DEVELOPMENT AND EVALUATION OF MODIFIED RELEASE ORAL IRON FORMULATIONS

Synopsis of the Ph.D. thesis

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1. Abstract:

Iron is an essential micronutrient, and if iron deficiency remains untreated, it leads to anaemia. Oral immediate-release iron supplements can treat iron deficiency without anaemia, but the problem persists. Iron gets absorbed only in the ferrous form, and due to its carrier-mediated absorption from the absorption window (duodenal enterocytes), poor iron bioavailability, and GI side effects due to unabsorbed iron are the reasons for poor patient compliance. Short gastric emptying time limits iron bioavailability from sustained-release iron products. A gastroretentive sustained-release iron delivery system may improve its bioavailability with lower GI side effects due to the fractional release of iron for a prolonged period at the absorption site. This research work aimed to develop a multi-particulate gastroretentive iron formulation (non-effervescent floating approach using low-density lipids) of commonly used dried ferrous sulphate and ferrous ascorbate (FA). Dried ferrous sulphate pellets were prepared with Gelucire® 43/01 and ETHOCEL® 100 cp as main excipients by extrusion spheronization technique, and the formulation was optimized using Central Composite Design. Formulation of Std run 3 (Formulation 1) yielded an average pellet size of 868 µm ± 30 and pellet roundness of 0.93± 0.02 with immediate floating and sustained drug release for 12 hr in 0.1 N HCl dissolution medium. FA mini-caplets were developed with Precirol ATO 5® and PVP K-30 as major excipients using the melt granulation method, and the formulation was optimized by applying a 3^2-factorial design. The F6 batch (Optimized Formulation 2) mini-caplets were found with instant floating and 12 hr floating duration in 0.1N HCl dissolution media. *In vitro drug* release (diffusion mechanism) from these minicaplets at 1 hr and 5 hr was 30-35% and 65-70%, respectively. Both formulations 1 and 2 were found stable for 3 months and were evaluated *in vivo* in healthy New Zealand white female rabbits, and were found to result in increased serum iron and decreased UIBC (parameters indicating iron absorption) significantly, in comparison to the immediate-release iron formulation. These formulations will address the problems of conventional iron products.

2. Brief description on the state of the art of the research topic:

This research study aimed at developing modified-release oral iron formulations in the form of a gastroretentive multiple-unit drug delivery system based on non-effervescent floating approach.

A gastroretentive drug delivery system is a novel drug delivery approach suitable for drugs like iron that get absorbed through carriers in the upper gastrointestinal region. The

gastroretentive formulations may overcome the problems associated with conventional oral formulations. Poor drug bioavailability can be a problem with conventional (immediate release) formulations, caused by short gastric emptying time as one reason, specifically for those drugs that are absorbed in the stomach or duodenum, and for those drugs that are poorly soluble in alkaline pH. Gastroretentive formulations solve this issue by keeping drugs in the stomach and releasing them slowly over time. This may increase the drug's bioavailability and therapeutic effectiveness.

Single or multiple-unit gastroretentive drug delivery systems (GRDDS) with increased gastric residence time can be developed using different approaches like floating, high density, mucoadhesive, expandable, magnetic, and super porous hydrogel systems based on the physiological conditions of the stomach, like gastric fluid density and pyloric sphincter size (1,2).

GRDDS with a low-density floating approach (Floating systems that result in less than 1 g/mL density) can be formulated with or without effervescent agents. A non-effervescent GRDDS may eliminate the possibility of premature evacuation faced by effervescent systems with the lag time involved before floating and can be developed using low-density polymers and lipids like Gelucire® and Precirol ATO 5®, which float immediately and sustain the drug release (3,4). Multiple-unit GRDDS are advantageous over single-unit systems as these are easy to administer due to their small size, with reduced risk of dose dumping, and dosage can be adjusted without changing the formulation (5,6).

3. Definition of the Problem:

Iron is an essential micronutrient. It plays a vital role in erythropoiesis and is required for cellular immune responses and oxidative metabolism(7). An iron deficiency indicating low iron stores is the most common nutritional deficiency and is one of the major causes of anaemia. Approximately 50% of all cases among pregnant and nonpregnant women and 42% of cases in children below five years of age globally are attributed to iron deficiency anaemia (8). In one global study, it was shown that about 1.2 billion people suffer from iron deficiency anaemia; double the population is estimated to suffer from iron deficiency without anaemia (IDWA) (9). Untreated iron deficiency leads to iron deficiency anaemia (10). IDWA can be treated with the marketed oral conventional iron products containing non-haem iron in the form of various ferrous and ferric salts (11). Most of the iron supplements are in rational or irrational combinations with other minerals or vitamins (12,13). Ferrous iron is more bioavailable than ferric iron, and hence ferrous products are preferred (14,15).

