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PREPARATION AND EVALUATION OF FERROUS GLYCINE SULPHATE TIMED RELEASE DOSAGE FORM

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ABSTRACT

Objective: Extrusion spheronization is a widely used multi-stage technique that can produce spherical particles of almost uniform size. In order to evaluate the effect of the substitution of microcrystalline cellulose with different amounts of ethyl cellulose on the mechanical properties and release properties of ferrous sulphate glycine matrix particles prepared by extrusion spheronization, this study was conducted**. Method**: Therefore, the ethyl cellulose used in the formulation test ranges from 0-40%, and the maximum MCC is 85%. The yield of pellets was in the range of 70-90.5% and was influenced by EC percentage. **Results:** Inclusion of EC changed the mechanical properties of pellet, and affected drug release rate and therefore was able to make sustained release matrix pellets up to 4 hr it was observed that, the ferrous glycine sulphate pellets, as an iron compound was found to produce an improved effect comparing to the standard. **Conclusion**: Results revealed that EC could not be used as a sole polymer in production of Ferrous glycine sulphate matrix pellets by extrusion-spheronization and at least 20% level of MCC was necessary in the process. Data between groups were compared statistically by t-test and values of $P<0.0001$ were considered statistically significant.

KEYWORD: Ferrous glycine sulphate, Pellets, Timed-release, Extrusion-Spheroinzation.

INTRODUCTION

Anaemia is a decrease in the total amount of red blood cells (RBCs) or haemoglobin in the blood, or a lowered ability of the blood to carry oxygen.[1] The diagnosis of anaemia in men is based on a haemoglobin of less than 130 to 140 g/L (13 to 14 g/dL); in women, it is less than 120 to 130 g/L (12 to 13 g/dL).[2,3] Iron-deficiency anaemia is anaemia caused by a lack of iron Children with iron deficiency anaemia may have problems with growth and development.[4] Iron-deficiency anaemia is caused by blood loss, insufficient dietary intake, or poor absorption of iron from food.[5] Women and young children are most commonly affected. Treatment of iron-deficiency anaemia includes dietary changes to incorporate iron-rich foods into regular oral intake and oral iron supplementation.[6,7,8] Sustained release formulation maintains a uniform blood level of drug with patient compliance as well as increased efficiency of drug.[9] Pellets are small free flowing; spherical particulates manufactured by the agglomeration of fine powder or granules. The most commonly use pelletization processes are Extrusion spheronization, [10,11] Spansules are defined as capsules containing medicines (in form of granules or Pellets), coated with materials having slow dissolving rates so that the medicament is delivered at different specific time.[12,13,14] Sustained release (SR) pellets using of cow ghee (CG) as an important hot-melt coating (HMC) agent [15] and using formulation of carbonyl iron also reported.[16] In order to evaluate the effect of the substitution of microcrystalline cellulose with different amounts of ethyl cellulose on the mechanical properties and release properties of ferrous sulphate glycine matrix particles prepared by extrusion spheronization, this study was conducted. This study also aims to find out the anti-anaemia potential of rat ferrous glycine sulphate pellets after causing anaemia and directly ingesting drugs by measuring haematological parameters.

MATERIALS AND METHODS

Ferrous glycine sulphate purchased from New Alliance Fine chemicals, Mumbai, Microcrystalline cellulose obtained from Indian Fine chemicals, Mumbai., Ethyl cellulose purchased from Feicheng Ruitai Fine Chemicals, China and Polyvinyl pyrrolidine from Chem fine Enterprises.

Preparation of Pellets by Extrusion and Spheronization

Eight different formulations of ferrous glycine sulphate-containing pills were prepared by extrusion and spheronization methods. The ingredients of the formula are listed in **Table: 1** Accurately weighed the solid powders of MCC, EC and PVP and mixed them by hand

in a polyethylene bag to obtain a uniform physical mixture. ferrous glycinate sulphate added and mixed well. The powder mixture was mixed with water to obtain the consistency of the wet mass. The wet mass was then passed through Single screw extruder (EXT 30, Rikon Pharma, Thane, Mumbai, India) with a 1.0 mm screen and a speed of 150 rpm. The extrudates were processed in a spheronizer (SPH 150, Rikan Pharma, Thane, Mumbai, India) fitted with a cross-hatched plate revolved at 300rpm for about 5 min. The obtained pellets were dried at 40ºC for 12 h.

