrs, bioavailability 60-70%, is unstable in alkaline pH, stable in acidic environment, and have absorption window in the upper part of GIT, thus Levamisole HCl is suitable candidate to give as GRDDS¹⁻¹².

Gastroretentive drug delivery system has been widely used to prolong the retention of dosage form in the stomach. The present study was an attempt to produce gas forming floating drug delivery system of Levamisole Hydrochloride for prolonging the retention of system in stomach¹³.

MATERIALS AND METHODS

Materials

Formulation of gastro retentive drug delivery system consist of swelling agent, gas generating agent, hydrophilic polymers, filler and lubricant.

The materials used were Levamisole HCl, Hydroxypropyl methylcellulose K4M, Carbopol 934,

Sodium bicarbonate, Microcrystalline cellulose, Magnesium stearate, Talc.

Equipment

Equipment used for formulation are UV/VIS Double Beam Spectrophotometer, Fourier Transform IR-Spectrophotometer, tablet press, Tablet Dissolution Tester, Monsanto hardness tester, Roche Friabilator, Balance, Stability Chamber.

Method

Preformulation Study

Identification test

Identification test for Levamisole HCl was carried out using IR spectroscopy and UV absorbance spectra.

- For IR spectroscopy KBr powder was dried out at 60 °C for one hour. The dried KBr powder was uniformly mixed with drug and IR spectra was taken for this mixture.
- For UV validation of Levamisole HCl the solution of concentration from 2-20 ug/ml was prepared in 0.1 N HCl. The solution was scanned from 200-400nm and a spectrum was observed for absorption maxima.

Solubility

The solubility of compound was carried out in water; the excess amount of drug was dissolved in 5ml of solvent. The solution was then subjected to ultrasonication for 30 min. It was then allowed to stand for 24 hours on room temperature in tightly closed vials to attain saturation equilibrium. After 24 hours the solution was filtered through whatman filter paper No 41. It was then diluted appropriately with the solvent and its absorption was observed through UV spectrophotometer at 216 nm.

Melting point

The melting point of Levamisole hydrochloride was recorded by capillary method using Thiele's tube melting point apparatus and was compared with the literature reported data.

Bulk density and taped density

The Bulk density and taped density of the drug was determined by using USP method. The specific weighed quantity of drug was added in measuring cylinder. Its volume was noted down to calculate bulk density.

After tapping the volume was again noted down to calculate tapped density. Bulk density and taped density was determined by using following formula.

$$\text{Bulk density (gm/cm}^3\text{)} = \frac{\text{Bulk volume}}{\text{Weight of powder}}$$

$$\text{Tapped density (gm/cm}^3\text{)} = \frac{\text{Tapped volume}}{\text{Weight of powder}}$$

Angle of repose

This parameter is useful to measure resistance of particle to movement. The static heap of powder, when only gravity acts upon it, will tend to form conical mound. One limitation exists; the angle to horizontal plane cannot exceed a certain value and this is known as angle of repose θ .

The angle of repose was determined by following formula.

$$\tan \theta = \frac{2h}{D}$$

Flow rates

A simple indication of the ease with which a powder can flow is given by application of a compressibility index (I). The compressibility was determined by the following equation.

$$I = [1 - V / V_0] \times 100$$

Loss on drying

The loss on drying (LOD) of drug was determined by removing the moisture in hot air oven.

For the determination of LOD, the preweighed drug was kept in hot air oven at 105 °C for 4 hours. After 4 hour the drug was removed from oven and its weight was noted down.

The LOD was determined by using following formula.

$$LOD = \frac{W_0 - W}{W_0}$$

Assay

Assay was carried out by dissolving 200 mg of Levamisole HCl in 30 ml of alcohol and adding 5 ml of 0.01 N HCl. The solution was then titrated with 0.1N sodium hydroxide. The volume of sodium hydroxide consumed was determined by potentiometrically.

Each ml of 0.1 N NaOH equivalents to 24.08 mg of $C_{11}H_{12}N_2S.HCl$ ¹²⁻¹⁸.

Study of Interaction Between Drug And Polymer

FTIR analysis

Physical mixtures comprising of drug and polymers in a ratio of 1:1 were prepared by triturating in mortar and pestle and stored at 40±1°C, 75% relative humidity for 4 weeks to accelerate the interactions between drug and polymers.

DSC analysis

Differential scanning calorimetry (DSC) analysis was performed for single drug using a DSC, Shimadzu TA 60WS, instrument. The Physical mixtures in the ratio 1:1 of Levamisole + HPMC K4M, Levamisole + Carbopol 934p were mixed thoroughly for 5 min in mortar and crushed tablet were then stored at 40±1°C, 75% relative humidity for 4 weeks to accelerate the interactions between drug and polymers¹⁹.

