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DESIGN AND DEVELOPMENT OF A NOVEL GASTRORETENTIVE MULTIUNIT PARTICULATE SYSTEM AND COLON TARGETED TABLET IN CAPSULE SYSTEM FOR EFFECTIVE MANAGEMENT OF ANAEMIA

B. Pendse [1], S. Sawarkar [*2] and M. Damani [2]

Long Island University [1], Brooklyn New York.
Department of Pharmaceutics, SVKM's Dr. Bhanuben Nanavati College of Pharmacy [2], University of Mumbai, Gate No. 1, Mithibai College Campus, V. M. Road, Navpada, Suvarna Nagar, Vile Parle West, Mumbai - 400056, Maharashtra, India.

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Correspondence to Author:
Sujata P. Sawarkar

Professor of Pharmaceutics, Department of Pharmaceutics, SVKM's Dr. Bhanuben Nanavati College of Pharmacy, University of Mumbai, Gate No. 1, Mithibai College Campus, V. M. Road, Navpada, Suvarna Nagar, Vile Parle West, Mumbai - 400056, Maharashtra, India.

E-mail: sujata.sawarkar@bncp.ac.in

ABSTRACT: Malnutrition is a condition in which there is an imbalance in the proportion of essential nutrients that the body requires to stay healthy. The most prevalent form of malnutrition is micronutrient deficiency and of this iron deficiency anaemia is the most common type, affecting over 2 billion people worldwide. Micronutrients are a special class of vital ingredients that are required in minuscule quantities to perform a wide range of physiological functions. The current therapy for the treatment of Iron deficiency anaemia has many drawbacks like gastrointestinal side effects, erratic absorption of iron, *etc*. To overcome the above side effects, we tried to develop and evaluate a site-specific sustained-release combined drug delivery system of Iron and Folic acid. Iron as Ferrous ions is preferentially absorbed in the jejunum. To facilitate the absorption of iron, the gastro retentive multiunit particulate system (GRMUPs) was developed comprising Ferrous Ascorbate is a source of iron. Colon targeted tablet was developed for Folic acid to localize and maximize the absorption of Folic acid in the colon. The objective was to avoid competitive absorption of two micro-nutrients in the jejunum. A factorial design was employed to optimize the formulation of GRMUPs effervescent system containing Ferrous Ascorbate. The GRMUPs were evaluated for floating lag time, floating time, and drug release profile. Folic acid core tablets were coated with colon targeted film and were evaluated for site-specific release in the simulated colonic fluid. Further, both the systems were filled in a capsule to provide once-a-day administration.

INTRODUCTION: Malnutrition is a condition in which there is an imbalance in the proportion of essential nutrients that the body requires to stay healthy.

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Imbalance in the normal levels of nutrients can result in a plethora of diseases that hamper normal physiological functions of the body. Anaemia is the most common nutritional deficiency disorder occurring worldwide.

According to the WHO report, anaemia is a condition in which the haemoglobin (Hb) content of blood is lower than average due to deficiency of one or more essential nutrients, regardless of the cause of such deficiencies. Anaemia is a syndrome

and not a disease, and hence, the treatment regimen and approaches should be focused on mitigating the cause rather than the symptoms. Globally, about 2 billion people are estimated to be affected by iron deficiency anaemia [1-4].

The pathological classification of disease condition is based on the reticulocyte count, which gives an idea about the efficiency of bone marrow functioning and thus the production of erythrocytes. Anaemia is classified as regenerative anaemia and hypo-regenerative type. Regenerative anaemia is characterized by an increase in the reticulocyte count that occurs in response to excessive bleeding or haemolysis. An increase in erythropoietin levels is also observed. In the case of hyporegenerative anaemia, there is an alteration in the number of bone marrow progenitor cells occurring at various stages of differentiation and maturation. Based on clinical manifestations, anaemia, anaemia is categorized as acute (due to bleeding of haemolysis) and chronic anaemia.

Red cell morphology and mean corpuscular volume (MCV) are used as parameters for diagnosis and classification of anaemia. Based on MCV, anaemia is classified into 3 types: Microcytic anaemia which consists of iron deficiency anaemia (IDA) as the primary type, which includes thalassemia, and anaemia of chronic disorders. Ferritin levels present in the blood is considered as a checkpoint for the diagnosis of IDA. Normocytic Anaemia is caused because of nutritional deficiency, haemolytic anaemia and renal failure.

Combined dietary deficiency of Vitamin B12, Folic Acid and iron is a characteristic feature of normocytic anaemia. Macrocytic anaemia involves megaloblastic anaemia wherein the red blood cells (RBCs) do not mature to the required size. The leading cause includes deficiency of Vitamin B12 and/or Folic Acid [5-9.]

One of the significant causes of anaemia across the globe is the deficiency of Iron either alone or along with other micronutrients like Folic Acid and Cyanocobalamin. Hence, the primary treatment of anaemia focuses on the use of an iron salt either alone or in a combination of Folic Acid and/or Cyanocobalamin. It should be noted, however, that effective treatment depends on the identification of the actual cause and accordingly the treatment [10].

The current therapy of iron deficiency anaemia involves the use of an iron source in the form of an iron salt or an iron complex that can be administered either by the oral or parenteral route. Conventional therapy, which is in clinical practice, is associated with several drawbacks like gastrointestinal side effects like nausea/vomiting, diarrhoea, abdominal discomfort, and constipation. Additionally, these conventional dosage forms exhibit erratic absorption of iron from the dosage form. The main reason is attributed to the inability of the dosage form to localize iron in the site of its maximum absorption. The Ferrous (Fe^{2+}) form of iron is preferentially absorbed over the ferric (Fe^{3+}) state. The commonly used iron source (Ferrous Sulphate) is administered at a high dose of 325mg thrice daily causing major side effects. Prevalence of side effects leads to non-adherence to the treatment and ultimately, patient non-compliance. In the case of parenteral iron therapy, the most common side effect is allergic reactions at the site of injection. The most severe form of response that occurs at the site of injection includes anaphylactic or allergy type reaction. These reactions can also be life-threatening in some cases. Parenteral iron can lead to an increase in oxidative stress that can lead to cardiovascular complications in rare cases[5-9, 11-13]. These drawbacks are mostly associated with the fact that iron is not being delivered efficiently and localized at the optimum site of absorption; thus, the amount of iron required to be administered is too high. Also, the main disadvantage of these dosage forms is the lack of site-specificity.

