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AN ATTEMPT TO UNDERSTAND AND VALIDATE THE FACTORS CONTROLLING *IN-SITU* RAFT FORMATION PROCESS

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Keywords:

In-situ raft forming system, *In-vitro In-vivo,* Process capability study, Process scale-up, Validation, Cause, and effect diagram

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ABSTRACT: In this study, *in-situ* raft forming Levofloxacin Suspension formulation was developed. *In-vitro* conditions like temperature, pH, and RPM simulating the *in-vivo* conditions like gastric pH, body temperature, and gastric motility respectively were identified as critical process parameters in system scale-up studies. Challenging and characterization were performed *in-vitro*. The working limits were identified and verified by calculation of process capability indices. Process Potential (Cp) and Process performance (Cpk) values greater than 2, and 1.33 respectively helped to select the conditions for the formation of raft system controlling the release up to 8 h with zero order release kinetics found to be significant (R^2=0.9930). The release mechanism was found to be Korsemeyer-peppas showing the mechanism of drug release by diffusion and relaxation of polymeric raft structure. The Suspension formulation subjected to stability studies (40 ºC ± 2 ºC / 75% ± 5% RH) were found to be stable for pH, viscosity, floating lag time, content uniformity and percentage drug release from the raft structure. Moreover, radiographic x-rays evaluation for optimized formulation for the validation of set *in-vitro* protocol revealed that the raft structure formed *in-vivo* elicits excellent gastric retention as proposed in observations and removed from the body within safe period of 24 h.

INTRODUCTION: Raft forming system (RFS) is one of the floating drug delivery system, which is retained in the stomach and is useful for drugs that are poorly soluble or unstable in intestinal fluids. RFS has a bulk density less than gastric fluids and so remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period. While the system is floating on the gastric contents, the drug is released slowly at the desired rate from the system.

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After the release of the drug, the residual system is emptied from the stomach. This results in increased gastric retention time and better control of fluctuations in plasma drug concentration. This interest has been sparked by the advantages shown by raft forming polymeric delivery systems such as ease of administration and reduced frequency of administration, improved patient compliance, and comfort. Raft formation occurs due to one or a combination of different stimuli like pH change, temperature modulation, and solvent exchange [1-5].

Levofloxacin is a synthetic fluoroquinolone antibacterial agent that inhibits bacterial DNA gyrase enzyme that is required for DNA replication and so causes bacterial lysis. It is L-isomer of ofloxacin. It has a half-life of 5-7 h, and the absorption of Levofloxacin is dose-dependent,

which increases with an increase in dose. It is effective against the treatment of *H. pylori*. The failure of antibiotic therapy can be avoided by providing the effective concentration of drug at the site of action [6, 7].

Literature till date on *in-situ* raft formulation reveals the focus on the composition of the system. Considering the process of *in-situ* raft formation, it has been found that there are numerous physiological parameters like gastric pH, body temperature and gastric motility which should be considered to generate raft structure with expected release profile *in-situ*. The proper system scale-up studies on a laboratory basis should be considered as a validated approach to justify the formulation, development, and optimization of raft-forming system to prove its reproducibility.

The proper process/system validation approach involves the generation of process flow diagram, identification of critical process parameters, cause and effect analysis, process capability study and process verification to prove it to be validated process. Considering these aspects, the need was to identify the critical parameter about *in-vivo* conditions and optimizes raft formation process *in-vitro*. Hence, the present study covers the approaches like formulation, development, optimization, and evaluation of raft formation process applying *in-vitro* conditions like temperature, pH and RPM simulating the *in-vivo* conditions mentioned above which were identified as critical process parameters. Process flow diagram was generated. Radiographic X-rays evaluation for the optimized formulation was also performed for the confirmation of set *in-vitro* protocol. Critical process parameters limits were identified and verified by process capability studies. System development protocol was generated finally as validated *in-situ* raft formation process.

EXPERIMENTAL:

Part A: Formulation and Development:

Drug and Excipients Characterization:

Determination of UV Absorbance Maxima of Levofloxacin: Stock solution of Levofloxacin in 0.1 N HCl was prepared and diluted till appropriate concentration. The solution was then scanned in UV visible spectrophotometer within the wavelength of 200-400 nm.

Preparation of Standard Calibration Curve of Levofloxacin in 0.1 N HCl: 10 mg of Levofloxacin was dissolved in 100 ml of 0.1N HCl. The solution was then diluted with 0.1 N HCl to obtain 2, 4, 6, 8, 10, and 12 µg/ml solution. It was then measured by UV visible spectrophotometer at 293 nm.

Experimental Design: A statistical model incorporating interactive and polynomial terms was used to evaluate the responses. A 3^2 full factorial design was constructed where the amounts of Sodium alginate (X_1) and Calcium carbonate (X_2) were selected as factors. The level of two factors was selected based on the preliminary studies carried out before implementing the experimental design. Percent drug release (%) and viscosity (cps) were considered as responses. All other formulations and processing variables were kept constant throughout the study [8].