Method of preparation of Levamisole HCl tablets

Preparation of powder blend

The formulation trials containing HPMC and Carbopol were prepared by direct compression method. In these trials drug along with HPMC and Carbopol were first sifted through sieve no. 30. These sifted ingredients were mixed well. Then separately sodium bicarbonate sifted through sieve no.60. Magnesium stearate and talc sifted through the sieve no. 40. The drug blend was mix with sifted sodium bicarbonate and subsequently mixed with Magnesium stearate and talc^{1-5 & 20}.

Preparation tablets

The blend of study drug obtained as above were compress into round shaped tablets on Karnavati eight station tablet press. The tablets were evaluated for different parameter like hardness and friability¹⁸.

Evaluation Parameter¹⁸

Hardness

Hardness of the tablet was tested using Monsanto hardness tester for each trial. The hardness range was determined for five tablet of each batch. The hardness of tablet was express in kg/cm.

Friability

Friability of the tablet of each tablet was determined by using Veego Friabilator. For this test ten tablets were weighed and placed in friabilator which was operated for 100 revolutions at the speed of 25 revolutions per minute. The tablets were then dusted and reweighed. The Friability of the tablet was calculated by using following formula.

$$LOD = \frac{W1 - W2}{W1} \times 100$$

Content uniformity test

Six tablets from each batch were weighed and powdered. Powder equivalent to the average weight of the tablet was accurately weighed and transferred into a 100-mL volumetric flask and dissolved in a suitable quantity of 0.1N HCl. The solution was made up to the mark and mixed well. A portion of the sample was filtered, diluted and analyzed by a UV/Visible Spectrophotometer at 216 nm.

In-vitro drug release study

In-vitro release study of Levamisole HCl was performed in USP dissolution apparatus Type 2. The dissolution medium was 900 ml of 0.1 N HCl. The dissolution was carried out at the rotation of 100 revolutions per minute. The temperature of bathe and medium was maintained at 37 ± 0.5 °C throughout testing. The 5 ml of aliquot was withdrawn at predetermined interval of 1, 2, 3, 4, and 5 upto 8 hours. The medium was replaced with 0.1 N HCl after each sampling.

The amount of Levamisole HCL dissolved at various time intervals was determined by measuring the absorption at the wavelength of 216 nm on test solution in comparison with standard solution.

In-Vitro Buoyancy study

The Buoyancy study is characterised by floating lag time and total floating duration. *In-vitro* Buoyancy study of the tablet was carried out by using dissolution apparatus Type 2. The medium was 900 ml of 0.1 N HCl. The testing was carried out at rotation of 100 revolutions per minute. The temperature of bathe and medium was maintained at 37 ± 0.5 °C throughout testing. The time required for the tablet to rise to surface of the dissolution medium and the duration of time the tablet constantly floated on the dissolution medium was noted for each formulation trial.

Swelling index

The swelling of the polymer can be measured by their ability to absorb water and swell enormously. The Swelling index is the ability of the polymer to swell by absorbing water. The water uptake study of the tablet was carried out by using dissolution apparatus Type 2. The medium was 900 ml of distilled water. The testing was carried out at rotation of 100 revolutions per minute. The temperature of bathe and medium was maintained at 37 ± 0.5 °C throughout testing. The tablet were withdrawn from the medium after selected time interval, excess water was removed by blotting and weighed^{1-4, 21}.

The swelling of the tablet was given by following formula.

$$\text{Swelling index (\%)} = \frac{\text{Weight of swollen tablet} - \text{Initial weight of tablet}}{\text{Initial weight of tablet}} \times 100$$

Formulation of Primary Trial batches

Formulation trial for Optimization of gas forming agent

For the floating of tablet sodium bicarbonate is used as gas forming agent. When it comes in contact with acidic environment (0.1 N HCl) endothermic reaction occurred with the formation of CO₂ gas. It allows tablet to float on the surface of simulated gastric

medium due to entrapment of CO₂ in the matrix of tablet.

Different formulation trials were taken with HPMC K4M and Carbopol 931K using different concentration of sodium bicarbonate. The tablets were made by using direct compression process. The compression was carried out on Karnavati eight station tablet press. The formula of these trials is given in table 1.

Formulation trial by using different concentration of HPMC K4M and Carbopol 934p

The formulation trials were taken with combination of HPMC K4M and Carbopol 934 P. Different concentration of polymer were taken to obtain optimized formulation.

In first three batches (A-C) concentration of Carbopol were kept constant and in last three batches (E-G) concentration of HPMC K4M were kept constant to obtained optimized formulation. The tablets were made by direct compression process. In table 2 the formulae of these trials are reported in terms of percentage composition.