Most of the drawbacks associated with conventional therapy can be mitigated by strategizing delivery of Iron and Folic acid at their optimum sites of absorption. Iron is absorbed in the ferrous form from the upper parts of the gastrointestinal tract, specifically the duodenum and jejunum. Among all the salts of iron, the Ferrous Ascorbate salt is the most tolerated form, which will help in reducing the gastrointestinal side effects during the long retention time of 8 h or more in the stomach [3]. Hence, a gastro retentive multiparticulate drug delivery system of Ferrous Ascorbate is ideal for localizing the drug at its site of absorption. Folic acid is absorbed throughout the entire gastro-intestinal act; however, the preferred location of absorption is colon [14-16].

Hence, it would be judicious to present Folic acid exclusively to the colon to avoid competitive absorption, and separation of two actives would also lead to increased overall absorption of both iron and folic acid at their respective sites.

In the present study, we have prepared colon-specific tablets of folic acid in combination with gastro retentive multiparticulate pellets of ferrous ascorbate enclosed in a capsule. We speculate that this spatially targeted dual drug delivery system comprising of iron and folic acid can provide effective and well-tolerated therapy for the management of anaemia. Moreover, by formulating the actives as separate entities, getting delivered at different sites across the gastrointestinal tract, we propose that the developed delivery system will enable efficient and complete absorption of actives, thereby providing an optimum therapeutic effect.

MATERIALS AND METHODS:

Materials: The active pharmaceutical ingredients Ferrous Ascorbate and Folic Acid were obtained as a gift samples from Indoco Remedies Pvt., Ltd. The polymers Eudragit FS 30D was obtained as a gift sample from Evonik, Methocel K15M (HPMC K15M), and Ethyl Cellulose 10P were obtained as a gift sample from Colorcon Ltd. Sodium Alginate (Protanol LFR 5/60) was obtained from FMC Biopolymer, Xanthan gum (Xanutral 75 CP Kelco) from Signet, Polyvinylpyrrolidone K30 (Kollidon 30) and Crospovidone (Kollidon CL) from BASF, Lactose monohydrate from DMV, Avicel PH 101 from FMC Biopolymer, Sodium Bicarbonate, Magnesium stearate, Talc, starch was procured from S.D. Fine Chemicals. All other excipients and reagents were purchased from a local vendor.

Methods: Formulation development was commenced with preformulation and compatibility study. The compatibility of drug and excipients was ascertained by performing Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared Spectroscopy (FTIR). DSC thermograms were recorded using Lab Mettler Toledo DSC apparatus at temperature range of 0°C to 300°C. FTIR graphs were recorded at wavenumber of 4100 cm^{-1} to 702 cm^{-1}. The drug content was analyzed by UV spectro-photometry using Shimadzu UV Spectrophoto-meter at a wavelength of 240.4 nm (Ascorbic acid) in pH 1.2 buffer (simulated gastric fluid) and 257 nm in purified water for Ferrous Ascorbate. For Folic acid, drug content was analyzed at a wavelength range of 293.4 nm, 279 nm, and 278 nm in pH 1.2 buffer (simulated gastric fluid), pH 6.8 phosphate buffer (simulated intestinal fluid) and pH 7.2 simulated colonic fluid, respectively. The analytical was validated as per ICH Q2B R1 guidelines. Ferrous Ascorbate is a weak salt consisting of one atom of Iron (as Fe^{2+}) and 2 molecules of Ascorbic Acid. In acidic conditions at a pH of about 1.2, it partially dissociates to release one molecule of ascorbic acid, and the iron atom remains complexed and associated with the other molecule of ascorbic acid [17]. In order to confirm this behavior, solution-state stability studies of Ferrous Ascorbate in pH 1.2 buffer (simulated gastric fluid) were conducted.

Gastroretentive Multiunit Particulates (GRMUPs) of Ferrous Ascorbate: Placebo pellets were prepared with an objective to optimize the concentration of sodium bicarbonate and polymers in order to achieve desired buoyancy or floatation time of 8 h and floatation lag time of less than 1 min using Extrusion-Spheronization technique. Various grades of Methocel® (Hydroxypropyl methylcellulose) K4M, K15M, and K100M were screened for their swelling and gas trapping properties. Sodium alginate (Protanol LFR 5/60 FMC Biopolymer) and Xanthan gum (Xanutral 75 CP Kelco) were explored as adjuvant gelling polymers along with HPMC for improving the gas entrapment.

The formulated batches were evaluated for their lag time (in seconds) and floating time (hour). 3^2 factorial design was employed at 3 different levels to optimize the quantity of sodium bicarbonate (X2) to obtain optimum lag time. In addition to sodium bicarbonate, the type of functional polymer (X1) at 10% level was varied. The details of the factorial design grid are mentioned in **Table 1**. Based on the factorial design, trial batches were taken as mentioned in **Table 2**.

TABLE 1: FACTORIAL DESIGN GRID

Levels Factors	-1	0	+1
X1	HPMC K4M (10%)	HPMC K15M (10%)	HPMC K100M (10%)
X2	5%	12.5%	20%

TABLE 2: COMPOSITION OF BATCHES AS PER FACTORIAL DESIGN

Formulation code of batches	Independent Variables (Coded)		Independent variables (Actual)	
	X1	X2	X1 (10%) (Methocel grade)	X2 (% of Sodium Bicarbonate)
F1	-1	-1	HPMC K4M	5%
F2	0	-1	HPMC K15M	5%
F3	+1	-1	HPMC K100M	5%
F4	-1	0	HPMC K4M	12.5%
F5	0	0	HPMC K15M	12.5%
F6	+1	0	HPMC K100M	12.5%
F7	-1	+1	HPMC K4M	20%
F8	0	+1	HPMC K15M	20%
F9	+1	+1	HPMC K100M	20%

Selection of Adjuvant Polymer in Combination with HPMC: In the case of placebo pellets formulated with HPMC singly, it was observed that floatation time was found to be less than 8 h. In order to improve floatation time, further trials were taken with HPMC K15M in combination with Xanthan Gum and Sodium Alginate at a constant ratio of 1:1 **Table 3**. The combination that showed promising results for floatation lag time and floatation time were considered for further optimization and formulation development.