Formula compositions are given as experimental matrix in **Table 1**

TABLE 1: EXPERIMENTAL MATRIX

S. no.	Ingredient	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Levofloxacin (%)	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
2	Sodium Alginate (%)	1.5	2.5	2	1.5	2.5	2	1.5	2.5	2
3	HPMC E15 (%)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
4	Calcium Carbonate (%)	1.5	1.5	1.5	2	2	2	1.75	1.75	1.75
5	Methyl Paraben (mg)	180	180	180	180	180	180	180	180	180
6	Propyl Paraben (mg)	90	90	90	90	90	90	90	90	90
7	Distilled Water	q.s.to 100ml	q.s.to 100ml	q.s.to 100ml	q.s.to 100ml	q.s.to 100ml	q.s.to 100ml	q.s.to 100ml	q.s.to 100ml	q.s.to 100ml

Preparation of Levofloxacin Suspension: Active material (Levofloxacin hemihydrates) was passed from sieve # 60 while other inactive ingredients like sodium alginate, calcium carbonate, HPMC E 15, methyl paraben, and propyl paraben were passed from sieve # 40. Sodium alginate was dissolved in sufficient quantity of water which was preheated not more than 60 ºC. The solution was

cooled to 40ºC. (Solution A). HPMC was dissolved in water in a separate beaker, then calcium carbonate and Levofloxacin hemihydrates (5 gm) were added to the above solution while stirring so that there was proper and homogenous dispersion of the active material in solution. (Solution B). This solution A was added to solution B or vice-versa and mixed well with the help of mechanical stirrer at 1000 RPM for 30 min. (Solution C).

Methylparaben and propylparaben were dissolved in water, and it was added to the above mixture (Solution C) and mixed well. Volume was adjusted to 100% with distilled water. Finally, the mixture was mixed well to get the final preparation (Final formulation).

Evaluation of Levofloxacin Suspension and the Raft Structure: Experimental formulations were evaluated for pH values, viscosity studies, *in-vitro* buoyancy study (Floating lag time and Floating Duration), measurement of percentage water uptake by the raft, drug content estimation. The results are discussed in **Table 2.**

Determination of pH Values: The pH of each of prepared RFS formulation was recorded using previously calibrated digital pH meter.

Viscosity and Rheology Studies: Viscosity determinations of the prepared RFS's were carried out on Brookfield Viscometer (Model No Brookfield DVE -LV viscometer Version 10.0) using an appropriate spindle. The viscosity of RFS was measured at different angular velocities (RPM) at a temperature of 37 ± 1 °C. The absolute viscosity of formulations was reported at a fixed torque value.

The averages of three readings were used to calculate the viscosity. The rheological behavior was explained by plotting viscosity against angular velocity. Prepared raft forming system solution was transferred in a sample cell, which was placed carefully within the adaptor. The guard leg was placed around the adaptor, and the volume of the sample was stirred slowly using a motor driven stirring element. The viscosity values were recorded from the display window. The run time was fixed as 30 seconds for each measurement. When the constant reading was observed at desirable torque, viscosity (cps) was noted down.

***In-vitro* Buoyancy Study (Floating lag time and Floating Duration):** *In-vitro* buoyancy study is characterized by floating lag time and total floating duration. *In-vitro* buoyancy study of the RFS was carried out using USP dissolution apparatus Type II. The medium used was 900 ml of 0.1N HCl. The temperature of the bath and medium was maintained at 37 ± 0.5 ºC throughout the study. 10ml of the RFS was transferred by using a syringe. The time required for the raft to rise to the surface of the dissolution medium (Floating Lag time) and the duration of the time for which the raft constantly floated on the dissolution medium (Floating duration) were noted for each formulation [9].

Measurement of Percentage Water Uptake by the Raft: The water uptake by the raft of the selected formulations of sodium alginate was determined by a simple method. In this study, the raft formed in 40 ml of 0.1 N HCl (pH 1.2) was used. From each formulation, the raft portion from the 0.1 N HCl was separated, and the excess HCl solution was blotted out with a tissue paper. The initial weight of the raft taken was weighed, and to this raft, 10 ml of distilled water was adde, and water was decanted, and the weight of the raft was recorded after 16 hours, and the difference in the weight was calculated and reported.[10]

$$\text{Water uptake} = W_2 - W_1 \times 100 / W_1$$

Where, W_1 = Initial weight of gel (10 ml)

W_2 = Final weight of the swollen matrix.

Drug Content Estimation by Spectro-photometric Analysis: Accurately, 1 ml of RFS (equivalent to 50 mg of levofloxacin) from all the batches were taken and to this 50 ml of 0.1 N HCl was added and sonicated for 30 min containing the strength of 1000 µg/ml. Complete dispersion of contents was ensured, and the contents were filtered using Whatman filter paper. From this solution, withdraw 1ml, dilute with 0.1N HCl up to 100 ml.

Resulting 10µg/ml solution was used and determined spectrophotometrically at 293 nm using double beam UV-Visible spectrophotometer. The concentration of Levofloxacin hemihydrate was determined from a previously prepared calibration curve [11].