Table 1: Formulation Components of Ferrous Sulphate Pellets.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Ferrous Glycine Sulphate (%)	10	10	10	20	30	30	20	30
Microcrystalline Cellulose (%)	85	4	65	40	65	30	35	35
Ethyl Cellulose (%)	-	40	20	35	-	35	30	30
Polyvinyl Pyrolidine (%)	5	5	5	5	5	5	5	5

Pilot mixing of Pellets

Pilot blend consisted of pellets of three different batches. These pellets were mixed equally after the determination of their drug content and mixed in proportion to the label claim. Therefore, approximately 33.33 % of each polymer drug pellets were mixed for dissolution test.

Pellet size analysis by sieve method

Using a combination of vertical movements (10, 14, 16, and 18), the ferrous glycine pellets to be analysed are vibrated through a series of successively decreasing sieves. The pellets in motion is finally oriented to the sieve (16) with its smallest two sizes, opens and passes through the sieve with the next smaller nominal opening (18). Upon completion of the sieving process, the weight of the sieves was measured and compared to the weight of the sieves before addition of the sample. Through addition of the mass fraction on each sieve, from the smallest to the largest sieve, a cumulative mass distribution of the test sample was calculated.

Micrometrics[17]

Angle of repose was determined by the fixed funnel method. Bulk density and tapped density was determined by carefully pouring pre weighed amounts of powders into 100-ml graduated cylinder and measuring the volume occupied by the powders. The tapped bulk density was determined by the volume of the powder bed after tapping the cylinder onto a wood surface fifty times from a height of approximately 2.5 cm at 2 s intervals, and the ultimate tapped density was calculated after continued tapping caused no further reduction in volume. The compressibility index was calculated using the bulk and ultimate tapped bulk density.

***In vitro* dissolution study**[18]

Using USP XIII dissolution test, the dissolution study of granules was carried out in triplicate apparatus- 1 (Electrolab, TDL- 08L, India) after determination of ferrous glycine sulphate equivalent by assay. Weighed quantities of the pellets were loaded into the basket of the dissolution apparatus and 900 ml of dissolution medium of Acetate buffer pH 4. The temperature of the dissolution fluid was maintained at 37°C±0.5°C with a stirring speed of 100 rpm. The samples withdrawn predetermined intervals 0, 1, 2, 3, 4 hrs and were filtered (0.22 µm, Millipore). The drug quantity was determined using spectrophotometrically at 279 nm.

Drug release kinetics

Several dissolution models were applied to investigate the release mechanism of the optimized formulations. The models included zero order, first order, Higuchi's, and Korsemeyer-peppas model. All formulation was tested with zero order release rate kinetics then the optimized formulation selected.

Morphological study of pellet

The shape and surface of pellets were investigated by a projection microscope. Pellets were placed on a dark background and a light source was used to reduce the influence of shadow on the image.

***In Vivo* Drug Efficacy Study (Analysis of Haematological Parameters)**

Anaemia were induced in female wistar rat by 2.5 % neutralized Phenylhydrazine Hydrochloride (Fisher Scientific company, USA) at a dose of 30 mg/kg body weight with a two maintenance dose of 10 mg/kg BW at the interval of 24 hours,(1,3,6 h).The rats were given with standard diets and the drug formulation were administered orally, after the diagnosis of anaemia 0.5 ml of blood sample were collected by the tail vein at predetermined time interval (at the end of 24hrs).Red Blood Cell (RBC) counts were done by haemocytometer. Haemoglobin (Hb) estimation and Packed Cell Volume (PCV) were determined by cyanomethanoglobin method using a spectrophotometer and microhaematocrit. Approval for animal experiments was obtained from committee for the purpose of control and supervision of Experiments on animal (CPCSE)/ Institutional Animal Ethical Committee (IAEC) Proposal No :**08/321/PO/Re/S/01/CPCSEA.**

The animals were divided into Two groups; each group consists of 6 rats (n=6) as follows:

Group –I (Control group): Anaemic rats treated with ferrous glycine sulphate (30mg/kg; po)

Group - II (Test group): Anaemic rats treated with the ferrous glycine sulphate formulation drug orally (30mg/kg; po). The percentage absorbance of ferrous

glycine sulphate formulation was calculated by comparing with that of control group.[19]

Statistical analysis[20]

The mean and standard deviation (SD) of the data was calculated. The results were analyzed by one-way analysis of variance (ANOVA) were probability (p value) of the model should be ($p < 0.05$). And student T-test were calculated.