Table 1: Optimization of gas forming agent Formulation trial G1-G4

Ingredients	G1		G2		G3		G4	
	%	mg	%	Mg	%	mg	%	mg
Levamisole HCl	20	50	20	50	20	50	20	50
HPMC K4M	25	62.5	25	62.5	25	62.5	25	62.5
Carbopol 934P	10	25	10	25	10	25	10	25
NaHCO ₃	16	40	18	45	20	50	22	55
Mg stearate	27	67.5	25	62.5	23	57.5	21	52.5
Talc	1	2.5	1	2.5	1	2.5	1	2.5
MCC	1	2.5	1	2.5	1	2.5	1	2.5
Total	100	250	100	250	100	250	100	250

Table 2: formulation of batches from A – C

Ingredients	A		B		C		D		E		F		G	
	%	mg	%	mg	%	mg	%	mg	%	mg	%	mg	%	mg
Levamisole HCl	20	50	20	50	20	50	20	50	20	50	20	50	20	50
HPMC K4M	25	62.5	35	87.5	30	75	33	82.5	35	87.5	35	87.5	35	87.5
Carbopol 934P	10	25	10	25	10	25	20	50	20	50	18	45	22	55
NaHCO ₃	20	50	20	50	20	50	20	50	20	50	20	50	20	50
MCC	23	57.5	13	32.5	18	45	5	12.5	3	7.5	5	12.5	1	2.5
Talc	1	2.5	1	2.5	1	2.5	1	2.5	1	2.5	1	2.5	1	2.5
Mg stearate	1	2.5	1	2.5	1	2.5	1	2.5	1	2.5	1	2.5	1	2.5
Total	100	250	100	250	100	250	100	250	100	250	100	250	100	250

Compression was carried out on Karnavati eight station tablet press using 8.5 mm punch.

Factorial Design

Instead of repeating experiment for each independent variable or factor, we can design a more efficient experiment that evaluates the effect of two or more factors at same time. These types of design are referred to as factorial design.

A 3^2 full factorial design was used in the study. In this design two factors were evaluated, each at three levels and experiment trials were performed at all nine possible combinations.

The amount of HPMC K4M (X_1) and Carbopol 934 P (X_2) were selected as independent variables. The time required for dissolution of drug and swelling selected as dependent variable (Table 3 & 4).

Table 3: Formulation trial with 3^2 full factorial design

Formulation code	Variable level code	
	X_1	X_2
F1	-1	-1
F2	-1	0
F3	-1	+1
F4	0	-1
F5	0	0
F6	0	+1
F7	+1	-1
F8	+1	0
F9	+1	+1

Table 4: Value codes of factorial design

Coded value	X_1	X_2
-1	82.5	53
0	87.5	55
+1	92.5	57

Analysis of Data By Design Expert Software

A 3^2 full factorial design was selected and the 2 factors were evaluated at 3 levels, respectively. The percentage of HPMC K4M (X_1) and Carbopol 934p (X_2) were selected as independent variables and the dependent variables were % drug release at 1 hr, 4 hr, 8 hr and % swelling. The data obtained were treated using Stat Ease Design Expert 8.0.7.1 software and analyzed statistically using analysis of variance (ANOVA). The data were also subjected to 3-D response surface methodology to study the interaction of HPMC K4M (X_1) and Carbopol 934p (X_2) on dependent variables (Table 5 and 6).

Release Kinetics

In order to investigate the drug release mechanism from the matrix tablet, the % drug release data was analysed with kinetic mathematical model for zero order, first order and korsmayer peppas etc.

Drug release kinetics and best fit model for all the batches was found out with the help of PCP Disso Software V 2.08

Stability Studies

The best formulation was kept for stability studies in a stability chamber (Thermo lab) for a period of three months at temperature $40^\circ\text{C} \pm 2^\circ\text{C}$ and RH $75 \pm 5\%$. The changes in physical appearance, weight, drug content, friability, hardness, in-vitro drug release was observed after intervals of one month upto three month.

RESULTS AND DISCUSSIONS

Characterization of Levamisole Hydrochloride

All the characterization of Levamisole were obtained as per Table 7 and FTIR studies are performed for Levamisole HCL and were found to be within specified range (Table 8).

Characterization of Excipients

HPMC K4M

All the characterization and FTIR studies are performed for HPMC K4M and were found to be within specified range.

Carbopol 934p

All the characterization and FTIR studies are performed for Carbopol 934p and were found to be within specified Table 8 and figure 1.

Interaction between Drug and Polymer**FTIR study**

IR was carried out for individual polymers and drug and then combination of polymers and drug.