***In-vitro* Drug Release Study for Raft System:** The release rate of Levofloxacin hemihydrate from formulation was determined using experimental conditions as mentioned. This speed should be slow enough to avoid the breaking of the raft and was maintained the mild agitation conditions believed to exist *in-vivo*. 10 ml of raft-forming formulation was added into dissolution medium with the help of a syringe. 10 ml of a sample of the solution were removed at the pre-determined interval for analysis and replaced with 10 ml of fresh 0.1N HCl.

The drug concentration of each sample was determined by spectrophotometrically [12]. USP Type II dissolution test apparatus with 50 RPM were the conditions selected, Results shown in **Table 3**.

Optimization by Statistical Analysis:

Effect of Experimental Variables on Response: There were 2 independent variables like sodium alginate and calcium carbonate, which can show effects on dependent variables (% release and viscosity). Effect of each variable individually and combination of variables were also studied to come up with an optimized solution.

Approaches for Optimum Solution: Based on experimental data analysis, optimum solution was generated.

Formulation of Optimized Solution: Procedure for the formulation of the optimized formulation is the same as mentioned for experimental batches.

Evaluation of Optimized Solution: Optimized solution was evaluated for various parameters like pH, viscosity, floating lag time content uniformity, percentage drug release at 5th h. Results of these evaluation mentioned in the result and discussion section.

Dissolution Kinetic Modeling: Release data obtained were fitted into various mathematical models then order and pattern of release were decided.

Stability Study: The selected optimized Formulation was evaluated for stability studies, which were maintained at 40 ºC ± 2 ºC / 75% RH ± 5% RH tested for 1 month and were analyzed. The formulations were evaluated mainly for their physical characteristics like appearance/clarity, pH, viscosity, and drug content at the predetermined intervals (8 days) of 1 month.

Part B: Raft Forming System Scale- Up Studies: Even though the process development activities typically begin after the formulation has been developed, they may also occur simultaneously. The majority of the process development activities occur either in the pilot plant or in the proposed manufacturing plant.

Design of Process: The steps included in formation raft forming process are as given in **Fig. 1**.

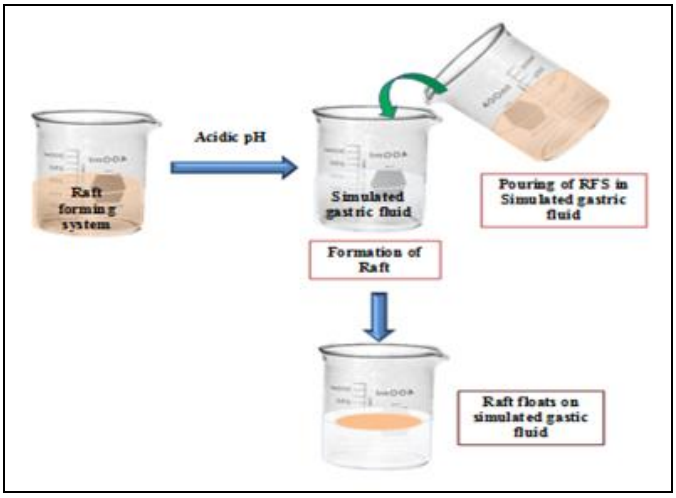

FIG. 1: PROCESS OF RAFT FORMATION

Challenging and Characterization Critical Process Parameters: There is 3 critical process parameter which affects the process of raft formation.

Temperature: Temperature control (thermo-regulation) is part of a homeostatic mechanism that keeps the individual at optimum operating temperature, as it affects the rate of chemical reactions. In humans, the average internal temperature is 37.0 °C (98.6 °F), though it varies among individuals. However, no person always has exactly the same temperature at every moment of the day. Temperatures cycle shows regular up and down the rhythm throughout the day, as controlled by the person's circadian rhythm.

Hypothermia:

Mild 35 to 32 °C: Shivering, vasoconstriction, liver failure, or hypo/hyperglycemia.

Moderate 32 to 28 °C: Pronounced shivering, sufficient vasoconstriction to induce shock,

cyanosis in extremities and lips, muscle miss-coordination.

Severe 28 to 20 °C: Heart rate, respiratory rate, and blood pressure fall to a dangerous level. Multiple organs failure and clinical death soon occur. However, as with most things in human biology, there is a wide scope for variation between individuals.

Hyperthermia: A body temperature of above 40°C is likely to be fatal due to the damage done to enzymes in critical biochemical pathways (*e.g.*, respiratory enzymes). Children and epileptics may be very likely to get convulsions at this point. When body temperature goes above 40 °C fainting, vomiting, severe headache, dizziness, confusion, hallucinations, delirium, and drowsiness can occur.

Variations Due to Outside factors: Many outside factors affect the measured temperature as well. "Normal" values are generally given for an otherwise healthy, non-fasting adult, dressed comfortably, indoors, in a room that is kept at a normal room temperature (22.7 to 24.4 °C or 73 to 76 °F), during the morning, but not shortly after arising from sleep [13].