Formulation of Ferrous glycine sulphate Timed release pellets

The optimized formulation prepared as capsule dosage form by semi-automatic capsule filling machine. The empty hard gelatine capsule (size 1) obtained as gift sample. After capsulation, it was analysed as per IP including content uniformity, weight variation, etc.

RESULTS AND DISCUSSION

Formulation Development of Timed-release dosage form

Formulation development starts with the challenging aspects of choosing API, efficacy cost, accessibility, and drug characteristics to choose ferrous glycine sulphate. Then, the excipients were selected based on the previous studies and compatibility.The formulation was developed using Extrusion and Spheronization technique. API is weighed and mixed with other excipients as a part for manufacturing process. EC were added to timed release. So, the study moved to the development of timed-release pellet form to treat anaemia.In the phase of development, ferrous glycine sulphate pellets and polymers were added in the proportions shown in **Table 1**. Ferrous glycine sulphate were prepared by adding EC, MCC and PVP. Water, as a granulating fluid, was added in an amount sufficient to prepare a wet blend which is suitable for extrusion. It has good Sphericity, low friability, high density and smooth surface properties. PVP K30 (5%) were used as binder in the investigation.

Evaluation of Ferrous Glycine Sulphate Pellets

Ferrous Glycine Sulphate pellets were prepared with different percentage weight gain (F1- F9) to produce ferrous glycine sulphate timed release pellets. The sizes of the prepared Ferrous Glycine Sulphate Timed Release Pellets was measured by using Sieve technique and found between 0.754 to 0.824 mm. The results were shown in **Table 2.** The average particle size, the yield of the pellets and the crushing strength of the pellets are satisfactory.

Table 2: Average Pellet Size of Ferrous Glycine Sulphate Pellets.

Formulation	Average pellet size (mm)	Production yield (%)	Tensile strength ($Kg/cm^{2)}$
F1	0.761	89.9	2.45
F2	0.882	89.1	2.75
F3	0.787	87.44	2.92
F4	0.837	85.13	3.05
F5	0.774	84.24	3.14
F6	0.824	81.65	3.45
F7	0.811	78.47	3.56
F8	0.796	71.53	3.75
F9 (Optimized Pilot mixing Formulation)	0.754	90.5	3.82

Micromeritics Evaluation

The angle of repose for the formulation was determined and was found to be within the limit. The Formulation (F9) had excellent flow and other formulation showed good flow.The Bulk density of the all formulations was found to be in the limit of 0.6 to 0.7 hence it was within the limit. The tapped density of the formulations was found to be within the limit.The Carr‟s index and Hausner‟s ratio was found to be within the limit the results were shown in **Table 3.** The density measurement was helpful to fix the total weight of the capsule. The yield of pellets was in the range of 70-90.5% and was influenced by EC percentage.

Table 3: Micrometrics of Ferrous Glycine Sulphate Pellet.

Formulation	Angle of repose (θ)	Bulk density gm/ml	Tapped density gm/ml	Hausner's ratio	Friability %	Caar's index (%)
F1	25.43	0.65	0.73	1.15	0.084	10.95
F2	25.12	0.66	0.74	1.21	0.089	10.81
F3	24.81	0.67	0.74	1.04	0.087	9.45
F4	23.11	0.69	0.75	1.08	0.096	8.01
F5	23.01	0.70	0.77	1.01	0.092	9.09
F6	22.48	0.72	0.79	1.09	0.084	8.86
F7	21.14	0.75	0.81	1.08	0.079	7.40
F8	20.68	0.76	0.83	1.09	0.086	8.43

Dissolution profile of Ferrous Glycine Sulphate

The Ferrous glycine sulphate Pellets consists of two polymers. Two hydrophilic Polymer coated pellets (EC: MCC 40:0, and 85:30). During dissolution, the dissolution medium penetrated into the layer, leading to the hydrophilic polymer hydration and swelling. A gel layer was formed around the pellet surface, and the drug began to release; the thickness of the gel layer increased while the drug was released continuously from the erosion gel or diffused through the diffusion channels; and as the study time went on, after 4 hrs. The EC-coated ferrous sulphate pellets continue to penetrate/ infiltrate into the dissolution medium until the drug is completely released. In addition, increasing the amount of EC does not cause a delay in drug release, especially after 4 hours of drug release. Therefore, membrane rupture or crack formation or pore formation results in complete drug release, which is 25% to 30% after 4 to 5 hours of dissolution.