But despite the availability of plenty of cost-effective oral iron products, iron treatment failure occurs, which is related to poor patient compliance with the prescribed iron treatment regimen, which may be long due to the poor bioavailability and gastrointestinal (GI) side effects of immediate-release iron formulations (16,17). The introduction of timed-release, enteric-coated, and sustained-release (18,19) iron products on the market, which are costlier than immediate-release products, claims better gastric tolerance due to a smaller fraction of iron exposure to gastric mucosa, but bioavailability remains questionable and not recommended (20–22). This is due to the limited gastric emptying time as iron gets absorbed only in ferrous form through DMT-1 (Divalent Metal Transporter-1) carriers from duodenal enterocytes (23). This research work aimed to address these problems associated with available iron treatment options.

4. Objective and Scope of work:

Objectives:

a) Formulation and evaluation of non-effervescent floating pellets of Dried Ferrous Sulphate by extrusion spheronization technique.

b) Formulation and evaluation of non-effervescent floating mini-caplets of Ferrous Ascorbate.

c) Stability study of optimized formulations.

d) Comparison of *in vivo* study data (Serum iron and UIBC) of optimized formulations with immediate-release iron formulation.

Scope of work:

A gastroretentive iron (ferrous) drug delivery system can address both gastric intolerance and poor bioavailability. The low pH of the stomach keeps iron in a soluble and hence absorbable form (23) available in smaller fractions for a longer period at the absorption (duodenum) site, which can minimize gastric side effects and carrier saturation and may improve bioavailability due to better utilization of the administered iron dose. Both ferrous sulphate salt (24–26) and FA chelate (27,28) are commonly preferred iron supplements.

5. Original contribution by the thesis:

The research work presented in this synopsis of the thesis is original. The literature review indicated that there are few research studies published on gastroretentive iron formulations. One study looked at high-density pellets that stayed in the stomach and were filled with zero-valent iron nanoparticles (29), and the others all looked at floating iron formulations that used

an effervescent method (30–37). The concept of developing non-effervescent floating pellets of dried ferrous sulphate and mini-caplets of ferrous ascorbate has not been investigated previously by any researchers.

6. Methodology of Research, Results / Comparisons:

6.1 Formulation 1-Non-effervescent Floating Pellets of Dried Ferrous Sulphate by Extrusion Spheronization Technique:

6.1.1 Calibration curve development (based on estimation of a red-coloured complex between ferrous ions and o-Phenanthroline) (38): Spectra run from 400 to 800 nm wavelength confirmed the 510 nm λmax of dried ferrous sulphate (drug). A calibration curve of absorbance (y) vs. concentration (x) of a drug (4-12 µg/ml) in 0.1N HCl at 510 nm λmax indicated linearity with R^2= 0.996. The resulting regression equation (y = 0.0674x+0.0033) was used for the drug content and *in vitro* drug release studies.

6.1.2 Drug-Excipients compatibility studies by Fourier Transform Infrared Spectroscopy (FTIR) (39)**:** FT-IR spectra of individual samples of the drug, Gelucire 43/01, HPMC K4M and a mixture of all three ingredients (1:1:1 weight ratio) (stored in an airtight container at 25°C±2°C temperature and 60±5 % relative humidity for 1 month) were obtained in wavenumber range of 4000-400 cm^{-1} (Shimadzu Corporation, Japan, Model: IR Affininty-15, ATR- Attenuated Total Reflectance technique). Characteristic peaks (cm^{-1}) of OH stretching of the HSO_4 group and S=O stretching related to the drug were observed at 3232 and 1074, 1014 in a pure drug sample and 3398 and 1058, 1016 in a mixture without significant change in peak position.

The drug-excipient compatibility study was also checked by comparing the drug content of the pure sample with that of a drug in a mixture (Drug, Gelucire®, and ETHOCEL® (Ethyl Cellulose (EC)) 100 cp (1:1:1 weight ratio) after storage. No significant difference was observed in drug content. These observations confirmed the compatibility of the drug with the excipient polymers used in the formulation.

6.1.3 Formulation and process optimization: The extrusion-spheronization method was selected as the pelletization technique (40,41). A basket (10 cm internal diameter)-radial sieve type extruder (Cronimach, India) was used.