RPM (Gastric Motility): There seem to be at least three different states of functional dyspepsia of the stomach: delayed gastric emptying (gastroparesis), impaired gastric accommodation and gastric hypersensitivity (functional dyspepsia). All these states seem to have an abnormal neuro-humoral component. For example, the normally rhythmic contractions of the distal stomach are disorganized in diabetic neuropathy [14].

Neural input from the central nervous system is a potent regulator of gastric motor activity and emptying. Anger increases phasic motor activity in the stomach, whereas fear and depression reduce gastric contractions. Intra-ventricular infusion of Thyrotropin-releasing hormone (TRH) accelerates, while opiates, tachykinins, somatostatin, atrial natriuretic factor, Gamma-Aminobutyric Acid (GABA) and calcitonin delays gastric emptying [14].

pH: Variation of gastric pH occurs because of several physiological factors and patient-related factors like diseases, diet, presence of gases, age, pathological conditions, drugs, as well as intra- and inter-subject variation. This variation in pH may significantly influence the performance of orally administered drugs. About 20% of the elderly people exhibit either diminished (hypochlorous-hydria) or no gastric acid secretion (achlorohydia). Pathological conditions such as pernicious anemia and Acquired immunodeficiency syndrome (AIDS) may significantly reduce gastric acid secretion leading to elevated gastric pH. These things affect the mechanism of the raft formation; it means that the formation of raft varies according to the stomach pH of the patient at the time of administration of the formulation.

***In-vivo: in-vitro* Correlation Study:** An efficient representation of complex relationships between raft formation process and formulation variables like gastric temperature, gastric pH and gastric motility and a single response (raft formation time) can be shown by using a cause-and-effect diagram. Critical process parameters were determined through *in-vivo* and *in-vitro* cause and effect diagram. *In-vivo* conditions of the variation in gastric temperature, gastric pH, and gastric motility correlated with *in-vitro* conditions and *in-vitro* cause and effect diagram were constructed, as shown in **Fig. 2A,** and **Fig. 2B**

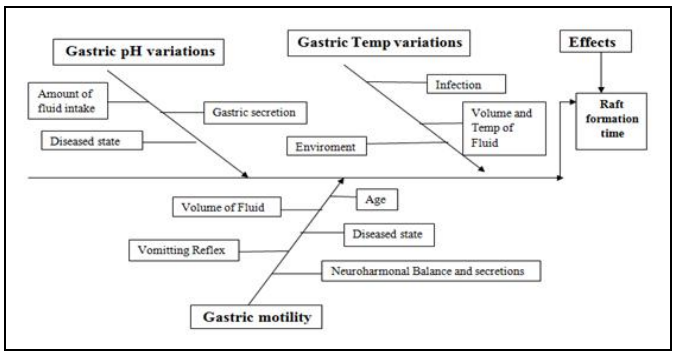

FIG. 2A: CAUSE AND EFFECT ANALYSIS CONSIDERING *IN-VIVO* CONDITIONS

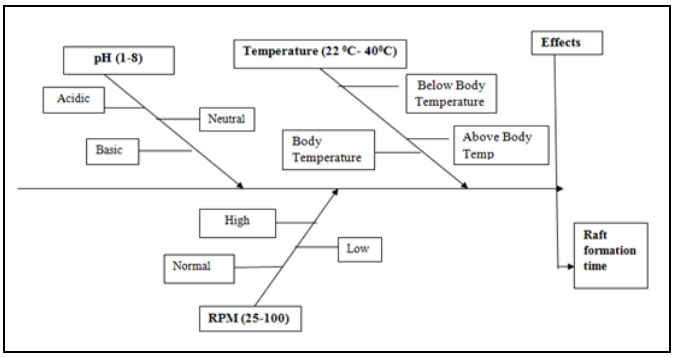

FIG. 2B: CAUSE AND EFFECT ANALYSIS CONSIDERING *IN-VITRO* CONDITIONS

For the determination of the process limit the following study was performed for the critical parameter.

Temperature Variation Study: Raft formation of the optimized formulation at various temperatures such as at 22 ºC, 27 ºC, 32 ºC, 37.5 ºC, and 40 ºC was determined with the help of dissolution apparatus. The effect of varying temperature condition on raft formation was noted down.

RPM Variation Study: Raft formation of optimized formulation at various RPM was studied, such as 25, 30, 40, 50, 60, 70, 80, 90 and 100, with the help of dissolution apparatus. USP apparatus type –II was used. The effect of varying in RPM on time required for raft formation is noted down.

pH Variation Study: Behavior of RFS in various pH conditions was studied. The optimized formulation in their appropriate dose was poured in the various pH conditions. Various pH selected for the study were 1.2, 3, 4, 5, 6, 7, and 8.