TABLE 3: BATCHES FOR SELECTION OF COMBINATION OF POLYMERS

Batch	Hydroxypropyl methyl cellulose K15M (mg)	Xanthan gum (mg)	Sodium alginate (mg)	Sodium bicarbonate (mg)	Polyvinyl pyrrolidone K30 (mg)	Avicel PH 101 (mg)	Starch (mg)
F10	37.5	37.5	-	62.5	50	175	37.5
F11	62.5	62.5	-	62.5	50	125	37.5
F12	62.5	-	62.5	62.5	50	125	37.5

Formulation Development of Gastroretentive Multiunit Particulate (pellet) Systems (GRMUPs) of Ferrous Ascorbate: Gastroretentive multiunit particulate (pellet) systems (GRMUPs) of Ferrous Ascorbate were prepared with optimized conc. of sodium bicarbonate and polymer combination as described in the previous section. Ferrous Ascorbate was found to have a bulk density of 1g/cc. Taking into consideration the bulkier nature of API, the quantity of sodium bicarbonate was further fine-tuned and modulated. Formulation trials of GRMUPs of Ferrous Ascorbate are described in detail in **Table 4**.

TABLE 4: FORMULATION BATCHES FOR GRMUPS OF FERROUS ASCORBATE

Batch code	FA (mg)	SB (mg)	HPMC K15M (mg)	SA (mg)	PVP K30(mg)	Avicel PH 101 (mg)	Starch (mg)	EC 10P (mg)	Unit weight / capsule (mg)
D1	100	62.5	62.5	62.5	50	187.5	37.5	-	500
D2	100	75	50	50	50	175	-	-	500
D3	100	75	62.5	62.5	50	150	-	-	500
D4	100	75	75	100	50	100	-	-	500
D5	100	100	100	62.5	50	87.5	-	-	500
D6	100	100	100	62.5	50	187.5	-	-	600
D7	100	100	75	25	50	150	-	-	500
D8	100	100	37.5	12.5	50	200	-	-	500
D9	100	100	37.5	12.5	50	200	-	-	500
D10	100	100	150	62.5	50	37.5	-	-	500
D11	100	100	62.5	12.5	50	150	-	25	500
D12	100	100	37.5	12.5	50	175	-	25	500
D13	100	100	62.5	12.5	50	165	-	10	500
D14	100	100	62.5	12.5	50	167.5	-	7.5	500
D15	100	100	50	12.5	50	180	-	7.5	500

FA- Ferrous Ascorbate; SB- Sodium Bicarbonate; SA- Sodium Alginate; PVP K30 – Polyvinyl Pyrrolidone K30; EC 10P- Ethyl Cellulose 10 Premium

Formulation Development of Colon-targeted Tablet of Folic Acid: Drug core tablet granules of Folic acid were prepared using excipients Avicel PH 101, Lactose monohydrate, Crospovidone, Starch, Polyvinyl Pyrrolidone K30. The granules were first mixed with talc and then lubricated with magnesium stearate **Table 5**. The tablets were compressed using 6.0 mm round biconvex punches.

TABLE 5: FORMULATION OF FOLIC ACID CORE TABLETS

Ingredients	Weight mg/ tablet (CT)	Weight mg/tablet (ST)
Folic Acid	5	5
Crospovidone	2.4	-
Starch	-	4
PVP K30	4	4
Avicel PH 101	33.7	32.9
Lactose monohydrate	33.7	32.9
Talc	0.8	0.8
Magnesium stearate	0.4	0.4
Total weight	80	80

The core tablets of Folic acid were the first seal coated with coating dispersion comprising of either mixture of HPMC E50 and Ethylcellulose 10P in combination or Ethylcellulose 10P alone **Table 6**. The coating process parameters maintained were Inlet temperature of 40 °C ± 5 °C, Pan speed of 40 ± 5 rpm, spray rate: 1 ml ± 0.5 ml per min, pump speed: 40 rpm. Core tablets were seal coated to obtain a weight gain of 2.0% - 3.0%. The seal coated tablets were evaluated for appearance, disintegration, and uniformity of weight.

TABLE 6: COMPOSITION OF SEAL COAT FOR FOLIC ACID TABLETS

S. no.	Ingredient	Composition 1	Composition 2
1	Ethyl cellulose 10P	20%	80%
2	HPMC E50LV	60%	-
3	Triethyl Citrate	12%	16%
4	Talc	4%	2%
5	Titanium dioxide	4%	2%

Seal coated tablets of Folic acid were further coated with pH-sensitive coating polymer Eudragit FS 30 D. The coating dispersion of Eudragit FS 30D contained triethyl citrate as plasticizer and talc as anti-tacking agent **Table 7**. The seal coated tablets were coated with Eudragit FS 30D to obtain a weight gain of 15%-35%. The adequacy of colon coating was ascertained by performing disintegration test and drug release by *in-vitro* dissolution testing in the simulated colonic fluid.

TABLE 7: EUDRAGIT FS 30D COATING DISPERSION COMPOSITION

S. no.	Ingredient	Percentage
1	Eudragit FS 30D	43.01%
2	Triethyl Citrate	0.65%
3	Talc	6.45%
4	Water	49.89%
	Total	100%

Evaluation of Ferrous Ascorbate Gastroretentive Multiunit Particulates: Ferrous Ascorbate GRMUPs were evaluated for dimensions, sphericity, and bulk and tapped density, angle of repose. For the floatation lag time test, the time is taken by the GRMUPs to rise to the surface when suspended in a simulated gastric fluid (*i.e.* pH 1.2 buffer) was determined. Additionally, floating time of pellets was assessed. Pellet formulations that showed floatation time of 8 hours or more were further taken ahead for *in-vitro* dissolution studies. *In-vitro* dissolution studies of GRMUPs were performed in simulated gastric fluid at 37 °C ± 2 °C using USP type I (Basket) apparatus at a rotational speed of 100 rpm. Aliquots were withdrawn at specific time intervals of 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, and 8 h, drug content was analyzed, and cumulative % drug release was estimated. The order of drug release kinetics was envisaged by computing regression coefficient of Zero order, First order, Higuchi, and Korsmeyer Peppas curve models.