a) Procedure for pelletization: Dry mixing of accurately weighed ingredients (all of 80 # size except EC in the T11 batch) except Gelucire 43/01 was done in a polybag for 10 minutes. This mixture was added to melted Gelucire 43/01, contained in a clean and dried mortar, at 43^0C on a thermostatic heating mantle, and kneaded for 5 minutes. TR1 batch was

extruded after this step (**melt extrusion)** (42,43). For all other batches**,** a measured amount of wetting solvent was added slowly to the above lipid mixture (for T1-T11 and Std run 1-21, the solvent contained PVP K-30) and kneaded continuously for 5 minutes to get the soft wet mass ready for the extrusion. For TR1-TR4 batches, mass was extruded (at high speed (120 RPM) through a 16# sieve) and extrudates were spheronized (at 2000 RPM speed for 15 min. with Friction Plate 1 (Cross-hatched, 12 cm diameter, grid height: grid width: groove width; 1:1:2 mm)). P1-P12 batches were prepared at different extrusion-spheronization process parameter values. The T1-T11 trial batches were processed at similar parameter values as the TR1-TR4 batch, except for a spheronization time of 5 minutes. Std run 1-21 experimental batches were processed as per the CCD design matrix. The spheronized pellets were collected on a paper sheet in a tray and air dried for 3 hr (at 30 ^{0}C room temperature, 60% RH). The dried pellets were stored in an airtight plastic bottle and evaluated.

b) Evaluation parameters (40,44): The mass extrudability, extrusion time, pellet appearance, average pellet size, and process loss based on product yield were checked for TR1-TR4 batches. P1-P12 batches were evaluated for selection of process and machine parameters based on average pellet size, roundness, flow rate, angle of repose, bulk and tapped density, and Carr's Index. Batches T1-T11 and Std run 1-21 were evaluated by average pellet size, roundness, floating behaviour, floating lag time, % drug content, and % *in vitro* drug release. Evaluation parameters (except roundness) were performed in triplicate and presented as the mean ± SD.

c) Preliminary screening (by trial and error) of pelletization Machine (friction plate)/Process (extrusion speed, spheronization speed, and time) parameters (45): It was done by selecting the suitable formulation out of the TR1-4 trial formulations. The nature and quantity of formulation ingredients significantly affected the process and pellet properties. TR4 formulation (Drug: Gelucire 43/01:HPMC K4M: MCC; 30:30:30:10 (in %) with Isopropyl Alcohol (IPA): Deionized water (D.W); 1:1(50% to total dry mass) was selected based on ease of extrudability, lower extrusion time (3±0.2 min), average 620±25 µm pellet size with normal size distribution, and 92.5±3.92 % product yield. TR4 formulation was processed at different values of the machine and process parameters (P1-12 batches) and evaluated based on micromeritics properties. The comparison was done between extrusion speed at 70 and 120 RPM, friction plates 1 and 2 (cross-hatched, 12 cm diameter, grid height: grid width: groove width; 1:2:4 mm), spheronization speed at 800-2500 RPM, and spheronization time of 5-20 minutes. Spheronization time and speed were found to be significant process parameters affecting pellet size and shape. These parameters were selected

for designing further formulation trial batches to get the desired sustained drug release profile and floating behaviour. Formulation changes were required because the P4 batch (spheronized at 2000 RPM for 15 min) with TR4 composition was found to have an immediate drug release (*in vitro)* of 98.99±3.5 % SD, in 0.1 N HCl at 1 hr.

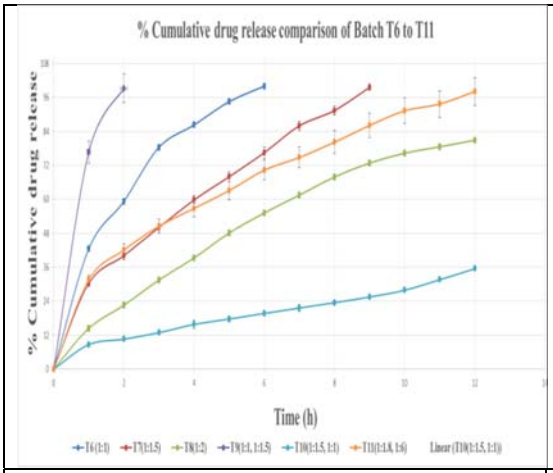
d) Preliminary trials for screening formulation excipients: T1-T12 batches were formulated as per Table 1.