Effect of EC: MCC in drug release

All formulations showed sustained release over 4 hours **(Fig 1).** The cumulative release or all the formulation were found within the range of 43-85%. The drug release directly depends on the polymers. EC and MCC percentage were differ in all formulation. In Formulation 1, 85.43% of EC: MCC (0:85) was released. In Formulation 2, 80.82% of the EC: MCC (40:45) drug was released. However, in the case of Formulation 3, the release rates of EC: MCC (20:65) 76.42% and in Formulation 4 were 76.42% and 69.08% (EC: MCC 35:40). In the case of higher EC: MCC, the drug release in formulations 5 and 6 was further reduced to 63.47% and 58.23%. In Formulation 7, EC: MCC (30:45) released 51.23% of the drug, but in Formulation 8, EC: MCC (30:35), 43.08% of the drug was released. Compared to all formulations, a high percentage of MCC gave a high percentage of cumulative drug release. A low percentage of EC produces a higher cumulative drug release percentage. While comparing formulation 1 produces high % cumulative drug release. Results revealed that EC could not be used as a sole polymer in production of Ferrous glycine sulphate matrix pellets by extrusion-spheronization and at least 20% level of MCC was necessary in the formulation. Inclusion of EC changed the mechanical properties of pellet, and affected drug release rate and therefore was able to make sustained release matrix pellets up to 4 hr it was observed that, the ferrous glycine sulphate pellets, as an iron compound was found to produce an improved effect comparing to the standard.

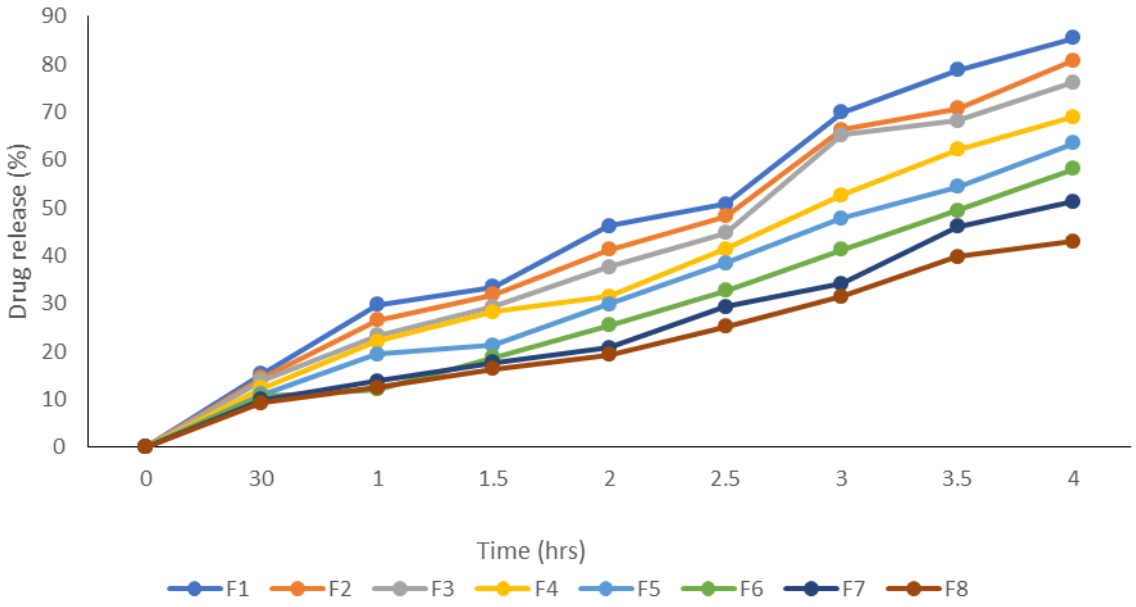


Fig. 1: Comparative *In-vitro* Drug release profile of Ferrous glycine sulphate pellet forms.