Verification of Developed Process Using Process Capability Studies: From above critical process was determined the process limit of the raft formation and the developed process was verified by process run of the optimized formulation given by design expert software. Process verified by the process capability study. Process potential and process performance were calculated, and the process was verified using the following equation given below

$$\text{Process potential (Cp)} = \text{USL} - \text{LSL} / 6\sigma$$

$$\text{Process performance (Cpu)} = \text{USL}-\mu / 3\sigma, \text{Cpl} = \mu-\text{LSL} / 3\sigma$$

$$\text{Cpk} = \text{Minimum } \{\text{Cpu, Cpl}\}$$

Key elements of process verification run were evaluated, which gives that process is capable of giving the desired results [15, 16].

Validation and Confirmation: *In-vivo* Evaluation of RFS:

Radiographic Study by X-Rays: To confirm gastric retention of dosage form *in-vivo* retention studies were carried out using rats as an animal model. Dose of raft-forming formulation with 1.5 ml of barium sulfate suspension was administered by oral rout using Ryle's tube. The retention was studied by taking X-Ray radiographs at a regular interval (after 6, 16 and 24 h) to ensure the retention of raft containing barium sulfate [17]. All the experiments and protocols described in this study were approved by the institutional animal ethical committee (IAEC-MET/IOP/M .pharm /2014-2015/IAEC/5) and all experiments were conducted as per the norms of the committee for control and supervision of experiments on animals (CPCSEA- 1344/ac/10/ CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

RESULT AND DISCUSSION:

Part A: Formulation and Development:

Drug Excipient Characterization:

Determination of λ_{max} of Levofloxacin Hemihydrate: The drug solution was scanned in the range of 400 nm to 200 nm. The spectrum indicated that the observed λ_{max} of Levofloxacin Hemihydrate was found to be 293 nm in 0.1N HCl using a double beam UV spectrophotometer.

Calibration Curves of Levofloxacin Hemihydrate in 0.1 N HCl: The calibration curve of Levofloxacin hemihydrate was prepared in 0.1N Hydrochloric acid solution at λ_{max} 293 nm. The straight line obtained in the 0.1N Hydrochloric acid solution had a regression coefficient of 0.998 (y = 0.088x + 0.073) Linearity was found in the concentration range of 2-12 µg/ml.

Optimization Using Experimental Design: Optimized batches formulated according to the procedure given above. The experimental batches evaluation and data analysis were performed to generate the optimized solution.

Evaluation of RFS (Factorial Batches): All the batches shown off-white color and floating duration >12 h. Tablet batches were evaluated for percentage water uptake, pH, and viscosity, floating lag time and drug content estimation. The results are reported in **Table 2**. The dissolution profile of formulation of F1 to F9 is shown a significant decrease in the rate and extent of drug release was observed with the increase polymer concentration in the formulation the release of the drug from these rafts was characterized by an initial phase of high release (burst effect). However, as raft formation proceeds, the remaining drug was

released at a slower rate followed by the second phase of moderate release. This biphasic pattern of release is a characteristic feature of matrix diffusion kinetics. Formulation F7 released the complete drug at the end of 5 h. While formulations F1, F3, F4, and F9 released complete drug at the end of 6 h. Moreover, F2 and F6 showed complete release at the end of 7 h. Only F5 and F8 formulations sustained the release of the drug up to 8 h. Results depicted in **Table 3.**

TABLE 2: EVALUATION EXPERIMENTAL MATRIX BATCHES OF LEVOFLOXACIN SUSPENSION AND THE RAFT STRUCTURE

Formulation code	Measurement of Percentage Water Uptake	pH	Viscosity in cps	FLT* in seconds	Drug content (%)
F1	28.63 ± 0.51	7.86 ± 0.30	867.33 ± 1.52	48 ± 2	97.2 ± 0.97
F2	31.94 ± 0.05	7.5 ± 0.1	2143.33 ± 1.52	37 ±2.6	97.4 ± 2.45
F3	34.15 ± 0.44	7.7 ± 0.17	936 ± 2.6	29 ±2	99.4 ± 0.48
F4	31.73 ± 0.15	8.07 ± 0.20	1019.33 ± 2.3	33 ±1	98.4 ± 1.06
F5	37.62 ± 0.24	7.55 ± 0.05	2765.66 ± 2.08	25.33 ± 0.57	99.7 ± 0.12
F6	26.08 ± 0.94	7.8 ± 0.1	1452 ± 2	29 ± 1	96.4 ± 0.54
F7	32.9 ± 0.78	7.73 ± 0.20	946.33 ± 1.52	24 ±2	97.3 ± 1.31
F8	37.15 ± 0.92	7.53 ± 0.05	2743.66 ± 1.52	33.67 ± 1.52	99.0 ± 0.73
F9	37.72 ± 0.89	7.96 ± 0.15	1095.66 ± 2.08	31 ± 1	97.0 ± 1.11

*Floating lag time, Note: All reading are averages of triplicate determination (Mean± standard deviation)