Evaluation of Folic Acid Colon Specific Tablets: Folic acid tablet granules were evaluated for pre-compression parameters like Carr's Index, Hausner's ratio and Angle of Repose. Core tablets, seal coated, and Eudragit FS 30 D coated tablets of Folic acid were subjected to disintegration testing. Integrity of colonic tablets in simulated gastric fluid and intestinal fluid was ascertained. In-vitro dissolution studies of colon-specific Folic acid tablets were performed using USP type I (Basket) apparatus at a rotational speed of 100 rpm in simulated gastric fluid pH 1.2 for 2 h, followed by simulated intestinal fluid pH 6.8 for the following 4 h and simulated colonic fluid pH 7.2. Aliquots were withdrawn at specific time intervals of 1, 2, 3, 4, 5, 6, 6.5, 7, 7.5, and 8 h, drug content was analyzed, and cumulative % drug release was estimated. The order of drug release kinetics was envisaged by calculating the regression coefficient of Zero order, First order, Higuchi, and KorsmeyerPeppas curve models.

Stability Studies: The capsules filled with Ferrous Ascorbate GRMUPs and Folic acid colon targeted were subjected to stability studies as per ICH Q1A (R^2) guidelines. The capsules were stored at 25°C ± 2 °C/60% RH ± 5% RH and 40 °C ± 2 °C/75% RH ± 5% RH. The stability studies were conducted for a period of 3 months, and the final product was characterized for appearance, assay, and drug release studies.

RESULTS AND DISCUSSION: The appearance of the drug excipient compatibility mixtures was unchanged at the end of 3 months for both Ferrous Ascorbate and Folic acid. The drug content was found to be in the range of 98% to 102% for both the drugs indicating compatibility of Ferrous Ascorbate and Folic Acid with excipients. The compatibility of drug and excipients was further confirmed as the FTIR graph showed the significant peaks of all the functional groups were found to be retained in both individual drug samples as well as drug excipient mixture samples as indicated in **Table 8**. The significant peaks for Ferrous Ascorbate included -OH Stretch (H-bonded), C-O Stretch (alcohol), and C-O stretch (acid) and were found to be in range in both Ferrous Ascorbate individual sample and its mixture with excipients. The significant peaks for Folic Acid included -OH Stretch (acid), C-O Stretch (acid), C=O stretch (amide), and N-H Stretch (amide) were found to be in range in both Folic acid individual sample and its mixture with excipients.

TABLE 8: FT-IR PEAKS OF BLEND OF FERROUS ASCORBATE AND FOLIC ACID WITH THEIR RESPECTIVE EXCIPIENT BLENDS STORED AT DIFFERENT CONDITIONS

Functional Groups	Wavenumber (cm^{-1})			Reported range (cm^{-1})
	5°C ± 3°C	25°C ± 2°C / 60% RH ± 5% RH	40°C ± 2°C / 75% RH ± 5% RH	
Ferrous Ascorbate with its excipients				
-OH stretch (Alcohol)	3372.22	3379.23	3362.22	3600-3200
C-O stretch (Alcohol)	1034.07	1098.36	1090.65	1200-1050
C-O stretch (Acid)	1168.23	1168.02	1168.14	1300-1100
C=C (Stretch)	1483.83	1417.92	1484.14	1600-1450
Folic Acid with its excipients				
-OH stretch (Acid)	2900.40	2900.10	2900.27	3300-2500
C-O stretch (Acid)	1168.57	1167.38	1167.92	1300-1100
C=O stretch (Amide)	1693.66	1692.72	1693.34	1695-1630
N-H stretch (Amine)	3332.95	3360.05	3384.02	3500-3300
-NH Stretch (Amide)	1605.29	1606.08	1606.14	1640-1550

The DSC thermogram confirmed the purity of the drug as it showed a sharp endothermic peak at 199.08 °C that represents a melting point of Ferrous Ascorbate in the mixture of drug and excipients stored at room temperature. A sharp endothermic peak was observed at 253.58 °C that represents a melting point of Folic Acid (248°C-250°C) in the mixture of drug and excipients stored at room temperature. The drugs, as well as the excipients, were found to be compatible since no endothermic peak other than that obtained for the drug. The thermographs of pure drugs and their respective excipient blends were similar. The compatibility of the drug with the excipients was maintained. The pH stability profile for Ferrous Ascorbate was determined in 0.1N hydrochloric acid, and it was observed that there was approximately a 50% reduction in the initial concentration of Ferrous Ascorbate when estimated at three different concentrations, as shown in **Table 9**. As mentioned in the preceding section, Ferrous Ascorbate splits in the presence of an acidic medium to release one molecule of free Ascorbic acid. The other molecule of Ascorbic acid exists in the complexed state with the ferrous ion [17].

TABLE 9: STABILITY OF FERROUS ASCORBATE IN 0.1N HYDROCHLORIC ACID

Conc. Taken (ppm)	Concentration Measured (ppm)				% Reduction in concentration		
	Initial	1hr	2hrs	3hrs	1hr	2hrs	3hrs
50	50.18	49.25	44.35	28.53	1.5%	11.3%	42.94%
100	98.12	90.89	77.59	55.32	9.11%	22.41%	44.68%
150	148.93	132.83	109.23	85.59	11.4%	27.18%	42.94%

Thus, the released molecule of Ascorbic acid was quantified, which was then used to quantify the total concentration of Ferrous Ascorbate released at a specific time point during dissolution studies.

Floatation Characteristics of Placebo Batches: The prepared placebo gastroretentive multiunit particulates were evaluated for floating lag time (in seconds) and floating time (in minutes). The results are tabulated in **Table 10**. The first three formulations (F1-F3) showed floating lag time in the range 95-103 seconds which was more than 60 seconds (1 minute). Formulations F4-F6 and F7-F9 exhibited the desired floating lag time of less than 1 minute; however, the desired floating time for pellets was still not achieved in any of these formulations. The floating time of all these formulations was less than 15 min.