Table 1 Preliminary trial batches for formulation screening

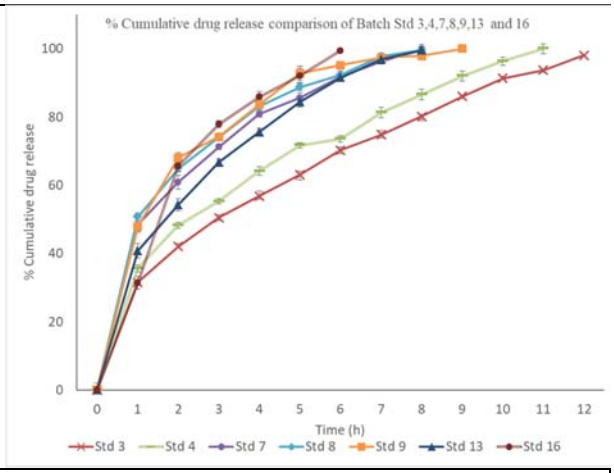
Ingredients(g)	Batch Code										
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11*
Dried Ferrous Sulphate	5	5	5	5	5	5	5	5	5	5	5
HPMC K4M	5	-	-	-	-	-	-	-	-	-	-
HPMC K100M	-	5	-	-	-	-	-	-	-	-	-
HPMC K200M	-	-	5	10	5	-	-	-	-	-	-
Gelucire®43/01	5	5	5	10	5	5	7.5	10	5	7.5	9
ETHOCEL™ 100 cp	-	-	-	-	5	5	7.5	10	7.5	5	8
Batches T1 to T5: Solvent system (mL) (50% of dry mix) contained a 1:1 ratio of IPA: D.W						T6 to T11: Formulated with IPA (50% of dry mix)					
PVP K-30 was added to 5 %W/W of the dry mix amount in all formulations as a solution in a solvent system.											

*Batch T11 was formulated with 44# size ETHOCEL™ 100 cp.

The evaluation data indicated the influence of formulation ingredients and their concentration on pellet characteristics and the type of wetting solvent system effective for pelletization. Only T6-T12 batches were found to have immediate floating and sustained drug release.



(a)



(b)

Figure 1 (a) % Cumulative Drug release comparison of preliminary batches T6 to T11
(b) % Cumulative drug release comparison of Std 3, 4, 7, 8, 9, 13, and 16 batches (16-22# pellets)

Based on the desired drug release criteria (Figure 1 (a)), batch T11 was considered for the selection of concentration levels of Gelucire and EC for further optimization.

e) Formulation optimization by Design of Experiment (46)**:** To understand the effect of multi factors, CCD (rotatable, α =1.68), as a response surface method, was applied (Design Expert® software version 11). Formulation batches (Std run 1-21) were prepared as per the design matrix for three levels of four factors A, B, C, and D (Table 2).

Table 2 Experimental formulation matrix as per CCD with response values

Std run	A: Time min	B: Speed RPM	C: Gelucire (g)	D: EC (g)	Response Y1: Average pellet size (µm ± SD)(n=3)	Response Y_2: Roundness ± SD (n=50)
1	1	1	1	-1	2360 ± 0	0.95 ± 0.03
2	1	1	**-1**	-1	1520 ± 50	0.65± 0.03
3	**1**	**-1**	**1**	**1**	**868 ± 30**	**0.93± 0.02**
4	-1	1	-1	1	1265 ± 45	0.85± 0.04
5	1	-1	-1	1	1932 ± 80	0.92± 0.04
6	-1	-1	1	-1	2150 ± 90	0.93± 0.02
7	-1	1	1	1	1256 ± 50	0.73± 0.03
8	-1	-1	-1	-1	1172 ± 40	0.55± 0.02
9	-1.68	0	0	0	830 ± 25	0.55± 0.02
10	1.68	0	0	0	2067± 85	0.91± 0.04
11	0	-1.68	0	0	1742 ± 60	0.9± 0.03
12	0	1.68	0	0	2360 ± 0	0.95± 0.02
13	0	0	-1.68	0	1183 ± 30	0.75± 0.03
14	0	0	1.68	0	1968 ± 85	0.94± 0.02
15	0	0	0	-1.68	2200 ± 100	0.93± 0.02
16	0	0	0	1.68	795 ± 30	0.65± 0.03
17	0	0	0	0	1890 ± 75	0.88± 0.04
18	0	0	0	0	1950 ± 70	0.9± 0.03
19	0	0	0	0	1830 ± 55	0.88± 0.04
20	0	0	0	0	1920 ± 80	0.9± 0.04
21	0	0	0	0	1800 ± 60	0.9± 0.03

The factor A reperesnts Spheronization Time (min):-1:0:1:-1.68:1.68;3:4:5:2.32:5.68, B as Spheronization Speed (RPM):-1:01:-1.68:1.68;2000:2125:2250:1915:2335, C Gelucire®43/01 Concentration (g): -1:0:1:-1.68:1.68;8:8.5:9:7.66:9.34, and D as ETHOCEL™100cp Concentration (g): -1:0:1:-1.68:1.68;7:7.5:8:6.66:8.34. Average pellet size and roundness (pellet shape) were selected as response variables 1 and 2 respectively.

