



MICROENCAPSULATION OF FOLIC ACID AND FERROUS ASCORBATE FOR ORAL ADMINISTRATION

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ABSTRACT

The aim of the present work was to formulate and evaluate the oral sustained release formulation of a model drug with a view to control the drug release for better patient compliance, to reduce the dose frequency and enhance the stability. Microparticles of ferrous ascorbate and folic acid release iron slowly, hence side effects are minimized. Increased absorption of iron & folic acid enhanced their bioavailability. The DSC & XRD studies show that both the drugs are amorphous in nature, there is no any chemical interaction occurs during the formulation and the microspheres encapsulate the drug properly. The melting point of ferrous ascorbate and folic acid was found 190°C and 250°C respectively. The surface viewed through SEM shows spherical and slightly smooth. Drug release kinetic study was performed, based on r^2 value & it was concluded that the release of drug from the microspheres complies zero order kinetics i.e. the release is concentration independent. Hence this is a controlled release formulation.

KEYWORDS: Microspheres, Scanning electron microscopy (SEM), ferrous ascorbate, folic acid, controlled release and Chitosan.

INTRODUCTION

Microsphere

Microspheres are solid spherical particles ranging in size from 1-1000 μm . Microspheres are free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature.^[1-5] There are two types of microspheres: microcapsules and micromatrices. Microcapsules are those in which entrapped substance is distinctly surrounded by distinct capsule wall and micromatrices in which entrapped substance is dispersing throughout the microspheres matrix. Solid biodegradable microspheres incorporating a drug dispersed or dissolved through particle matrix have the potential for the controlled release of drug. They are made up of polymeric, waxy, or other protective materials, that is, biodegradable synthetic polymers and modified natural products.^[6-9]

TYPES OF MICROSPHERES

- Bioadhesive microspheres
- Magnetic microspheres
- Diagnostic microspheres
- Floating microspheres
- Radioactive microspheres
- Mucoadhesive microspheres
- Polymeric microspheres: - Biodegradable polymeric microspheres & Synthetic polymeric microspheres.

ADVANTAGES

- □ Microspheres provide constant and prolonged therapeutic effect.
- □ Reduces the dosing frequency and thereby improve the patient compliance.
- □ They could be injected into the body due to the spherical shape and smaller size.
- □ Better drug utilization will improve the bioavailability and reduce the incidence or intensity of adverse effects.
- □ Microsphere morphology allows a controllable variability in degradation and drug release.¹⁰⁻¹² □

LIMITATION

Some of the disadvantages were found to be as follows:

- □ The modified release from the formulations.
- □ The release rate of the controlled release dosage form may vary from a variety of factors like food and the rate of transit though gut.
- □ Differences in the release rate from one dose to another.
- □ Controlled release formulations generally contain a higher drug load and thus any loss of integrity of the release characteristics of the dosage form may lead to potential toxicity.
- □ Dosage forms of this kind should not be crushed or chewed.^[13-15]

MATERIAL AND METHOD

Preformulation studies were carried out on the obtained sample of drug for identification and compatibility studies. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients.

Organoleptic properties

The organoleptic studies like general appearance like nature, color, odor etc. were performed by visual observations.

Color

Small quantity of drug was taken in butter paper and viewed in well illuminated place.

Odor

Very less quantity of drug was smelled to get the odor.

Melting point

Melting points of Ferrous ascorbate and Folic acid were determined using capillary tube method. Two capillaries filled with drug were placed in the holes of melting point apparatus. A thermometer was inserted in the hole. Adapter was connected to the power plug and the temperature started to increase slowly. The temperature at which the drugs started to melt was noted which the melting points of the drugs.

Solubility Studies

The spontaneous interaction of two or more substances to form a homogenous molecular dispersion is called solubility. For solubility determination, excess of drug was added in each solvent (3.0 ml) in separate test tubes. These test tubes were occasionally stirred for 24 hours at room temperature. After 24 hours each sample was filtered and filtrate was suitably diluted and analyzed for the drug content using UVVisible spectrophotometer.