TABLE 10: FLOATATION BEHAVIOUR OF PLACEBO BATCHES OF GRMUPS COMPRISING OF HPMC

Formulation code	Floating lag time (sec)	Floating time
F1	102-103	5-6 minutes
F2	100-102	Up to 6 minutes
F3	90-95	7-8 minutes
F4	25-30	6-8 minutes
F5	28-30	8-10 minutes
F6	22-25	9-10 minutes
F7	15-20	6-7 minutes
F8	17-19	7-9 minutes
F9	15-18	8-12 minutes

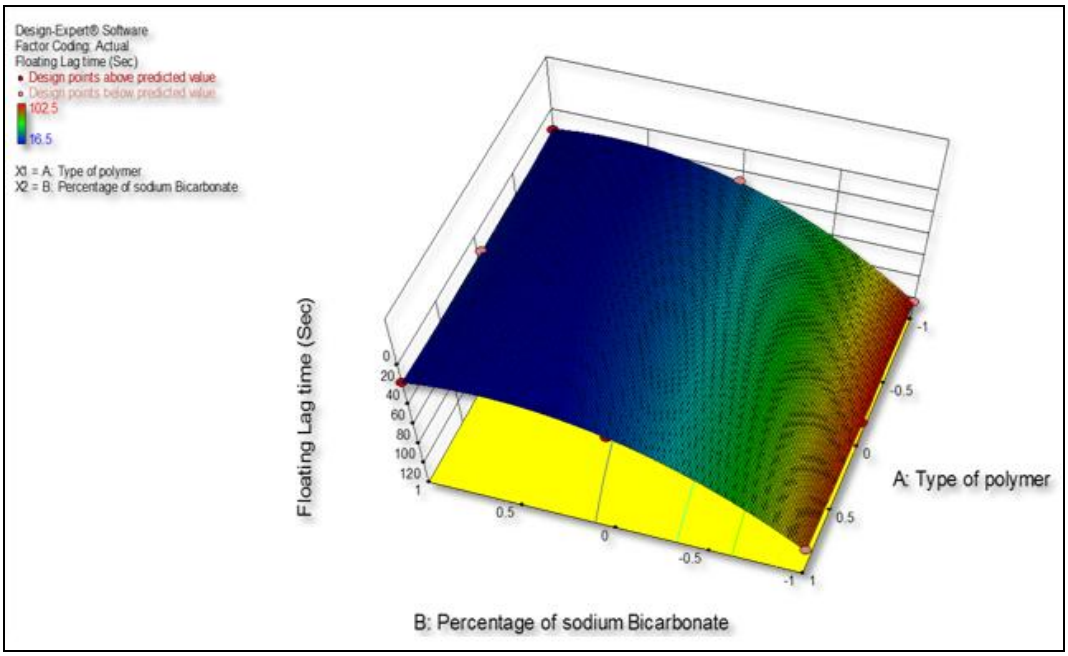

FIG. 1: CONTOUR PLOT FOR FLOATING LAG TIME

TABLE 11: ANOVA FOR RESPONSE 1 (FLOATING LAG TIME)

ANOVA for Response Surface Quadratic model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	11958.19	5	2391.64	2033.83	< 0.0001	significant
A-Type of polymer	37.50	1	37.50	31.89	0.0110	
B-Percentage of sodium Bicarbonate	9922.67	1	9922.67	8438.17	< 0.0001	
AB	20.25	1	20.25	17.22	0.0254	
A^2	14.22	1	14.22	12.09	0.0401	
B^2	1963.56	1	1963.56	1669.80	< 0.0001	
Residual	3.53	3	1.18			
Cor Total	11961.72	8				

Polynomial Equation: Floating Lag time = +28.44 – 2.5*A - 40.67*B + 2.25 AB – 2.67* A^2 + 31.33* B^2

The Contour plot **Fig. 1** suggested a negative relation between both the independent variables and floating lag time. It was observed that floating lag time decreases with a corresponding increase in

the second variable (% of sodium bicarbonate). It can be observed from the contour plot of **Fig. 1** that, with a change in the type of polymer (Variable 1) and increase in the percentage of sodium bicarbonate (Variable 2), there is a significant decrease in the floating lag time (Dependent variable). The minimum floating lag time was observed with Methocel® K100 M and at highest level (20%) of sodium bicarbonate. The polynomial equation suggested a similar pattern in **Table 11**. The negative sign with both the independent variables indicates an inverse relationship between the floating lag time and both the factors. The floating lag time with the squared quantity of the type of polymer shows an inverse relationship, but that of the squared quantity of % of Sodium Bicarbonate shows a direct relation indicating that any further increase in Sodium Bicarbonate levels will have minimal or no significant effect on floating lag time. The selected factors had no significant effect on the floating time as the placebo gastroretentive multiunit particulates remained buoyant for almost the same amount of time. All the plots and equations were plotted and tabulated respectively with the help of Design-Expert Software. Based on the results obtained, it was observed that the optimum level for sodium bicarbonate to make the formulation buoyant was 12.5% and above. The various grades of Methocel® that were screened could not maintain the floating ability of multiunit particulates. All the grades of Methocel® showed a similar ability to maintain the buoyancy of the placebo gastroretentive multiunit particulates. However, the Methocel® K4M grade was unable to retain the integrity of placebo GRMUPs. Methocel® K100M, on the other hand, formed a very thick mass on granulation which led to problems in the extrusion process. Hence, Methocel® K15M was selected as one of the polymers that required to be combined with other polymers to improve the floating time. Hence, in further trials, Methocel® K15M was combined with Xanthan gum (1:1) and Sodium alginate (1:1) at different levels. The results showed a similar range of floating lag time, but the combination of HPMC K15M with Sodium alginate showed promising improvement in the floating time.

Drug Loaded Batches: The GRMUPs showed a bulk density of 0.48 to 0.50 g/ml and a tap density of 0.650 to 0.750 g/ml. The angle of repose was found to be between 25° to 30°, which indicated good flowability for capsule filling. All the drug-loaded batches showed the required value of floating lag time (less than 1 min); however, only bathes D7, D8, and D10 to D15 showed the desired floating time (of more than 8 h) **Table 12** and **Fig. 2**.