Statistical analysis was done by applying analysis of variance (ANOVA) at a level of 0.05. Polynomial equations for coded terms were generated for the average pellet size response

Y_1= 1874.05+367.762A+183.73B+151.221C-417.709D-182.584AB-149.125AC+114.625BC

+ 263.137BD -361.375CD-147.47A^2-102.568 C^2-130.146D^2 and for pellet shape response Y_2=0.888+0.107A+0.065C-0.083D-0.127AB+0.034AD-0.026BC+0.058BD-0.099CD-0.053A^2+0.016B^2-0.012C^2-0.032D^2 with A, B, C, and D as variable factors. (P-values less than 0.05 indicating significant model terms are included.) A design space for the desired response criteria was generated.

f) Drug content, drug release study, and release mechanism of experimental batches under CCD: All 21 Std run batches were found to have drug content within the 5 % limit. All batches floated immediately, and they floated throughout the drug release study. The % *in vitro* drug release profiles were collected for only those batches that are presented in Figure 1 (b) which yielded an average pellet size of 759-1300 µm. Std run 3 was found to meet all desired criteria. Mathematical modelling of Std run 3 drug release profile was found to be best fitted to the Higuchi and Korsmeyer-Peppas models. Based on the higher (0.9989) R^2 and lower MSE (0.9459) values, the Korsmeyer-Peppas model was considered with a value of n = 0.48 confirming the diffusion mechanism of drug release.

6.1.4 Stability study: Stability study was carried out as per the conditions specified in ICH guideline (47), after 3 months of storage in an airtight HDPE bottle at 30^0C±2^0C temperature and 65±5 % RH. The formulation contained Gelucire 43/01 with a melting temperature of 43^0C; hence, real-time stability study conditions were selected. Drug content, Cumulative (%) drug release, and floating behaviour of Std run 3 were compared before and after the stability study storage period, and no significant difference in their values was observed. The Std run 3 was considered stable for 3 months at specified storage conditions.

6.2 Formulation 2- Non-effervescent Floating Mini-caplets of Ferrous Ascorbate:

6.2.1 Calibration curve development (38)**:** The calibration curve of absorbance (y) vs. concentration (x) of FA (10-30 µg/ml), in 0.1N HCl at 515 nm λmax (48) indicated linearity with R^2= 0.9996. The resulting regression equation (y = 0.0193x-0.0019) was used for drug content and *in vitro* drug release studies.

6.2.2 Drug characterization and drug-polymer compatibility study by FTIR (IRSpirit FTIR spectrometer, ATR, Shimadzu, Japan)**:** FTIR spectra (in the wavenumber range of 4000-400 cm^{-1}) of FA showed a broad peak of the hydroxyl group: carbonyl group: ether linkage c-o-c; in the 3600 to 3000 cm^{-1} range: around 1754 cm^{-1}: near 1100 cm^{-1} that are the characteristic peaks of FA (39,49). FTIR spectra of a mixture of the drug: Precirol ATO5®: Eudragit RSPO; 1:1:1 ratio, stored in an airtight container for one month at 25°C±2°C

temperature and 60±5 % RH, showed drug characteristic peaks with no significant change in their characteristics, and hence confirmed the compatibility of these polymers with the drug.

6.2.3 Formulation development and optimization:

a) Preparation of floating Ferrous Ascorbate mini-caplets:

TR0 batch was prepared by direct compression and all other batches by melt granulation (42,43). For T1-T12 batches (Table 3)- All the ingredients (22# undersize) except Precirol were mixed for 5 minutes, in a poly bag, and the dry mixture was added to melted lipid (at its melting point) and kneaded for 4 minutes. The soft mass was screened through 16# and granules were cooled in the open air for 10 minutes and again screened through 16# and 22#. Granules undersize to 16 and oversize to 22# were collected. Pre-compression parameters of granules like bulk and tapped density and angle of repose were measured for trial batches. Bulk granules were compressed to 100 mg weight/mini-caplet using a 7.50*3.50 mm tooling set on a rotary compression machine. (Precirol facilitates solvent-free granulation and acts as a binder as it is a low-density meltable lipid. It may influence mini-caplet floating and control the drug release from a dense lipid matrix (50)). To accommodate a larger amount of bulky drug FA, a capsule shape of a mini-tablet with a 100 mg target weight was selected (51,52).

b) Evaluation parameters (50)**:**

The thickness of all the mini-caplets of all batches was maintained at 4.6± 0.2 mm (to avoid the effect of compression force variability on the porosity and, hence, the floatability of the mini-caplets of similar weight (53)).

Post-compression parameters- weight variation, % friability loss, % drug content, % drug release, floating lag time, and floating duration were measured for all trial and factorial batches. An *in vitro* drug release study was done using a USP type 2 Paddle apparatus (50 RPM, 900 ml 0.1 N HCl, at 37 ^{0}C ± 0.5^{0}C) by introducing mini-caplets equivalent to 100 mg FA. A 5 ml sample was withdrawn at every hour (replaced with 0.1 N HCl) and was analyzed (floating lag time and floating duration were observed during this time interval.). All experiments were performed in triplicate and presented as mean ± SD.