Study of drug-polymer interaction (FTIR Spectroscopy)

It is a useful analytical technique used to check the interaction between drug and the polymers used in formulation. FTIR spectrum was recorded for ferrous ascorbate and folic acid powder, blank chitosan microspheres, physical mixture of blank chitosan microspheres and drug; and optimized formulation were obtained by Perkia-Elmer Fourier Transform Infrared spectrophotometer. Samples were prepared in KBR disk (2mg sample in 200mg KBr) with a hydrostatic press at a force of 40 psi for 4 min. The Scanning range was 400-4400cm⁻¹ of FTIR Spectrophotometer and the resolution was 4 cm⁻¹. The IR spectrum of samples was compared with that of physical mixture to check any possible drug-excipients interaction.

Preparation of ferrous ascorbate and folic acid loaded chitosan microspheres:

FAS and FA loaded chitosan microspheres were prepared by chemical crosslinking method. 75 ml of light liquid paraffin and 75 ml of heavy liquid paraffin oil was placed in a 250 ml beaker. 1% w/w of span 80 was mixed with the oil with stirring and heated upto 800 C. To this, 10 ml of chitosan solution of different concentration (prepared by dissolving chitosan in 2% v/v glacial acetic acid) was added drop wise using a 22-gauge hypodermic syringe. This addition was accompanied by stirring of paraffin oil at different speeds (1000-4000 rpm) with the help of a high-speed stirrer with propellers (Remi Motors, India). Stirring was continued for 1h after the complete addition of chitosan solution into oil. After 1 hour stirring, 10 ml of gluteraldehyde solution saturated with 30 ml of toluene was added dropwise to the mixture with continuous stirring at 500 rpm for next 1 h at 50-550c. Stirring was stopped after 1 hour of the final addition of gluteraldehyde. A suspension of chitosan microspheres in paraffin oil was obtained which was allowed to stand for 1 hour to settlement the microspheres under gravity. Clear supernatant was decanted and microspheres were washed three times with diethyl ether. After the final wash, microspheres were allowed to dry in desiccator at room temperature.

Table 2: Formulation of microspheres of Ferrous ascorbate and Folic acid

S.no.	Formulation Code	Folic Acid (mg)	Ferrous Ascorbate (mg)	Chitosan (%w/v)	Rotation Speed (rpm)
1	F1	2	500	2	1000
2	F2	2	500	2	1000
3	F3	2	500	3	2000
4	F4	2	500	4	2000
5	F5	2	500	6	2000
6	F6	2	500	6	2000
7	F7	2	500	8	4000
8	F8	2	500	8	4000
9	F9	2	500	10	4000

PREPARATION OF REAGENTS

The reagents and solutions were prepared as follows.

1) Solution of ammonium sulfate and 0.1M cerium

65g of ammonium sulfate and cerium was dissolved and mixed with 30mL of concentrated sulfuric acid and

500mL of water. The mixture was cooled and a further 1000mL of water was added. Then, 25mL of ammonium sulfate and cerium was added to 2g of potassium iodide and 150mL of water. This was titrated immediately with 0.1M sodium thiosulfate, using 1mL of starch solution as an indicator.

2) Solution of ammonium sulfate and 0.01M cerium

50mL of ammonium sulfate and 0.1M cerium was diluted with 500mL water. Starch solution: 1.0g of soluble starch was ground with 5mL of water and poured, stirring constantly, into 100mL of boiling water, to which 10mg of mercuric iodide was added.

3) Gastric Fluid (pH 1.4)

90mL of concentrated hydrochloric acid and 84mL of 10M sodium hydroxide were transferred to a 10L container. This mixture was stirred, and approximately 9L of water was added until the pH reached 1.50–0.05. The solution was then made up to 10L with water.

4) Intestinal Fluid (pH 4.5)

8.7g of mono- potassium phosphate was added to a 10L container. Water was added to the mixture, which was stirred and diluted to 1L. 38mL of 10M sodium hydroxide and 30mL of concentrated hydrochloric acid were then added. The solution was stirred and adjusted until the pH was 4.50–0.05.

5) Intestinal juice (pH 6.8)

The same procedure was used as described in preparation of the intestinal juice pH 4.5, except the pH was adjusted to 6.90–0.05.

CHARACTERIZATION OF MICROSPHERES OF FERROUS ASCORBATE AND FOLIC ACID

1) Density and flow measurement

Accurately weighed 10g of the drug was loaded into a cylinder and placed on a bulk densitometer. The bulk and the tap density of the drug were determined. Hausner's ratio and %Compressibility were also calculated.