Table 5: Design Summary

Factor	Name	Units	Type	Low Actual	High Actual	Low Coded	High Coded
A	HPMC K4M	mg	Numerical	82.5	92.5	-1	+1
B	Carbopol 934p	mg	Numerical	53	57	-1	+1

Table 6: Response Summary

Response	Name	Units	Observations	Analysis
Y1	% Drug release at 1 st hr.	%	9	Polynomial
Y2	% Drug release at 4 th hr.	%	9	Polynomial
Y3	% Drug release at 8 th hr.	%	9	Polynomial
Y4	% Swelling	%	9	Polynomial

Table 7: Characterization of Levamisole hydrochloride

Test	Specifications	Results
Colour ⁶¹	White	Confirms
Odour ⁶¹	Odourless	Confirms
Physical state ⁶¹	Crystalline	Confirms
Identification	FTIR	Positive
Melting point ⁶²	230-235	230
pH of 10% water solution ⁶³	3.0 to 4.5	4.5
Loss on drying ⁶³	Not more than 0.5%	0.45%
Solubility ⁶²	Freely Soluble in water	208.64 mg/ml
λ max ⁶²	215	216
Assay ⁶³	NLT 95% - NMT 105%	98.728%

In IR study it was found that all prominent peaks which are present in individual graphs of Levamisole HCl and polymers were also present in the IR of physical mixture of drug and polymers (Table 8).

DSC study

DSC thermogram of drug, polymers + drug and formulation F₁ were obtained as per figure 2 & 3

Table 8: Characteristics of IR absorption of functional group for Levamisole HCl + Carbopol 934 p and HPMC

Particulars	Wavenumber(cm ⁻¹)	Vibration mode
Levamisole HCl	3067	CH=CH
	1307	C-S
	1380	C-N
Carbopol	2933	-OH
	1716	C=O
	1246	C-O-C
HPMC	3393	-OH
	2872	CH ₂

Angle of repose

The angle of repose for all the batches was determined by following equation.

$$\tan \theta = \frac{2h}{D}$$

The angle of repose values for the blends from A-G were within the range of 25° – 31° after calculation. Thus blends having required flow properties (Table 9).

The angle of repose values for the blends from F1 – F5 were within the range of 25° – 30° after calculation and having excellent flow properties. The angle of repose for blends from F6 – F9 were in the range of 30° – 35° and it has good flow.

Flow rates

The values of compressibility index from batches A – G for optimization is given in table 9. The values of Compressibility index ranged between 14% - 25%.

Characterization of Formulation Blends

Figure 1: FTIR Spectra of Carbopol 934p

From the results given in table. We can say that the blend containing Carbopol 10% show good flow properties and blend containing Carbopol 20% and above show fairly good flow properties.

The compressibility index of formulation with factorial design is given in table 9 that shows fairly good flow property for these blends. The compressibility index values for all the blends were found to be ranged between 20%-25%.

Tablet Evaluation Parameters

Optimization of gas forming agent

Optimization was carried out by taking different concentration of sodium carbonate as shown in following table 10. From the results observed it was decided to use 20 % of sodium bicarbonate for further development of formulations as this concentration gives required floating properties.

Tablet evaluation parameters of trial with HPMC K4M + Carbopol 934p

The tablet parameter like Hardness, Friability, Floating lag time, Floating duration and swelling index for these trials were noted down. All the values of evaluation parameters are given in table 11.

Tablet evaluation parameters of trial with 3² full factorial design

The tablet parameter like Hardness, Friability, Floating lag time, Floating duration and swelling index for factorial design were noted down. All the values of evaluation parameters are given in table 12 & table 13.

Table 9: Angle of repose of formulation trial batches A-G

Formulation code	Height of powder heap (h)(cm)	Diameter of powder heap (D)(cm)	Angle of repose	Bulk vol.(ml)	Tap vol.(ml)	Bulk density	Tap density	Compressibility index (%)
A	2	8.8	24.44	41.6	35.6	0.450	0.532	14.4
B	2.4	9.1	27.81	43.2	36	0.442	0.531	16.66
C	2.2	9.2	25.1	44.8	36.8	0.419	0.510	17.8
D	3	11.2	28.17	55.2	43.2	0.384	0.437	21.73
E	3	10.5	29.74	52.8	41.2	0.354	0.454	21.96
F	3	10.6	29.50	51.6	40	0.364	0.470	22.58
G	3	10.1	30.62	54.4	41.6	0.349	0.456	23.52
F1	2.4	9.3	27.29	50	39.2	0.380	0.485	21.6
F2	3	10.2	30.46	51.6	40.4	0.368	0.471	21.7
F3	2.4	9	28.072	52.4	40.8	0.358	0.460	22.138
F4	2.6	9.18	29.5	53.6	41.6	0.357	0.460	22.33
F5	3	10.3	30.22	54.4	42	0.348	0.451	23.53
F6	2.8	9.18	31.38	53.6	41.5	0.352	0.453	22.389
F7	2.6	8.60	31.15	55.2	40.8	0.342	0.462	23.88
F8	3	9.38	32.60	56.4	42.8	0.334	0.440	24.114
F9	3	9.22	33.05	57.6	44	0.328	0.430	23.618

IN-VITRO Release Profile

The trial batches A, B, C were formulated by keeping concentration of Carbopol constant. For the optimization of HPMC K4M were carried out by

taking different concentration from 25- 35 %. The trial batch D was formulated by increasing concentration of both polymers to minimize the burst release.