Effect of pilot mixing in drug release

The pilot blend (mixture) consisted of three different batches of pellets. These pellets were mixed equally after the determination of their drug content and mixed in proportion to the label claim. Hence, close to 33.33 % of each polymer drug pellets were mixed for dissolution test. For the formulation 1 (82.03%) drug was released and for the formulation 2(80.13%) drug was released for the formulation 3 (85.43%) drug was released **(Fig 2).** Comparing to the three-batch 3rd formulation produced higher % cumulative drug release. It contains 20% of each (F1-F5) batch.

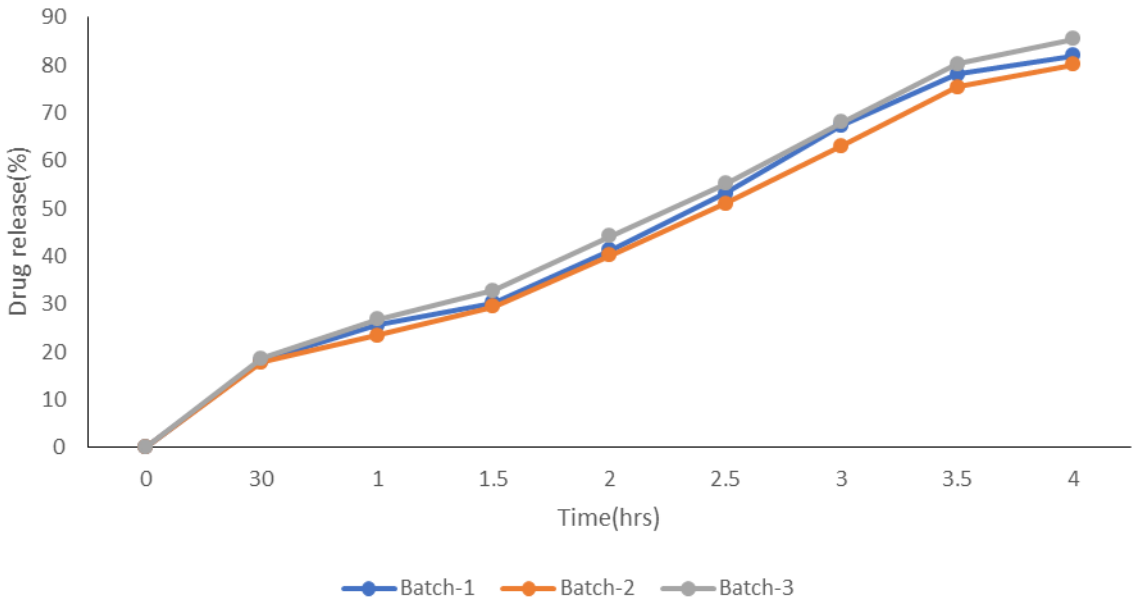


Fig. 2: Comparative *In-vitro* Drug release profile of Ferrous glycine sulphate pellet pilot (Pilot mix)

Effect of optimized formulation in drug release

Final optimized batch were mixed from the 3 batches of pilot mixing by using % drug release and for the optimized batch 85.12% of drug were released **(Fig 3).**