Hausner's ratio = Tap density/Bulk density

Carr's index (or) % compressibility = (Tap density- Bulk density) x100/Tap density

The angle of repose was determined by keeping mouth of funnel at 5 cm above the surface of ground, which is indicative of the flow properties of the drug.

2) Percentage yield

The percentage yield of the microspheres was determined for drug and was calculated using the following equation.

% Yield = (M/M₀) X 100

Where M = weight of microspheres

M₀ = total weight of drug and polymer.

3) Determination of Mean Particle Size

Particle size analysis of unloaded and drug- loaded chitosan microspheres was performed by optical microscopy using a compound electron microscope. A small amount of dry microspheres was suspended in glycerin. The suspension was sonicated for 5 seconds. A small drop of suspension, thus obtained, was placed on a clean glass mounted on the stage of the microscope and diameter of at least 500 particles was measured using a calibrated ocular micrometer.

4) Drug entrapment efficiency

The amount of drug entrapped was estimated by digesting microspheres in 50 ml of distilled water. The suspension was warmed for few min, filtered with 0.2µm membrane filter (MDI, India) and an aliquot of the filtrate was diluted appropriately with respective solvent system and the absorbance is measured by spectrophotometer against appropriate blank. The amount of drug entrapped in the microspheres was calculated according to the standard regression. And the % age of entrapment efficiency was calculated using the following formula.

% Entrapment

$$\text{Efficiency} = \frac{\text{Amount of drug entrapped}}{\text{Amount of drug used}} \times 100$$

5) Scanning electron microscopy (Morphological Study of Microspheres)

The shape and surface morphology of the microspheres was investigated using scanning electron microscopy. The sample for the scanning electron microscopy (SEM) analysis was prepared by sprinkling the microspheres one side of double adhesive stub. The stub was then coated with gold using Jeol JFC 1100 sputter coater. The SEM analysis of the microspheres was carried out by using Jeol JSM 5300, Japan. The microspheres were viewed at an accelerating voltage of 15 kV.

6) Study of thermal behaviour of drug and microspheres

The thermal behaviour of Ferrous ascorbate and Folic acid, blank chitosan microspheres, physical mixture and drug loaded chitosan microspheres was examined with DSC 7 (Perkin-Elmer) Thermal analyzer. Argon was used as a carrier gas and the DSC analysis was carried out at a heating rate of 10°C/min and an argon flow rate of 35cc/min. The sample size was 5mg and curves were recorded at a temperature range of 60–300°C.

7) Study of crystallinity of the drug using powder X-ray diffraction analysis (PXRD)

PXRD was carried out to investigate the effect of microencapsulation process on crystallinity of the drug. PXRD patterns were recorded on a RIGAKU, Rotaflex, RV 200 (Rigaku Corporation, Japan) powder XRD using Ni-filtered, CuK-α radiation, a voltage of 60kV and a current of 50 mA. The scanning rate was 10/min over

the 100 to 400 diffraction angle (2θ) range. The XRD patterns of Ferrous ascorbate and folic acid crystals, blank chitosan microspheres and drug loaded microspheres were recorded. Microspheres were triturated to get fine powder before taking the scan.

8) Comparative study of release kinetics of the formulation batch

In order to determine the release mechanism of drug from the prepared microspheres of the formulations, the results of the dissolution study were examined in accordance to the kinetic models. The mechanism of release was determined by fitting the release data to the various kinetic equations such as zero order, first order, Higuchi and Korsmeyer-Peppas and finding the R² value of the release profile corresponding to each model.

a) Zero Order Kinetics

The zero order rate describes the systems where the drug release rate is independent of its concentration. A zero-order release would be predicted by the following equation;

$$A_t = A_0 - K_0t$$

where A_t is the amount of drug released in time t ,
 A_0 is the initial concentration of drug (most times, $A_0=0$) and,
 K_0 is the zero order release constant expressed in units of concentration/time.

To study the release kinetics, data obtained from *in vitro* drug release studies were plotted as cumulative amount of drug released versus time, if the plot is linear then the data obeys zero-order release kinetics, with a slope equal to K_0 .

b) First Order Kinetics

The first order describes the release from system where release rate is concentration dependent. A first-order release would be predicted by the following equation

$$\log C = \log C_0 - Kt/2.303$$

Where C is the amount of drug released in time t ,
 C_0 is the initial concentration of drug and;
 K is the first order rate constant.