Table 3 Formulation compositions of trial batches TR0 to TR12

Batch No.	**Ingredients Quantity (%)**						
	Ferrous Ascorbate	Precirol ATO 5®	PVP K-30	HPMC (METHOCEL®)	Eudragit RSPO	Lactose	Aerosil
TR0	46.5	46.5	5	-	-	-	2
TR1	45	45	10	-	-	-	-
TR2	45	45	5	-	-	5	-

TR3	50	30	5	-	-	15	-
TR4	45	45	5	2.5(K100M)	-	2.5	-
TR5	45	45	5	2.5(K200M)	-	2.5	-
TR6	50	40	5	2.5(K200M)	-	2.5	-
TR7	45	45	5	3.5(K200M)	-	1.5	-
TR8	45	45	5	-	2.5	2.5	-
TR9	45	47.5	5	-	-	2.5	-
TR10	42.5	45	5	-	-	7.5	-
TR11	42.5	45	10	-		2.5	-
TR12	42.5	45	12.5	-	-	0	-

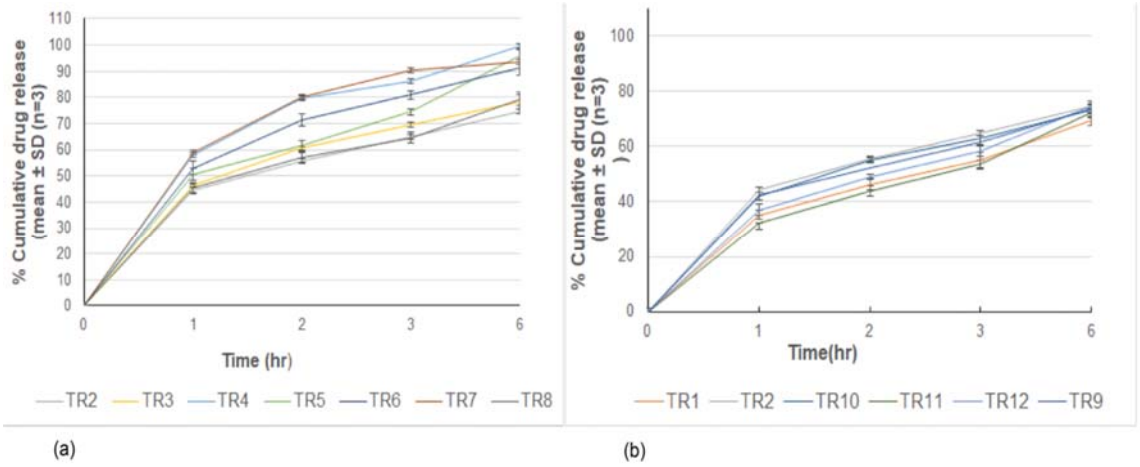


Figure 2 *In vitro* % Cumulative drug release-time profile comparison of (a) Trial batches TR2 to TR8 (b) Trial batches TR1, TR2, and TR9 to TR12

TR0 batch, based on the mean % Cumulative drug release ± SD found as 65.4±2.1 at 1 hr and 71.2±3.5 at 2 hr, failed to sustain the drug release. Melt granulation was found effective to sustain the drug release (TR1) which also exhibited an excellent flow due to the granular structure of the formulation blend. As the formulation of TR2 contained only two excipients, Precirol, and PVP K-30, lactose was added as a diluent to understand the effect of concentration of these excipients and further optimization.

As per Figure 2 (a) and (b), it was evident that concentration of Precirol affected drug release. Increased Precirol decreased drug release. Adding different grades or amounts of swellable polymer HPMC could not sustain the drug release, possibly due to its hydrophilicity and hence the higher permeability of dissolution media in the dosage form. The addition of low-permeable Eudragit RSPO also could not sustain drug release, which may be due to the lowering matrix effect of Precirol on the drug in its presence.

As per Figure (b), the binder, PVP K-30, in optimum amount retarded the drug release, but low (due to less binding effect) and high amount (higher permeability due to its

hydrophilicity) increased drug release. Precirol and PVP K-30 were considered as two significant formulation factors for the optimization as they affected the drug release profile. The Batch TR11 formulation, which yielded prolonged drug release for 12 hr, was considered for the selection of levels of factors for further optimization.

c) Formulation optimization by DOE: Response surface randomized method of 3-level factorial design (42) with nine runs was applied with quadratic model analysis (Design Expert Software, version 11). PVP K-30 was varied at 7.5, 10, and 12.5% and Precirol at 40, 42.5, and 45% concentration. FA was kept constant at 42.5%, and lactose was added in quantity sufficient to make 100% of the formulation blend of F1 to F9 as per Table 4, which shows the values of the post-compression parameters of these batches.