TABLE 12: LAG TIME AND FLOATING TIME OF DRUG LOADED BATCHES

Formulation code	Floating lag time	Floating time
D1	42-45 sec	About 1.5 hours
D2	38-40 sec	About 1 hours
D3	40-42 sec	About 1.5 hours
D4	38-40 sec	About 4 hours
D5	30-34 sec	6-8 hours
D6	35-38 sec	About 6 hours
D7	32-34 sec	> 8 hours
D8	35-38 sec	> 8 hours
D9	38-40 sec	About 1 hour
D10	30-32 sec	> 8 hours
D11	34-36 sec	> 8 hours
D12	32-36 sec	> 8 hours
D13	35-37 sec	> 8 hours
D14	33-35 sec	> 8 hours
D15	35-38 sec	> 8 hours

FIG. 2: FLOATING BEHAVIOUR OF GRMUPS OF FERROUS ASCORBATES (A): THE GASTRORETENTIVE MULTIUNIT PARTICULATE (GRMUPS) IS INTRODUCED INTO A BEAKER CONTAINING 0.1N HYDROCHLORIC ACID (B): FLOATING LAG TIME WHERE THE GRMUPS BEGIN TO FLOAT (C): THE GRMUPS FLOATING AT THE SURFACE IN THE BEAKER CONTAINING 0.1N HYDROCHLORIC ACID

The *in-vitro* dissolution studies of the GRMUPs were performed and simulated gastric fluid pH 1.2 for 8 h. The results are computed in T**able 13**.

TABLE 13: *IN-VITRO* DISSOLUTION STUDIES OF GRMUPS OF FERROUS ASCORBATE (N=3)

Time (Hrs)	% Cumulative Drug Release (%CDR)					
	D7	D11	D12	D13	D14	D15
1	40.906	30.964	33.888	36.818	40.822	46.891
2	47.566	31.721	37.579	46.418	48.024	54.346
3	61.572	34.82	39.541	49.596	51.211	57.276
4	75.946	37.059	42.975	52.206	55.876	63.241
5	92.738	38.139	45.842	58.825	60.274	71.479
6	-	39.224	50.185	64.796	67.325	77.812
7	-	41.776	52.797	67.193	72.952	81.254
8	-	45.802	54.545	71.356	78.316	90.074

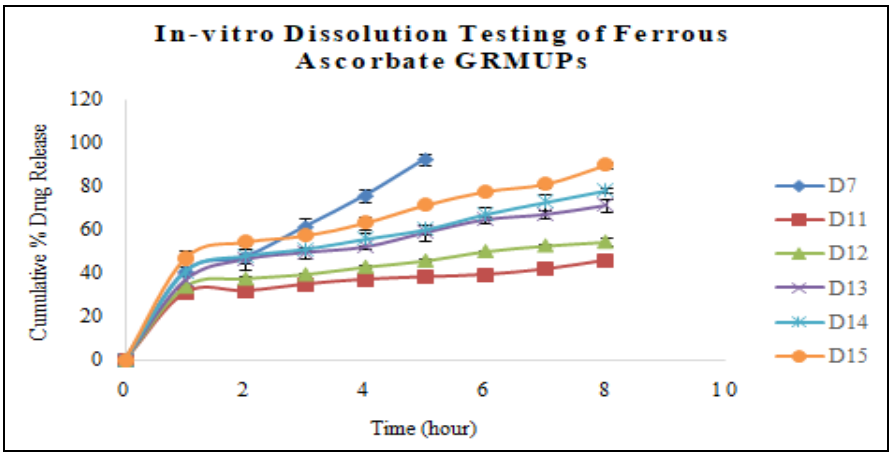

FIG. 3: *IN-VITRO* DISSOLUTION STUDIES OF GRMUPS IN SIMULATED GASTRIC FLUID pH 1.2

Formulations D1 to D6 displayed a floating time of less than 8 h, and hence they were not further considered for evaluation of drug release by in-vitro dissolution studies. Pellets of formulation D7 comprising of HPMC K15M (15%) and Sodium alginate were subjected to dissolution studies in simulated gastric fluid pH 1.2. The formulation showed a cumulative drug release of about 92% at the end of 5th hour. In order to prevent the initial burst release as observed in D7 and prolong the drug release, in subsequent batches D11-D15, additional release retardant Ethylcellulose 10P was incorporated at levels of 1.5% - 5% of fill weight. Formulation D11 comprising of HPMC K15M 15%, Sodium alginate 2.5%, and Ethylcellulose 10P 5% showed a cumulative drug release of 45% at the end of 8 hours. Hence, in subsequent formulation D12, HPMC K15M level was reduced to 7.5%, and the release was about 54% at the end of 8 h. In formulations D13 and D14, concentrations of Ethylcellulose 10P were reduced to 2% and 1.5%, respectively. Cumulative drug release of about 71% and 78% at the end of 8 h was obtained for D13 and D14, respectively.

Formulation D15, comprising of HPMC K15M 10%, Sodium Alginate 2.5%, and Ethylcellulose 10P 1.5% yielded cumulative drug release of about 90% at the end of 8 h **Fig. 3**. The drug release data were subjected to regression analysis in order to determine the drug release kinetics. The release pattern from the optimized formulation was observed to follow Higuchi kinetics with a regression coefficient R^2 value of 0.9621. The drug release is predicted to follow the process of diffusion through a hydrophilic matrix **Table 17**.

Evaluation of Colon Specific Tablets of Folic Acid: The pre-compression parameters of granules of both the batches (CT and ST) were found to show acceptable values for flow and good compressibility as per the compendial standards. The core tablets were evaluated for post-compression parameters. The hardness of the compressed tablets was maintained between 6-10 kg/cm^2 with a thickness range of about 4.90 to 5.10 mm. The Friability of tablets was found to be in the range of 0.1 - 0.2%, which was much less than the limit of 1%.

The disintegration of tablets was found to be 6-8 minutes which was less than the upper limit of 15 minutes. The post-compression parameters of the core tablets were found to be within the compendial limits. Core tablets seal coated with HPMC E5, and Ethylcellulose in combination yielded tablets with a rough appearance. Tablets seal coated with ethyl cellulose alone had smooth film. Core tablets seal coated with Ethylcellulose to obtain weight gain in the range of 2.5 - 5%. In order to confirm the adequacy and suitability of the seal coat, these tablets were further coated with colon targeting polymer Eudragit FS30D and subjected to disintegration test in the simulated colonic fluid. For starch-containing seal coated tablets with weight gain of 2.5% Ethylcellulose disintegrated in simulated colonic fluid in 30 minutes; however, tablets with weight gain in the range of 3-4.5% failed to disintegrate in simulated colonic fluid even after 2 h. In the case of Crospovidone-based core tablets, tablets with 2.5% weight gain softened on Eudragit FS 30D coating, and those with 5% weight gain failed to disintegrate in simulated colonic fluid even after 2 hours. Hence, starch-based core tablets with a seal coat of 2.5% were considered for further processing and evaluation. The disintegration behaviour of seal coating. Folic acid tablets are tabulated in **Table 14**.