When the data is plotted as log cumulative percent drug remaining versus time yields a straight line, indicating that the release follows first-order kinetics. The constant ' K ' can be obtained by multiplying 2.303 with slope values. (87,88)

c) Higuchi's Model

The first example of a mathematical model aimed to describe drug release from a matrix system was proposed by Higuchi in 1961. Initially conceived for planar systems, it was then extended to different geometries and porous systems.

This model is based on the hypothesis that:

- (i) Initial drug concentration in the matrix is much higher than drug solubility;
- (ii) Drug diffusion takes place only in one dimension (edge effect must be negligible);
- (iii) Drug particles are much smaller than system thickness;
- (iv) Matrix swelling and dissolution are negligible;
- (v) Drug diffusivity is constant; and
- (vi) Perfect sink conditions are always attained in the release environment.

Higuchi was the first to derive an equation to describe the release of a drug from an insoluble matrix as the square root of a time-dependent process based on Fickian diffusion. Simplified Higuchi equation is following;

$$Q_t = KH(t)^{0.5}$$

Where, Q_t is the amount of drug released in time t and;
 KH is the release rate constant for the Higuchi model.

When the data is plotted as cumulative drug released versus square root of time, yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to ' KH '.

d) Korsmeyer and Peppas Model

The release rates from controlled release polymeric matrices can be described by the equation proposed by Korsmeyer et al.

$$Q = Kt^n$$

Where, Q is the percentage of drug released at time ' t ', K is a kinetic constant incorporating structural and geometric characteristics of the tablets and ' n ' is the diffusional exponent indicative of the release mechanism. For Fickian release, $n=0.45$ while for anomalous (Non-Fickian) transport, n ranges between 0.45 and 0.89 and for zero order release, $n = 0.89$.

To study the release kinetics, data obtained from *in vitro* drug release studies were plotted as log cumulative percentage drug release versus log time.

RESULTS AND DISCUSSION

Preformulation Studies

The main objective of a preformulation study is to characterize the chemical and physical properties of drug substance.

FT-IR, UV-Visible spectrophotometry, melting point and solubility were used for identification of chemical and physical properties of Ferrous ascorbate and folic acid. The first requirement of any preformulation study is the development of a simple analytical method for quantitative estimation in subsequent steps.

Melting Point

Melting point determination is one of the analytical techniques applied to check the purity of pharmaceutical drugs. Capillary tube method widely used for

the determination of the melting point. Melting point was found to be 1900 C and 2500 C for Ferrous Ascorbate and Folic Acid respectively. Theoretical melting point and practical melting point was found to be same. So we can say that drug was in pure form.

Solubility

The solubility of drug is an important physicochemical property because it affects the bioavailability of the drug, the rate of drug release into dissolution medium and consequently, the therapeutic efficiency of the pharmaceutical product. Solubility of the Ferrous Ascorbate was determined in various solvents at room temperature. The solubility of Ferrous Ascorbate was found to be 178.587 ± 1.935 mg/ml in water, $79.047 \pm$

0.128 mg/ml in 0.1 N HCl, and 77.127 ± 0.218 mg/ml in phosphate buffer (pH 6.8). Solubility of the ferrous ascorbate shows that it is freely soluble in water and soluble in 0.1 N HCl, soluble in phosphate buffer pH 6.8. Drug is found to be freely soluble in water and so mixture of both these solvents was used for formulation of the microspheres as no single solvents can dissolve both drug and polymers.

FTIR studies

FTIR spectrum of Ferrous Ascorbate

The infrared (FT-IR) spectra were obtained using a perkinelmer FT-IR spectrometer at resolution from 4000 to 400 cm^{-1} .

Table 3: Infrared spectral assignment of Ferrous Ascorbate

Sr.No.	Frequency(cm^{-1})	Groups
1	3750,3629,3571	O-H Stretching
2	3282	O-H Stretching (Chelate Compounds)
3	2361,2341	C-H Stretching
4	1710	C=O Stretching(α - β unsaturated five membered ring)
5	1312	C=C Stretching
6	1022	C-O Stretching
7	849	C-H Deformation
8	821	C-O Stretching