The trial batches E, F, G were formulated for optimization of Carbopol by changing concentration from 18-22%.

In-vitro release profile of trial batches A-G

The details of release profile of these trials are performed for A-G batch and given in table 14.

Table 10: Optimization of gas forming agent

Parameters	G1	G2	G3	G4
NaHCO ₃	16%	18%	20%	22%
Floating lag time	5.30 min	2.48 min	47 sec.	5 sec.
Total floating time	Submerged after 5 hrs	Submerged after 12.30 hrs	24 hrs.	Burst after 30-40 min.

Tablet 11: Evaluation parameters of formulation from A – G

Parameters	A	B	C	D	E	F	G
Floating lag time (sec)	46	47	50	45	50	42	43
Floating duration (Hrs)	24	24	24	24	24	24	24
Swelling index (%)	114.2	1430.4	127.3	126.8	139.4	134	145
Hardness (kg/cm ³)	5.5	6	5.5	5.9	5.4	5.9	5
Friability (%w/w)	0.26	0.33	0.21	0.36	0.22	0.37	0.35

Table 12: Swelling index from F1-F9

Time(Hrs)	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)	F7 (%)	F8 (%)	F9 (%)
1	25	29.23	30.55	40	44.85	46.58	59.65	60.54	62
2	33.98	38	39.99	58	61.2	68.8	71.56	73.12	79.59
3	50.66	55.6	60	64.8	67.6	76	88.42	96	100.58
4	58.67	61.58	71	80.46	90.4	99.4	101.94	120	130.42
5	70	74.1	81.6	98	114.6	121.4	125	140.73	151.46
6	74.44	81.2	90.4	110	128.4	135.4	142.01	160.25	172.52
7	90	100	110.4	125.6	139.67	150	165.89	175.1	187.48
8	99.5	110.8	120.5	131	143.2	156.8	175.8	189.65	198.98

Dissolution medium: 0.1N HCl

Speed: 100 rpm

Apparatus: USP type 2 (Paddle)

Volume of dissolution medium: 900 ml

In-vitro release of 3² factorial design

3² factorial design was carried out on optimized batch. Formulation trial with 3² full factorial design given in table 15 Value codes of factorial design give Table 16 Release profile of formulation F₁-F₉ given in table 17 & Figure 4.

Multiple Regression Analysis For 3² Factorial Design

A statistical model incorporating interactive and polynomial terms was utilized to evaluate the responses.

$$Y=b_0+b_1 X_1+b_2 X_2+b_{12} X_1X_2+b_{11}X_1^2+b_{22}X_2^2$$

Tablet 13: Evaluation parameters of formulations with 3² full factorial design

Parameters	F1	F2	F3	F4	F5	F6	F7	F8	F9
Appearance	Off white	Off white	Off white	Off white	Off white	Off white	Off white	Off white	Off white
Floating lag time (Sec)	49	55	42	50	46	47	50	45	44
Floating duration (Hrs)	24	24	24	24	24	24	24	24	24
Swelling index (%)	99.5	110.8	120.5	131	143.2	156.8	160.02	168	178
Hardness (kg/cm ³)	5.4	5.6	5.7	5.5	6.3	5	5.5	5.6	5.5
Friability (%w/w)	0.39	0.35	0.35	0.27	0.27	0.33	0.25	0.24	0.23
Drug content (%)	99.60	98.37	101.80	99.50	99.90	102.70	103.20	97.30	98.50

Table 14: Release profile of formulation batch A-G

Time (Hrs)	% drug release						
	A	B	C	D	E	F	G
1	15.099±1.09	13.489± 0.72	12.177± 0.54	19.034± 0.90	18.736± 0.57	19.093± 0.99	17.9613±0.72
2	22.695± 0.90	22.984± 0.47	28.522± 1.29	36.847± 0.91	36.428± 1.29	37.503± 1.29	33.9203±1.35
3	30.392± 1.44	39.448± 1.37	46.268± 0.57	47.366± 0.47	42.473± 0.90	46.058± 0.82	42.3953±0.90
4	69.374± 1.10	34.896± 1.87	48.849± 0.10	53.172± 1.29	52.723± 0.91	57.521± 1.34	53.4803±0.65
5	93.844± 1.47	49.993± 0.88	52.039± 1.66	63.898± 0.57	59.154± 0.89	65.349± 0.19	60.4523±0.89
6	52.865± 1.17	56.527± 0.90	61.565± 1.16	73.847± 0.83	70.866± 1.17	75.307± 0.55	69.6073±0.67
7	50.051± 1.82	62.202± 1.77	67.685± 0.91	83.134± 0.89	80.017± 0.89	84.781± 0.98	77.7983±0.89
8	44.120± 1.82	74.764± 1.36	97.089± 0.49	90.563± 0.98	87.131± 0.91	92.337± 0.76	84.8993±0.45

Multiple regression analysis of 3² factorial batches for Y1- percent Drug release at 1st hr in table 18 and figure 5, Multiple regression analysis of 3² factorial batches for Y2- percent Drug release at 4 hr given in table 19 figure 6, Multiple regression analysis of 3²

factorial batches for Y3- percent Drug release at 8 hr given in table 20 and figure 7, Multiple regression analysis of 3² factorial batches for Y4- percent swelling at 8 hr given in table 21.