Table 4 Formulation and comparative evaluation of standard experimental runs F1 to F9

Run no.	Factor 1 A: Precirol ATO 5	Factor 2 B: PVP K-30	Weight variation (mg) mean± % D (n=20)	% Friability	Drug content (%) mean± SD (n=3)	Floating lag time (Sec) mean± SD (n=3)	R1:% Cumulative drug release at 1 hr mean± SD (n=3)	R2:% Cumulative drug release at 5 hr mean± SD (n=3)
F1	-1	-1	95±5	0.2	98±3	Zero	45.7±1.2	78.5±2.1
F2	0	-1	94±6	0.2	98±5	Zero	44.1±1.5	77.8±1.3
F3	1	-1	95±2	0.4	99±2	Zero	41.1±0.9	75±2.0
F4	-1	0	92±6	0.2	98±5	Zero	38.5±1.2	75.8±1.5
F5	0	0	101±3	0.2	97±4	Zero	36.4±1.7	72.1±2.4
F6	**1**	**0**	**96±3**	**0.2**	**98±3**	**Zero**	**32.2±2.1**	**66.7±1.2**
F7	-1	1	91±3	0.2	97±5	Zero	48.4±2.0	89.4±3.1
F8	0	1	105±2	0.2	97±3	Zero	43.6±1.8	76.5±2.1
F9	1	1	92±2	0.2	99±4	Zero	37.2±0.5	69.8±2.3

The comparison of % Cumulative *in vitro* drug release from F1 to F9 confirmed the findings of the trial batches. Batch F6, which met the desired criteria for response variables, was considered an optimized batch.

d) Statistical analysis: The overlay plot with design space was generated for the range of Precirol ATO5® and PVP K-30 concentration in the formulations to get the value of R1:R2;30-35:65-70 %. The design space region (54) suggested a checkpoint batch, which was prepared and evaluated. The actual response values were similar to the predicted values by the model, which validated its use. The regression analysis of the data generated polynomial equations for the % Cumulative drug release at 1 hr as $R1 = 36.27 - 3.68A - 1.65AB + 7.65 B^2$ and for the % Cumulative drug release at 5 hr as R2 =71.27-5.37A-

4.0AB+6.3 B^2, where A and B represent Precirol ATO5® and PVP K-30 concentration, respectively.

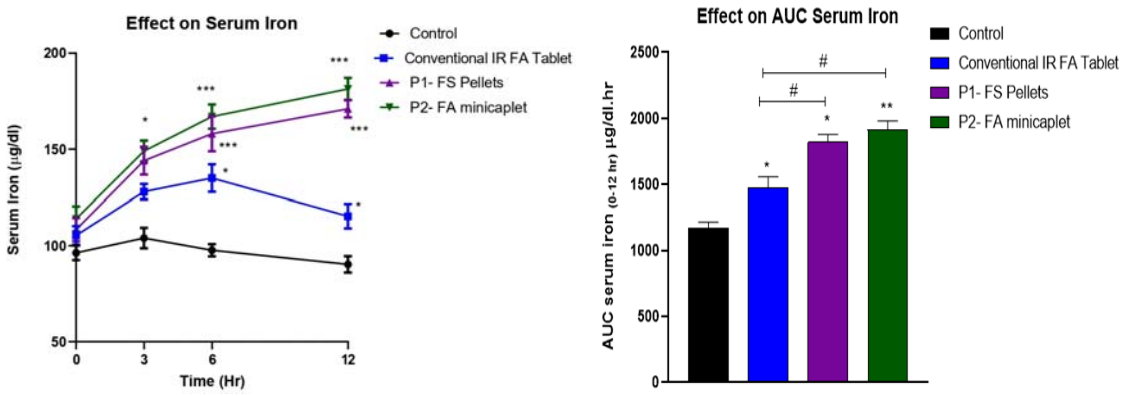
e) Drug release kinetic: Model fitting (DD Solver 1.0 Program) of the % Cumulative drug release profile of the F6 batch was done for various models (55) and the Korsmeyer Peppas was found to be the best-fit model, based on R^2=0.999. The observed value of exponent n (0.444)< 0.5 confirmed the diffusion mechanism of drug release from the lipid matrix (56).

6.2.4. Accelerated stability studies: The mini-caplets of the optimized formulation (F6) were evaluated for post-compression parameters before and after three months of storage in an airtight high-density polyethylene bottle (HDPE), kept in a stability chamber, at 40°C±2°C temperature and 75±5% RH, as per the accelerated stability studies conditions specified in the ICH guideline (47). F6 was considered stable as no significant changes in the value of parameters were observed after storage.