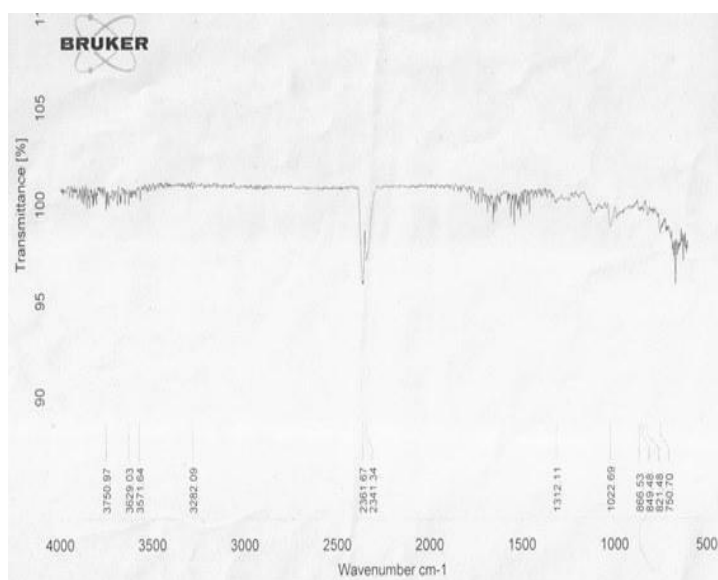


Fig.1 FTIR spectrum of Ferrous Ascorbate

FTIR spectrum of Folic Acid

The infrared (FT-IR) spectra were obtained using a perkinelmer FT-IR spectrometer at resolution from 4000 to 400 cm^{-1} .

Table 4:- Infrared spectral assignment of Folic Acid

Sr.No.	Frequency(cm^{-1})	Groups
1	3500	N-H Stretching
2	~ 3400	N-H Stretching (Amines)
3	2365,2355	C-H Stretching
4	1725,1700	C=O Stretching(α - β unsaturated six

		membered ring)
5	1604	C=O Stretching
6	1486	C=C Stretching (in Aromatics)
7	1418	C-C Stretching (due to Aromatic ring)
8	817	C-H Deformation out of plane
9	764	C-C Bending

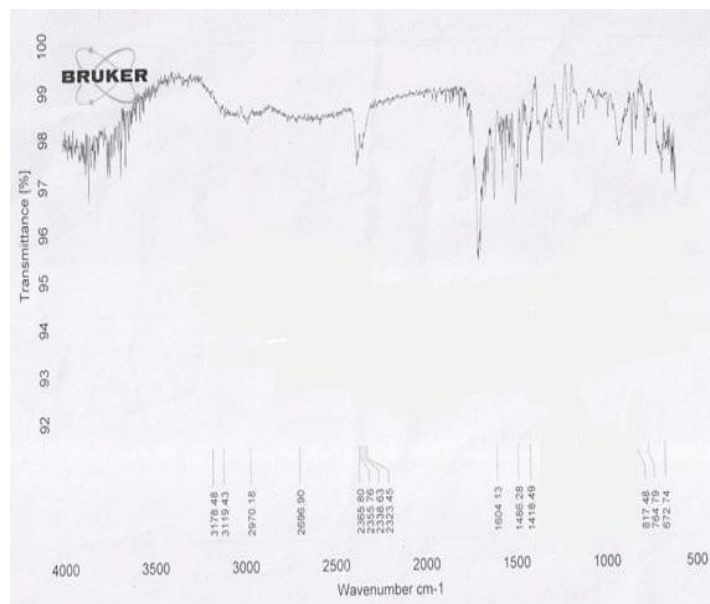


Fig: 2 FTIR Spectrum of Folic Acid

FTIR spectrum of plane chitosan microspheres

The infrared (FT-IR) spectra were obtained using aperkinelmer FT-IR spectrometer at resolution from 4000 to 400 cm⁻¹.