TABLE 3: % CUMULATIVE DRUG RELEASE FOR EXPERIMENTAL BATCHES

Time (Hrs)	% Cumulative drug release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	66.25 ± 0.35	65.13 ± 0.26	65.66 ± 0.79	66.65 ± 0.26	67.06 ± 0.26	63.96 ± 0.30	71.03 ± 0.86	66.19 ± 0.82	63.15 ± 0.90
2	72.12 ± 0.90	68.84 ± 0.44	70.13 ± 1.23	71.66 ± 1.26	71.31 ± 0.53	67.13 ± 0.30	76.81 ± 0.59	69.73 ± 0.97	70.04 ± 1.05
3	79.63 ± 1.85	72.92 ± 0.41	79.78 ± 0.53	81.09 ± 0.30	74.84 ± 1.06	72.19 ± 0.73	79.39 ± o.87	75.11 ± 0.82	77.82 ± 1.16
4	83.93 ± 0	75.65 ± 0.45	88.48 ± 0.61	85.43 ± 0.71	77.88 ± 0.54	75.84 ± 0.31	81.08 ± 0.20	78.03 ± 0.65	83.40 ± 0.89
5	87.28 ± 0.35	78.80 ± 0.54	91.48 ± 0.93	87.81 ± 0.31	81.64 ± 1.42	81.62 ± 0.35	97.22 ± 0.50	80.82 ± 0.43	89.1 ± 0.35
6	97.62 ± 1.51	84.55 ± 0.77	99.76 ± 3.76	99.49 ± 2.31	88.47 ± 0.24	90.09 ± 0.66	-	88.20 ± 0.20	97.18 ± 3.94
7	-	97.72 ± 0.68	-	-	93.44 ± 1.60	97.07 ± 0.76	-	92.18 ± 0.28	
8	-	-	-	-	99.74 ± 1.45	-	-	99.10 ± 2.65	

Note: All reading are averages of triplicate determination (Mean± standard deviation)

Kinetic Treatment to Dissolution Data: Data of the *in-vitro* release were fit into different equations and kinetics models to explain the release kinetics of Levofloxacin hemihydrate from raft forming system. The order of drug release of formulation F1, F6, and F7 were found to be first order, the mechanism of drug release were found to be Korsemeyer-Peppas as indicated by the highest R^2 value. The order of drug release of formulation F2, F3, F4, F5, F8, and F9 were found to be zero order, the mechanism of drug release were found to be Korsemeyer-Peppas as indicated by highest R^2 value. It indicates that drug released behavior for all the batches is both diffusion and relaxation of polymer chain [18].

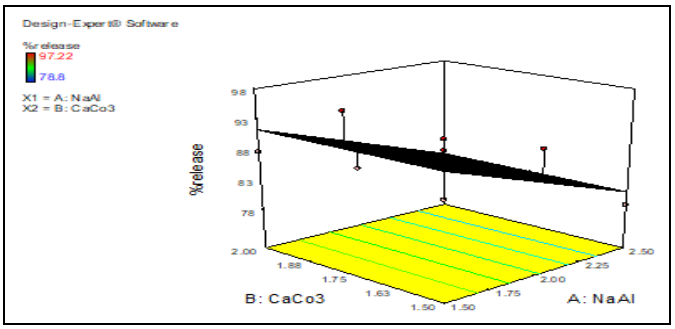

FIG. 3: THREE DIMENSIONAL PLOT SHOWS EFFECT OF SODIUM ALGINATE AND $CACO_3$ ON % CDR

Effect of Experimental Variables on Responses: Three Dimensional Graphical Presentations (3D PLOT): The 3D response surface plots of the factorial model were drawn to show the effect of the variables on the % CDR. The effect of the amount of sodium alginate and $CaCO_3$ on the % CDR was shown in **Fig. 3**. It is demonstrated that the % CDR depends on sodium alginate.

Approached for Optimum Solutions:
Criteria for Selection of Optimized Batch and its Evaluation: Based on analysis of experimental variables the effect of variables on response was judged and selection criteria were set, and optimized formulation was selected which consist of 2.42% sodium alginate and 1.90% calcium carbonate.

These selected optimized batches were further evaluated for the pH, Viscosity, floating lag time, content uniformity and percentage drug release which given the result 7.56 ± 0.056, 2462.33 ± 1.20, 26.33 ± 1.52, 97.85 ± 0.88, 80.38 ± 1.09 respectively.

Predicted Values and Observed Values of Optimized Formulation: The order of drug release of the formulation was found to be zero order, the mechanism of drug release was found to be Korsemeyer-Peppas as indicated by highest R^2 value with diffusion and relaxation of the polymer chain.

***In-vivo* Evaluation:**
Radiographic X-Rays Evaluation: Results obtained from X-Rays studies was found to be as shown in **Fig. 4** (From left 1, 2, 3, 4).

Radiograph 1: It was taken for control animal.

Radiograph 2: It was clear that raft formed was floated in the gastric fluid at the end of 6 h.

Radiograph 3: We could see that raft formed was found in the intestine.

Radiograph 4: There was no raft found in X-Rays studies. As a result, we concluded that raft structure eliminated after 24 h from the body.