TABLE 14: DISINTEGRATION BEHAVIOUR SEAL COATED TABLETS OF FOLIC ACID

Ethyl Cellulose (Weight gain in %)	Crospovidone tablets (CT)	Starch tablets (ST)
5.0%	Tablets were easily coated using Eudragit FS 30D but did not disintegrate in the Simulated Colonic Fluid after 2 h	-
4.5%	-	Tablets were easily coated using Eudragit FS 30D but did not disintegrate in the Simulated Colonic Fluid after 2 h
4.0%	-	Tablets were easily coated using Eudragit FS 30D but did not disintegrate in the Simulated Colonic Fluid after 2 h
3.0%	-	Tablets were easily coated using Eudragit FS 30D but did not disintegrate in the Simulated Colonic Fluid after 2 h
2.5%	Tablets became soft during Eudragit FS 30D coating	Tablets were easily coated using Eudragit FS 30D and disintegrated in the Simulated Colonic Fluid (Colonic phase) after 30 min

The seal coated tablets were further coated with Eudragit FS 30 D at weight gains in the range of 15% to 35%. The tablets were then subjected to disintegration test in 0.1N Hydrochloric acid representing the gastric stage, followed by phosphate buffer pH 6.8 representing the intestinal stage and finally in Simulated Colonic Fluid pH 7.2. The results are depicted in **Table 15**. Tablets at all the weight gains were found to be intact in simulated gastric fluid. Tablets with 15% weight gain disintegrated in 4 h in simulated intestinal fluid, whereas those with 20%, 25%, and 30% weight gain disintegrated within 5 h and 30 min. Considering average small intestinal transit time of 4 to 6 h, weight gains of 15-30% were found to be insufficient to deliver the tablet intact to the colon. Tablets with a weight gain of 35% were found to be intact in small intestinal fluid; however, when subjected to simulated colonic fluid, tablets disintegrated within 30 min. These results were further confirmed by performing drug release studies by *in-vitro* dissolution testing in simulated gastric fluid for the first 2 h followed by simulated small intestinal fluid from 3-6 h and finally in simulated colonic fluid **Table 16, Fig. 4**.

TABLE 15: DISINTEGRATION TEST OF COLON SPECIFIC FOLIC ACID TABLETS

Weight gain	Acid Stage (Gastric transit: 2 hours)	Phosphate buffer pH 6.8 (Small intestinal transit: 3-6 hours)	Simulated Colonic Fluid pH 7.2 (30 minutes)
	-	Total 6 hours	Total 7.5 hours
15%	No disintegration	Disintegrated within 4 hours	-
20%	No disintegration	Disintegrated within 5 hours	-
25%	No disintegration	Disintegrated within 5 hours	-
30%	No disintegration	Disintegrated within 5.5 hours	-
35%	No disintegration	No disintegration	Disintegrated within 7.5 hours

TABLE 16: *IN-VITRO* DISSOLUTION STUDIES OF COLON SPECIFIC FOLIC ACID TABLETS

Time (Hrs)	% Cumulative Drug Release (%CDR)					
	Weight Gain	15%	20%	25%	30%	35%
1	Simulated Gastric fluid	1.040	0.860	0.000	0	0
2	(pH 1.2)	4.293	2.138	2.976	3.320	2.795
3	Simulated intestinal	5.918	4.496	2.521	5.924	4.203
4	fluid (pH 6.8)	21.051	6.546	2.589	6.363	5.311
5		54.513	36.949	16.988	7.966	6.248
6		73.423	58.328	35.759	29.707	12.788
7	Simulated Colonic	80.666	69.019	66.320	66.060	92.740
8	Fluid pH 7.2	92.720	87.574	88.530	85.030	98.460

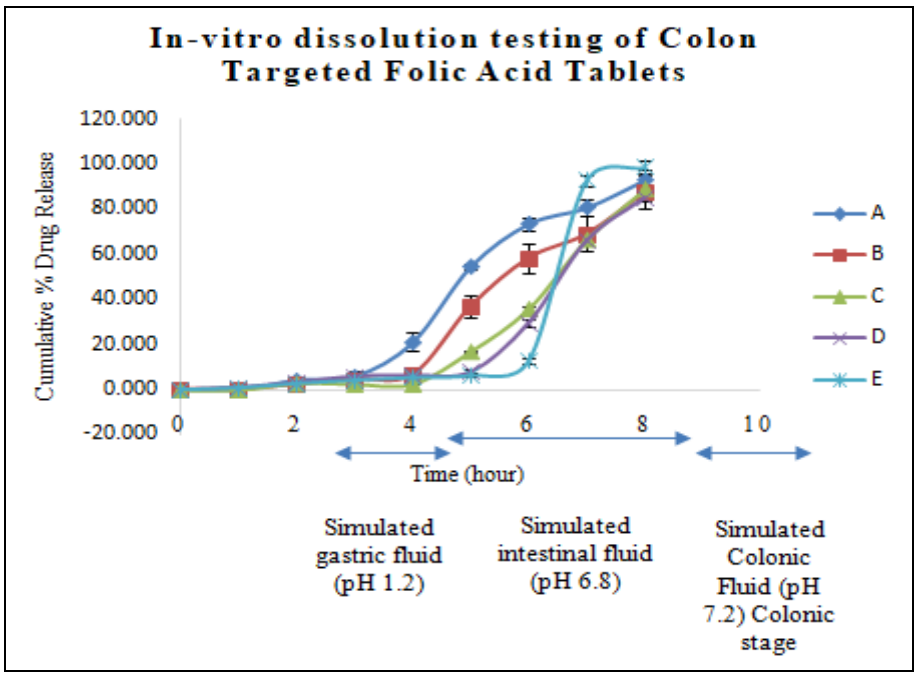

FIG. 4: *IN-VITRO* DISSOLUTION STUDIES OF COLON SPECIFIC FOLIC ACID TABLETS A- 15% weight gain; B- 20% weight gain; C- 25% weight gain; D- 30% weight gain; E- 35% weight gain