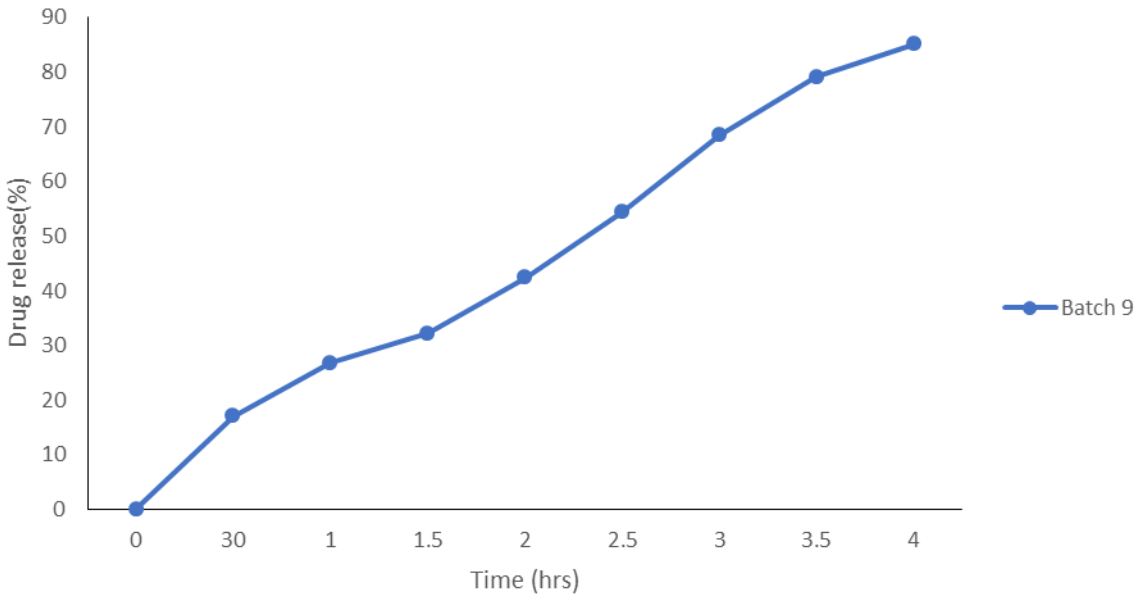


Fig. 3: *In-Vitro* drug release profile of Ferrous glycine sulphate Pellet pilot mixing from Optimized Formulation.

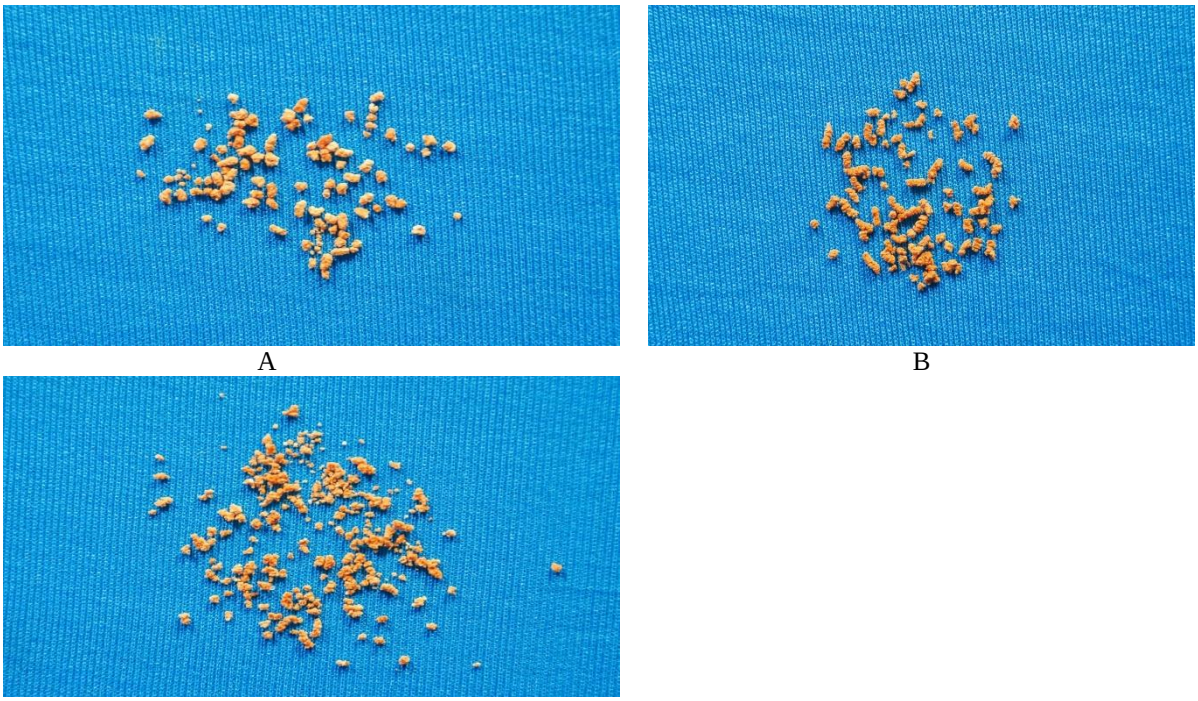
Drug release kinetics

Dissolution data of the optimized formulation was fitted to various mathematical models (zero-order, first-order, Higuchi and Korsemeyar-Peppas) in order to describe the kinetics of drug release. Accordingly, optimized formulation fitted with all dissolution model and the values found to be followed zero-order and Korsemeyer-Peppas kinetics**.** The release exponent of Peppas model (n=1) indicate caseII transport drug release mechanism and rates as a function of time follows zero order release.Similarly adjusted R^2, AIC, some of square residues (SSR) and mean selection criterion were satisfied with Korsemeyer-Peppas model.