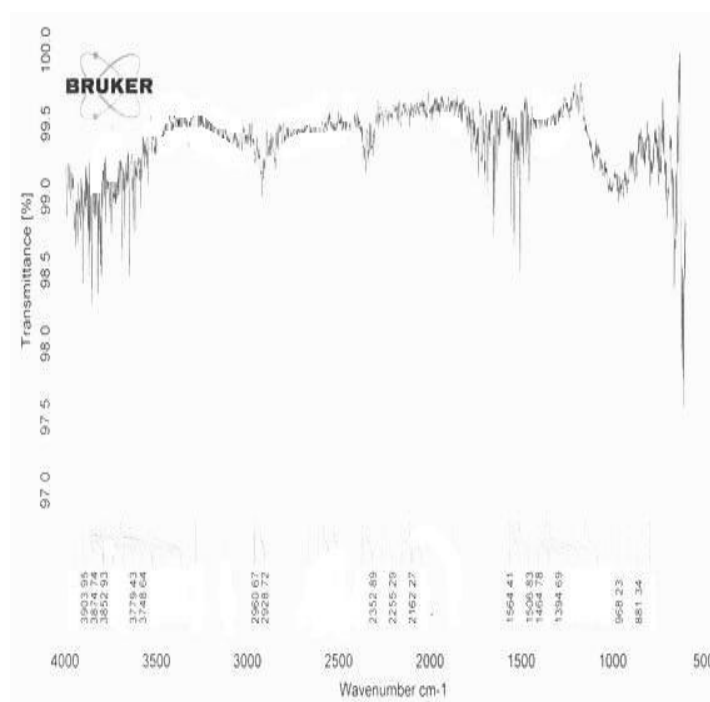


Fig.3 FTIR Spectrum of Chitosan Microsphere

FTIR spectrum of Ferrous ascorbate and Folic acid loaded Chitosan microspheres

The infrared (FT-IR) spectra were obtained using aperkinelmer FT-IR spectrometer at resolution from 4000 to 400 cm⁻¹.

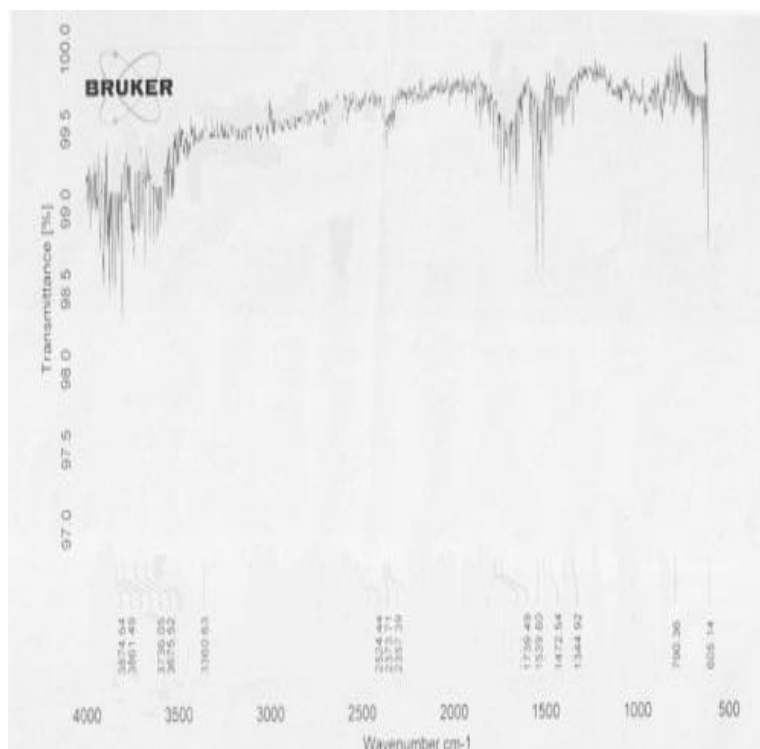


Fig.4 FTIR Spectrum of formulation

Table 5: Infrared spectral assignment of Formulation

Sr. No.	Frequency(cm^{-1})	Groups	Peak due to
1	3667	Free O-H Stretching	Ferrous Ascorbate
2	3360	N-H Stretching (Amines)	Folic Acid
3	2524, 2373	C-H Stretching	Ferrous Ascorbate
4	1739	C=O Stretching(α - β unsaturated five membered ring)	Folic Acid
5	1539, 1472	C=C Stretching(due to aromatic Ring)	Both
6	1344	C-N Stretching	Folic Acid
7	790	C-O Stretching	Both

The above peaks are due to both Ferrous ascorbate and folic acid which shows that there is no any chemical reaction occur between the two drugs, they are compatible with each other. Both the drugs do not affect the stability of each other. So the method of preparation and the combination of drugs is reliable and accurate and satisfactory.

In conclusion, the FT-IR spectrum, and melting point results suggested that Ferrous ascorbate and Folic acid

were pure and good in quality and the estimation procedure was found to be quite reliable, accurate and suitable for formulation development.

DSC (Differential Scanning Calorimeter)

The glass transition temperature of microspheres was found to be 84.94°C because the material was amorphous which became crystalline at 242.4°C showing crystallization temperature.