ANOVA Study

Evaluation and interpretation of research findings are utmost important and the p-value serves a valuable purpose in these findings. It shows ANOVA

for the dependent variables % drug release at 1sthr, 4thhr, 8thhr, and % swelling respectively. The coefficients of X₁ and X₂ were found to be significant at p<0.05, hence confirmed the significant effect of both the variables on the selected responses.

Figure 2: DSC of Levamisole HCl

Table 15: Formulation trial with 3² full factorial design

Formulation code	Variable level code	
	X1	X2
F ₁	-1	-1
F ₂	-1	0
F ₃	-1	+1
F ₄	0	-1
F ₅	0	0
F ₆	0	+1
F ₇	+1	-1
F ₈	+1	0
F ₉	+1	+1

Response surface plot

The response surface plot was generated using Design Expert 8.0.7.1 software presented in figure

5,6 & 7 to observe the effects of independent variables on the response studied such as % drug release at 1sthr, 4thhr, 8thhr, and % swelling respectively.

Table 16: Value codes of factorial design

Level	X1- conc. Of HPMC K4M	X2- conc. Of Carbopol 934p
Lower (-1)	82.5 mg	53 mg
Middle (0)	87.5 mg	55 mg
Upper (+1)	92.5 mg	57 mg

In-Vivo buoyancy study

The best formulation was subjected for *in-vivo* assessment of gastro-retentivity.

The best formulation was made X-ray opaque by replacing 50 mg of the drug with barium sulphate and all other ingredients were kept constant.

X-ray of rabbit at 0.5 hr (A) and 1hr. (B) in figure 8 and at 4th hr in figure 9. Rabbit were used for this study since gastric motility and stomach emptying

between humans and rabbit comparability shows no big differences.

Figure 3: DSC of Tablet

Figure 4: Comparative drug release of F₁-F₉

Stability Study

Stability study of formulation F₅ given in table 22, Percentage Drug release of stability batch F₅ given in table no 23.

The value of n with regression coefficient for all the formulation is shown in table 24 The value of all the formulations was to be in range of 0.5- 1 which indicate the anomalous transport.

Release Kinetics

Therefore, drug release occurs by three mechanisms diffusion, swelling and erosion.

Table 17: Release profile of formulation F₁-F₉

Time (Hrs)	% drug release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	34.356±	33.224±	24.638±	22.909±	21.299±	20.763±	18.378±	15.397±	11.581±
	1.72	0.81	0.55	0.47	0.47	0.89	1.37	0.89	0.99
2	44.862±	43.425±	40.157±	38.955±	36.979±	35.128±	28.377±	24.605±	24.404±
	1.39	1.80	1.19	0.64	0.89	1.50	0.77	0.94	0.89
3	47.793±	47.481±	52.423±	50.320±	49.943±	46.948±	41.651±	37.559±	38.789±
	1.45	1.46	1.72	1.69	0.99	0.63	0.81	1.72	0.81
4	52.826±	54.718±	68.750±	64.609±	62.321±	60.145±	53.924±	52.255±	48.543±
	0.82	1.17	1.91	0.90	0.64	0.47	0.45	1.84	1.52
5	66.888±	60.026±	78.966±	71.940±	69.998±	67.333±	64.536±	60.770±	56.382±
	1.81	0.17	0.30	0.72	1.09	0.91	0.81	0.79	1.80
6	88.598±	81.937±	85.540±	80.622±	78.013±	76.704±	71.567±	67.424±	64.442±
	1.24	1.71	1.84	0.95	0.89	0.74	1.44	1.29	1.28
7	89.858±	87.214±	90.837±	85.831±	81.539±	82.430±	74.642±	71.608±	69.207±
	0.67	0.93	1.11	0.59	0.83	1.77	0.83	0.58	0.57
8	97.263±	94.247±	93.477±	90.293±	87.885±	86.277±	78.864±	75.039±	73.698±
	0.77	0.90	1.28	0.93	0.52	1.44	1.18	1.36	0.58

Table 18: Multiple regression analysis of 3² factorial batches for Y1- percent Drug release at 1st hr.