Morphology study of pellet

Microscopic examination of the particles showed that the addition of EC affected the surface properties of the particles and resulted in the formation of particles with a rougher surface. The surface characteristics of the pellets with 40% and 30 % and 20 % of EC with drug are shown

in **Fig 4** as an example (formulations 2 and 3, 7, 8). As it can be seen the substitution of MCC with EC resulted in formation of pellets with rougher surfaces.Similar findings reported [21] that higher amount of MCC increased strength.



A B C

Fig. 4: Surface morphology of ferrous glycine sulphate pellet (A-20% of EC, B-30% EC, C- 40 %EC).

***In-Vivo* studies**

The method has been used to estimate haematological parameters by inducing anaemia. 2.5% phenyl hydrazine hydrochloride were given oral dosing 30 mg.After a period of time 15mg of ferrous glycine sulphate optimized formulation and marketed formulation were administered to rat. After the administration of the formulations, where as in test Haemoglobin for the marketed formulation was found to be 17.4gms% and for the ferrous glycine sulphate 15.03gms% and Red Blood Cell count for the marketed product was found to be 7.7mill. /cumm and for the ferrous glycine sulphate 6.6mill. /cumm and its Packed cell volume of marketed formulation were found to be 52.3% and for the formulation 47.3% were found and it's shown on the **Table 4.**

Table 4: Anti-Anaemic Property of Haemoglobin, RBC and Packed Cell Volume Values.

Treatement	**Day1**	**Day8**	**Day15**
Drug	15.2±0.35	16.8±0.51	17.4±0.37
Formulation	13.2±0.67	13.8±0.28	15.03±0.23
Treatement	**Day1**	**Day8**	**Day15**
Drug	5.7±0.29	6.1±0.19	7.7±0.34
Formulation	5.6±0.26	5.8±0.30	6.6±0.39
Treatement	**Day1**	**Day8**	**Day15**
Drug	44.4±2.16	43.4±2.63	52.3±1.72
Formulation	38.4±2.78	39.6±2.07	47.3±2.58

Stastical analysis

The mean and standard deviation (SD) of the data was calculated. The results were analyzed by one-way analysis of variance (ANOVA). Analysis of variance were calculated probability (P<0.0001) of the model was lower than 0.05 were found to be statistically significant as shown on the table 4, and for the Student T-test p value is 0.7397 as shown on the **Table 5,** which indicates that the selected model was significant. Data between groups were compared statistically by t-test for comparisons.

Table 5: Statistical Analysis and Student T Test Value of the optimized Formulation.

Source	Sum of Square	df	Mean Square	F Value	P value
Treatment	2114	2	1057	129.6	0.0001
Residual	48.95	6	8.158	-	-
Source	Sum of Square	df	Mean Square	F Value	P value
Treatment	-	2	-	2.627	0.0005

CONCLUSION

The average particle size, the yield of the pellets and the crushing strength of the pellets are satisfactory. Inclusions of EC led to the formation of larger pellets and it was found that the mean particle size of pellets was dependent on the percentage of EC in the formulation. The yield of pellets was in the range of 70-90.5% and was influenced by EC percentage. Results revealed that EC could not be used as a sole polymer in production of Ferrous glycine sulphate matrix pellets by extrusion-spheronization and at least 20% level of MCC was necessary in the formulation. Inclusion of EC changed the mechanical properties of pellet, and affected drug release rate and therefore was able to make sustained release matrix pellets up to 4 hr it was observed that, the ferrous glycine sulphate pellets, as an iron compound was found to produce an improved effect comparing to the standard. Data between groups were compared statistically by t-test for comparisons. Values of $P<0.0001$ were considered statistically significant.

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