Tablets at all the weight gains showed a % drug release of less 5% in simulated gastric fluid. In simulated small intestinal fluid, tablets of weight gain 15-30% showed cumulative % drug release of more than 25%. It was observed that as the weight gain increased from 15% to 30%, there was a progressive decrease in % drug release from 73.4 % to 29.7%, indicating that an incremental increase in weight gain was retarding the drug. Tablets with 35% Eudragit FS 30 D coat showed drug release of about 12.8% at the end of 6 h and complete release in simulated colonic fluid at the end of 8 h. The drug release data were subjected to regression analysis in order to determine the drug release kinetics. The release pattern from the optimized formulation was observed to follow Korsmeyer Peppas kinetics with an R^2 value of 0.8039 **Table 17**. Anamalous behavior of the Korsmeyer Peppas model indicated drug release occurring due to erosion of polymer coat.

TABLE 17: PREDICTION OF FRUG RELEASE KINETICS BY REGRESSION COEFFICIENT R^2

Regression coefficient	Ferrous Ascorbate Gastroretentive Multiunit Particulate Pellets	Folic Acid Colon Targeted Pellets
% Drug Release *vs.* Time (Zero order kinetics)	0.815	0.6128
Log % Drug Retained *vs.* Time (First order kinetics)	0.94	0.5478
% Drug Release *vs.* Square root of time (Higuchi)	0.9621	0.4168
Log % Drug release *vs.* Log of time (Korsmeyer Peppas)	0.9425	0.8039

The developed capsule formulation containing Ferrous Ascorbate GRMUPs and Folic acid colon targeted tablets was found to be stable. The physicochemical properties, assay, and drug release for Ferrous Ascorbate and Folic acid were found to be similar to the initial values.

CONCLUSION: Among the various types of diseases that fall under the category of micronutrient malnutrition, one of the most common and prominent nutritional deficiency disorders in the world is anaemia. Gastroretentive dosage forms are intended to localize in the stomach or in the upper part of the small intestine, which ensures prolonged drug release in the stomach near the site where Iron gets absorbed primarily. Also, a colon-specific dosage form localizes the drug in the colon where Folic Acid is absorbed. By using these two techniques, an attempt was made to develop a combination of gastroretentive multiunit particulates of Ferrous Ascorbate and colon-specific tablet of Folic Acid to be administered simultaneously in a capsule. Combination of various polymers consisting of different grades of HPMC like HPMC K4M, HPMC K15M, HPMC K100M, and Sodium alginate was tried in the formulation of matrix gastro retentive multiunit particulates. A core tablet of Folic Acid was prepared using wet granulation technique followed by subsequent layers of seal coat (Ethylcellulose 10P) and functional coat (Eudragit FS 30D) at different weight gains were tried. Preformulation studies were carried out to establish compatibility between the drug and the excipients by DSC and FT-IR.

Investigations revealed that drugs and excipients were satisfactorily compatible. A 32 factorial design was evaluated to select the type of gas trapping polymer and optimize the Sodium bicarbonate level required to get a lag time of less than 1 min and a floating time of 8 h or more. Contour plots were plotted, and the ANOVA test was performed to check the significance of the applied model. The optimum level of sodium bicarbonate was established, while the selection of polymer required a further trial batch. Fifteen different formulations were tried (D1-D15) in which the levels of HPMC K15M and Sodium alginate were varied to get the optimum floating lag time, floating time, and drug release. The optimum batch showed a drug release of about 90-91% at the end of 8 h and followed Higuchi kinetics. Core tablets of folic acid were prepared using a wet granulation technique followed by subsequent layers of seal coat (Ethyl Cellulose IP) and functional coat (Eudragit FS 30D) at different weight gains. Core tablets consisting of crospovidone and starch were formulated and seal coated. Based on the disintegration behavior of tablets in simulated colonic fluid, starch-based tablets with 2.5% weight gain were considered for functional coating of Eudragit FS 30D. Tablets coated with Eudragit FS 30D with weight gain in the range of 15-35%, and each was subjected to dissolution testing. The optimized batch comprising of 35% weight gain showed a cumulative drug release of about 98% at the end of 8 hours and followed Korsmeyer-Peppas kinetics. From the results of stability studies data, it was observed that there was no significant change in the drug content and *in-vitro* drug release behaviour of the formulations. The formulations were found to be stable.

Hence, the developed combination of gastro-retentive multiunit particulates of Ferrous Ascorbate and Colon specific tablets of Folic Acid together in the capsule can be used in the management and treatment of anaemia by localizing the dosage forms at their desired site of absorption within the GIT and reducing the dosing interval. In this study, the factorial design results aided the selection of optimum sodium bicarbonate level (12.5% or more). Further trials helped in the selection of a combination of gas trapping polymers (HPMC K15M and Sodium alginate). Gastroretentive multiunit particulates of Ferrous Ascorbate were successfully formulated by using Sodium alginate (2.5%) and HPMC K15M (7.5%) as gas trapping agents and release retardants along with Ethylcellulose 10 P (1.5%) as release retardant. The optimized formulation D15 showed a floating lag time of less than 1 minute and a floating time of greater than 8 hours. The *in-vitro* drug release studies of optimized formulation showed a sustained release for about 8 h. Colon specific tablets of Folic acid-containing starch as a disintegrant were formulated. The tablets were seal coated with Ethylcellulose for an optimum weight gain of 2.5%.

These were then coated with Eudragit FS 30D as a functional coat. The optimized weight gain was 35%, and these tablets were subjected to *in-vitro* dissolution studies. The release study showed a delay in release for 6 h (2 h in 0.1N Hydrochloric acid and 4 h in phosphate buffer pH 6.8) followed by a complete release at the end of 8 h.

The present work demonstrated a satisfactory preliminary study in developing a site-specific sustained release formulation of Ferrous Ascorbate gastro retentive multiunit particulates (As iron source), and Folic acid colon targeted tablet to be administered together in the capsule.

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