Source	Degree of freedom	Sum square	Mean square	F-value	Prob>F
Model	5	432.31	86.46	13.56	0.0285
X ₁	1	366.01	366.01	57.41	0.0048
X ₂	1	58.04	58.04	9.10	0.0569
X ₁ X ₂	1	2.13	2.13	0.33	0.6035
X ₁ ²	1	3.24	3.24	0.51	0.5276
X ₂ ²	1	2.89	2.89	0.45	0.5489

Equation	$Y_1 = 22.46 - 7.81X_1 - 3.11X_2 + 0.73X_1X_2 + 1.27X_1^2 - 1.20X_2^2$				
$R^2 = 0.9576$	$\text{Adj}R^2 = 0.887$	$\text{Pred}R^2 = 0.515$	$\text{AdeqPrecisior} = 10.5$	$\text{SD} = 2.53$	$\text{CV} = 11.22$

Table 19: Multiple regression analysis of 3² factorial batches for Y2- percent Drug release at 4 hr.

Source	Degree of freedom	Sum square	Mean square	F-value	Prob>F
Model	5	306.35	61.27	3.60	0.1604
X ₁	1	77.56	77.56	4.56	0.1225
X ₂	1	6.16	6.16	0.36	0.5899
X ₁ X ₂	1	113.48	113.48	6.67	0.0816
X ₁ ²	1	103.36	103.36	6.07	0.0905
X ₂ ²	1	5.79	5.79	0.34	0.6007

Equation	$Y_2 = 61.22 - 3.60X_1 + 1.01X_2 - 5.33X_1X_2 - 7.19X_1^2 + 1.70X_2^2$				
$R^2 = 0.8571$	$\text{Adj}R^2 = 0.6190$	$\text{Pred}R^2 = 0.705$	$\text{AdeqPrecisior} = 5.29$	$\text{SD} = 4.13$	$\text{CV} = 7.17$

Table 20: Multiple regression analysis of 3² factorial batches for Y3- percent Drug release at 8 hr.

Source	Degree of freedom	Sum square	Mean square	F-value	Prob>F
Model	5	593.99	118.80	1026.79	<0.0001
X ₁	1	548.95	548.95	4744.73	<0.0001
X ₂	1	28.05	28.05	242.44	0.0006
X ₁ X ₂	1	0.48	0.48	4.14	0.1346
X ₁ ²	1	14.81	14.81	128.00	0.0015
X ₂ ²	1	1.70	1.70	16.66	0.0314

Equation	$Y_3 = 87.54 - 9.57X_1 - 2.16X_2 - 0.35X_1X_2 - 2.72X_1^2 + 0.92X_2^2$				
$R^2 = 0.9994$	$\text{Adj}R^2 = 0.9984$	$\text{Pred}R^2 = 0.9950$	$\text{AdeqPrecisior} = 84.452$	$\text{SD} = 0.34$	$\text{CV} = 0.39$

Table 21: Multiple regression analysis of 3² factorial batches for Y4- percent swelling at 8 hr.

Source	Degree of freedom	Sum square	Mean square	F-value	Prob>F
Model	5	9977.13	1995.43	797.24	<0.0001
X ₁	1	9097.16	9097.16	3634.63	<0.0001
X ₂	1	816.20	816.20	326.10	0.0004
X ₁ X ₂	1	1.19	1.19	0.47	0.5413

X_1^2	1	61.35	61.35	24.21	0.00158
X_2^2	1	1.24	1.24	0.49	0.5326

Equation $Y_4 = 144.46 + 38.94X_1 + 11.66X_2 - 0.54X_1X_2 - 5.54X_1^2 + 0.79X_2^2$
 $R^2=0.9992$ $AdjR^2=0.9980$ $PredR^2=0.9919$ $AdeqPrecisor=78.346$ $SD=1.58$ $CV=1.07$

Table 22: Stability study of formulation F₅

Parameter	Initial	1 month	2 month	3 month
Appearance	Off-white	Off-white	Off-white	Off-white
Weight	260.58	261.50mg	265.75mg	270.54mg
Friability	0.27	0.3	0.39	0.56
Hardness	6.3	5.8	5.4	4.5
Drug content	99.90%	99.18%	98.56%	97.85%
Microbial growth	Nil	Nil	nil	Nil

Table 23: Percentage Drug release of stability batch F₅

Time (Hrs)	<i>In-vitro</i> % drug released for F5 formulation			
	Initial	1 month	2 month	3 month
1	21.299 ±0.47	24.547 ±1.017	26.584 ±1.64	31.025 ±1.5
2	36.979 ±0.89	37.548 ±1.89	40.56 ±1.9	44.825 ±2.1
3	49.943 ±0.99	50.01 ±1.57	51.954 ±1.24	56.214 ±2.9
4	62.321 ±0.64	64.25 ±2.1	67.018 ±0.56	69.4 ±1.4
5	69.998 ±1.09	71.89 ±2.58	74.65 ±2.45	75.312 ±3.2
6	78.013 ±0.46	78.012 ±1.09	80.75 ±1.10	83.56 ±2.9
7	81.539 ±0.83	82.155 ±1.8	83.904 ±1.6	87.0 ±1.65
8	87.885 ±0.52	88.107 ±2.4	89.845 ±0.95	92.588 ±1.1