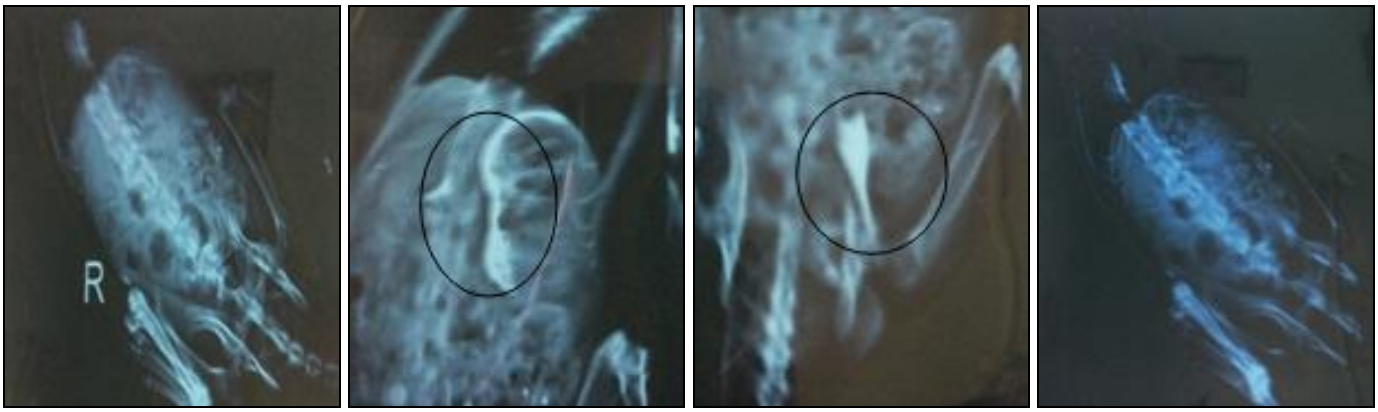

FIG. 4: RADIOGRAPH OF CONTROL ANIMAL AND AFTER 6, 16, 24 h OF DOSE ADMINISTRATION RESPECTIVELY (FROM LEFT RADIOGRAPHS 1, 2, 3, 4)

Part B: Process Scale-Up Studies Process Development: Process design for the process was validated and optimized based on the performance at varying experimental conditions which may appear *in-vivo*. The process was carried out *in-vitro* under simulated conditions. Simulated conditions were considered as challenges.

Challenging Critical Process Parameters: Critical process parameters identified were temperature, pH, and RPM then subjected to challenge studies. Results of these critical parameter experiments were found to be as follows.

Temperature Variation Study: Temperature variation study performed in the dissolution apparatus and raft formation time was noted down. There was no significant change in the raft formation time between temp 32 ºC – 40 ºC. But as temperature goes on the decreasing time required for raft formation was increased.

RPM Variation Study: As the RPM (Gastric Motility) increases the time required for raft formation increases slowly. But not too much difference seen in it.

pH Variation Study: Formation of raft occurs only in pH range 1.2-4. As we go above this range at pH 5-6, there was no raft formation. Formulation settled down and at pH 7-8 raft was not formed formulation moreover disperse in the solvent. So the pH range 1-4 is found to be ideal for the formation of the raft. The raft formation process is under varying pH conditions are shown in **Fig. 5**.