6.3 *In vivo* study:

a) Experimental Design: Healthy adult (New Zealand white) female rabbits (3-3.5 Kg body weight) were used to study the *in vivo* effect of the optimized formulations 1 and 2 in comparison to the immediate release (IR) FA formulation. The rabbit intestine behaviour matched that of the human duodenal mucosa for iron absorption (57–59). The experiments were conducted after animal protocol approval (LJIP/IAEC/2022-23/04) and as per the guidance of CCSEA, India. Iron absorption was checked by measuring the iron biomarkers, serum iron, and the UIBC (unoccupied binding sites on transferrin) using a serum Iron & TIBC kit (Coral Clinical Systems, India) (60). Based on the human equivalent dose of 46.8 mg elemental iron, each of the formulations, equivalent to elemental iron of 2 mg/kg (3kg body weight) was given orally in a single dose after 12 hr of overnight fasting (n=3). Minicaplets administered were of 3.5*7.5*2 mm size.

b) Statistical analysis of *in vivo* result data: It was done using GraphPad Prism software 8.0, by applying a two-way or one-way ANOVA followed by Dunnet's post hoc test to determine a significant difference between each treatment and the others. A student t-test was applied to AUC serum iron and UIBC to compare floating formulations with IR formulation. Both formulations, non-effervescent floating dried ferrous sulphate SR pellets and non-effervescent floating FA SR mini-caplets, increased serum iron (Figure 3) and decreased UIBC, significantly compared to IR FA tablet and were found to have a with higher relative bioavailability than conventional IR FA tablet specifically non-effervescent floating FA SR mini-caplets.



(a) (b)

Figure 3 Effect of different iron formulations on (a) Serum iron at different time intervals (b) AUC of serum iron (0-12 hr) (µg.dl.hr). Each line and bar represents mean±s.e.m., Statistical significance *p<0.05; **p<0.01; ***p<0.001 vs Control; #p<0.05 vs. Conventional IR FA Tablet

7. Achievements with respect to objectives:

Both formulations were successfully developed and were found stable after 3 months at the specified storage condition. The first optimized formulation, Std run 3 (Gelucire® 43/01:drug; 1.8:1 ratio, i.e., 39 % w/w; ETHOCEL™ 100cp: drug;1.6:1 ratio, i.e., 34.6 % w/w; Dried Ferrous Sulphate 21.65 % w/w; and PVP K-30 in 4.75 % w/w of total pellet weight.) yielded an average pellet size of 868 µm±30 and a pellet roundness of 0.93±0.02. Pellets floated immediately and sustained drug release for 12 hr in a 0.1 N HCl dissolution medium. The second optimized formulation of F6 mini-caplets (42.5% FA, 45% Precirol ATO 5®, 10% PVP K-30, and 2.5% lactose) was found to have instant floating and a 12 hr floating duration in 0.1N HCl dissolution media. *In vitro drug* release was found to be30-35% at 1 hr and 65-70% at 5 hr. Both formulations were found to have better absorption in terms of increased serum iron and decreased UIBC compared to IR FA in an *in vivo* study in rabbits.

8. Conclusion:

Based on the experimental data, it was concluded that non-effervescent floating (*in vitro*): pellets of dried ferrous sulphate and mini-caplets of FA can be developed successfully with low-density lipids like Gelucire®43/01 and Precirol ATO 5®. The *in vivo* results of increased iron absorption from these developed iron formulations in comparison to immediate iron formulation confirmed the hypothesis that floating gastroretentive sustained-release formulations may improve the bioavailability of ferrous iron (soluble in acidic pH) that has carrier-mediated absorption from the duodenum. Both formulations have the potential to be considered as better oral iron treatment options than conventional iron products in the future.

9. Copies of papers published and a list of all publications arising from the thesis:

Sr. No.	Paper Description
	Papers published
1.	Shital Trivedi, Shreeraj Shah, Riya Patel. Review on novel oral iron formulations with enhanced bioavailability for the treatment of iron deficiency. J Drug Deliv Sci Technol. 2023;90(December):105181. (Clarivate Analytics-2023 Impact Factor: 5)
2.	Trivedi S, Shah S. Optimization of Extrusion-Spheronization Process Parameters Affecting Micromeritics of Dried Ferrous Sulphate Pellets. J Pharm Sci Biosci Res. 2023;11(2271):165–74. (ISRA JIF-2015, Impact Factor: 2.6)
	Paper accepted
3.	Ms. Shital Trivedi, Dr. Shreeraj Shah. Formulation Development of Non-effervescent Floating Pellets of Dried FerrousSulphate by Extrusion-Spheronization Technique. Research Journal of Pharmacy and Technology. It will be published in year 2024, Vol: 17, Issue: 4 of the journal. (ISSN-0974-3618 (Print, 0974-360X (Online), Scopus Indexed, SJR-2022:0.27)
	Paper in communication (under review)
4.	Shital Trivedi, Vijay Kevlani, Shreeraj Shah. Ferrous Ascorbate non-effervescent floating mini-caplets as an oral iron supplement. Paper is submitted to 'Journal of Pharmaceutical Investigation'(Clarivate Analytics-2023 Impact Factor: 5.5)

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