Table 24: Release kinetics of batches with 3² factorial design

Formulat -ion	Release exponent (n)	Regression coefficient (R ²)				
		Zero order	first order	matrix	Kornsmayer-peppas	Higuchi model
F1	0.5184	0.9329	0.9163	0.9748	0.954	0.9643
F2	0.5064	0.9301	0.9386	0.9787	0.9619	0.9699

F3	0.6663	0.9384	0.9896	0.9879	0.9941	0.9973
F4	0.6680	0.9439	0.994	0.9899	0.9957	0.9979
F5	0.6835	0.9441	0.9962	0.9892	0.9944	0.9965
F6	0.6988	0.9555	0.9948	0.9864	0.9965	0.9972
F7	0.7407	0.9622	0.9901	0.9784	0.9937	0.9934
F8	0.8093	0.9698	0.9914	0.9693	0.9911	0.9926
F9	0.8945	0.9769	0.9937	0.9676	0.9902	0.9967

SUMMARY AND CONCLUSION

Gastroretentive drug delivery system has been widely used to prolong the retention of dosage form in the stomach. The present study is an attempt to produce gas forming floating drug delivery system of Levamisole Hydrochloride for prolonging the retention of system in stomach.

In the initial stages formulation trials were carried out by taking different concentration of sodium bicarbonate. It was found that as concentration of sodium bicarbonate increases upto 20% of tablet weight the floating lag time decreases, further increase in concentration lead to bursting of tablet.

In the study optimization of drug release were carried out by taking different concentration of HPMC K4M and Carbopol 934p.

It was found that HPMC and Carbopol with concentration 35% and 22% in tablet give satisfactory release and batch was selected for 3² factorial design.

3² full factorial design were carried out by considering HPMC and Carbopol as independent variable while % drug release and % swelling as dependent variable.

Design-Expert® Software
Factor Coding: Actual
% Drug rel. at 1st hr
● Design points above predicted value
○ Design points below predicted value
34.356
11.581
X1 = A: HPMC K4M
X2 = B: Carbopol 934p

Figure 5: Response Surface Plot showing the effect of variables on % drug release at 1st hour

Design-Expert® Software
Factor Coding: Actual
% Drug rel. at 4 hr
● Design points above predicted value
○ Design points below predicted value
68.75
48.543

X1 = A: HPMC K4M
X2 = B: Carbopol 934p

Figure 6: Response Surface Plot showing the effect of variables on % drug release at 4 hour

Factorial design were carried out with three level and nine all possible combination.

In this study it was observed that as polymer concentration increases the release of Levamisole decreases while as concentration of polymer increase % swelling increases.

In this study all the formulation were subjected to physical evaluation parameter such as Hardness, Friability, content uniformity all were found to be within limits.

Design-Expert® Software
Factor Coding: Actual
% Drug rel. at 8 hr.
● Design points above predicted value
○ Design points below predicted value
97.263
73.693

X1 = A: HPMC K4M
X2 = B: Carbopol 934p

Figure 7: Response Surface Plot showing the effect of variables on % drug release at 8 hour

Statistical analyses were carried out by design expert software on the data obtained in 3² factorial

design. Multiple regression analysis, ANOVA, response surface plot and desirability effect were

studied. In the analysis it was found that HPMC K4M and Carbopol 934 have positive coefficient for swelling while negative coefficient for drug release.

From the response surface plot of desirability formulation F_5 shows highest value of desirability.

Figure 8: x-ray of rabbit at 0.5 hr (A) and 1 hr. (B)

Figure 9: x-ray of rabbit at 4th hr. (C) and 8 hr. (D)

Hence Formulation F_5 was considered as optimized formulation and gave release of 87.885% in 8 hours. The kinetic study of formulation F_5 shows that it

follow Fickian transport ($n = 0.6835$) and swelling, diffusion, erosion is the mechanism of drug release.

In-vivo study F₅ formulation was carried out to check the buoyancy of tablet. Tablet float in stomach of rabbit for 8 hours without adhering to gastric mucous.

Stability studies of F₅ formulation indicated that formulation is stable for 3 months at 40° C and 75% RH. The formulation retained its integrity and *In-vitro* performance.

CONFLICTS OF INTEREST

None.

AUTHOR'S CONTRIBUTIONS

SMS, KG and ZSF alike collaborated for the completion of work. All authors reviewed and approved the final manuscript for publication.

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