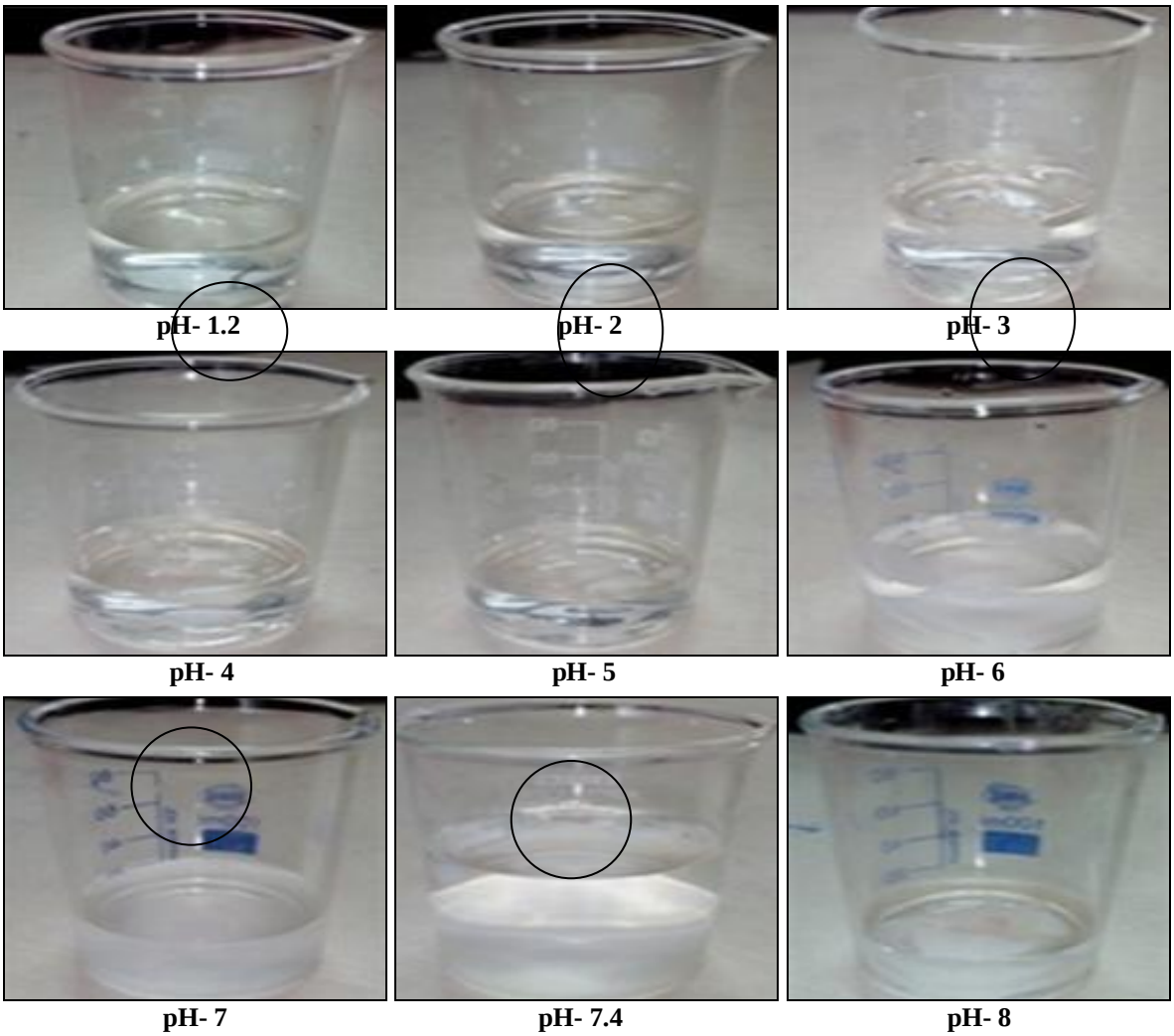

FIG. 5: BEHAVIOR OF RAFT FORMING SYSTEM IN DIFFERENT pH

Significance of the Critical Parameters: Upper and lower limit found for critical process parameters. Temperature- 32-40 ºC, RPM- 25-100 and pH -1-4.

Process Capability Indices: From the above study, the process limits of raft formation were determined, and the developed process was verified by process capability studies using parameters like process potential and process performance [19, 20, 21]. Results obtained from process verification studies are mentioned in **Table 4**. From this, we were ensured that process could sustain simulated production conditions and proved to be reproducible. Key elements of process verification run were evaluated, which shows that process was capable of giving desired results.

TABLE 4: VERIFICATION RESULT OF TEMP, RPM, AND pH ON THE RAFT FORMATION

S. no.	Temp (ºC)	Raft formation time (seconds)	Cp	Cpk
1	Temp (ºC)			
	32	26 ± 1.15	3.33	2
	37.5	24.66 ± 0.57	5.77	2.69
	40	25 ± 1	3.33	1.66
2	RPM			
	25	24 ± 1	3.33	1.34

	30	25 ± 1	3.33	1.66
	40	25 ± 1	3.33	1.66
	50	25 ± 1	3.33	1.66
	60	25 ± 1	3.33	1.66
	70	26.33 ± 1.52	2.18	1.38
	80	26.66 ± 1.15	2.88	1.92
	90	26.66 ± 1.52	2.18	1.45
	100	27.33 ± 1.52	2.18	1.38
3	pH			
	1.2	25 ± 1	3.33	1.66
	2	34 ± 1	3.33	2
	3	36.33 ± 1.15	2.88	1.85
	4	45.66 ± 2.08	1.60	-0.90

Interpretation: Process Potential (Cp) and Process Performance (Cpk) greater than 2 and 1.33, respectively for temperature range 32-40 ºC, RPM range between 25-100. And pH range between 1.2 - 3. Indicates that the raft formation process is highly capableof the mentioned ranges. Wherein at pH 4, Cp value less than 2 and cpk value less than 1 indicates the incapability of the process.

Validation by Radiographic X-Rays Evaluation: From the animal studies performed, it can be concluded that the raft structure formed *in-vivo* elicits excellent gastric retention as proposed in observations and removed from the body within 24 hours. As depicted in **Fig. 4**.

CONCLUSION: The present study was a successful attempt of (product/process) scale up studies for *in-situ* raft forming system optimization. The optimized formula for raft formation was evaluated at various parameters which were identified as critical process parameters. To develop any *in-situ* formulation system, it should not only be optimized for product performance but also the performance of the system considering the *in- vivo* variations is also equally important to optimize the functional performance of the dosage form. The objectives of the present research work were successfully fulfilled.

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CONFLICT OF INTEREST: Nil

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