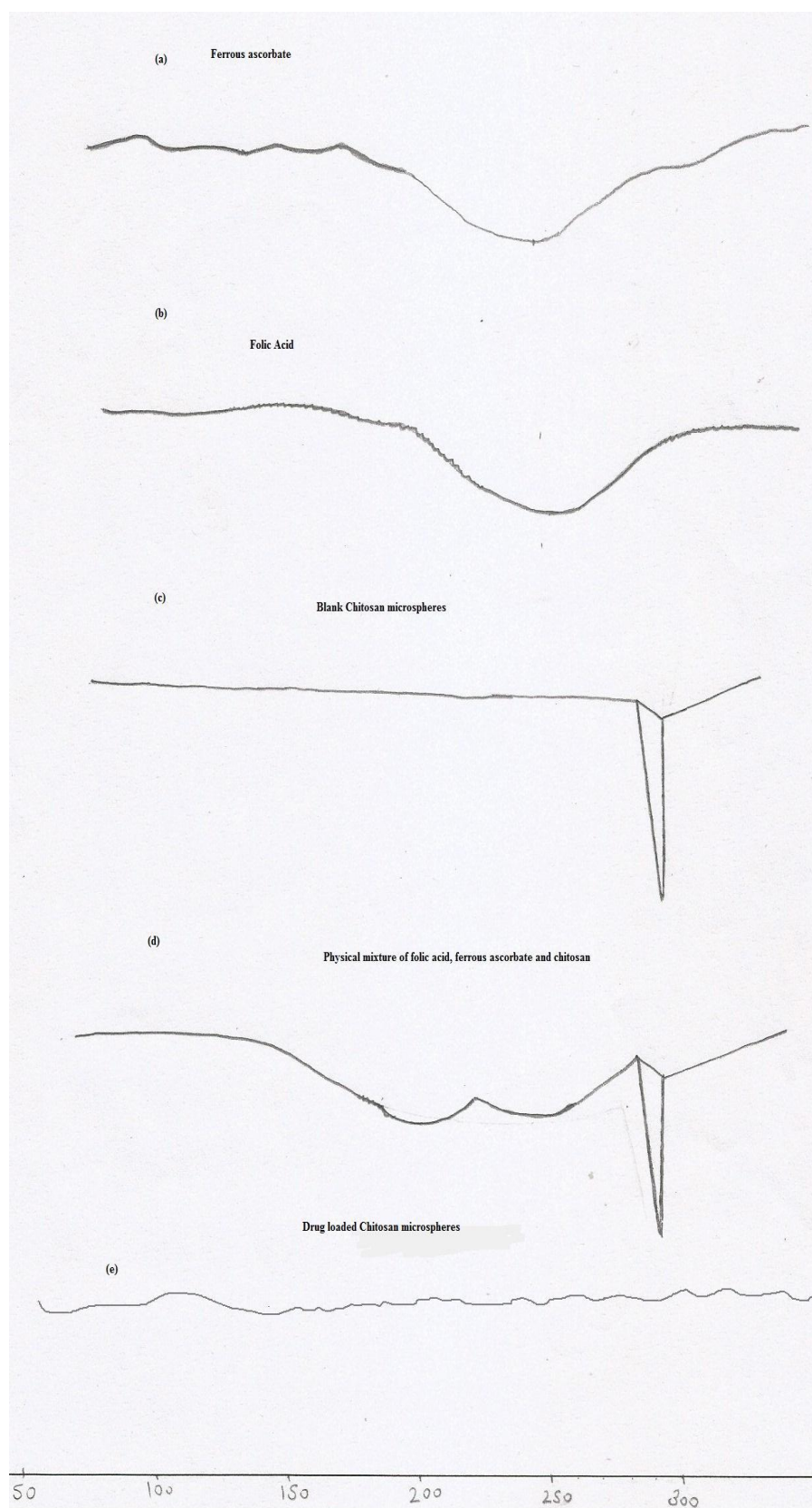


Fig.6 DSC spectra of various formulations

PXRD(Powder X-Ray Diffractometry)

The PXRD pattern of ferrous ascorbate showed no sharp peaks at 2θ scattered angles 0° to 80° , indicating the

amorphous state of ferrous ascorbate. The total crystallinity was found to be 1.904, which indicated that material was amorphous.

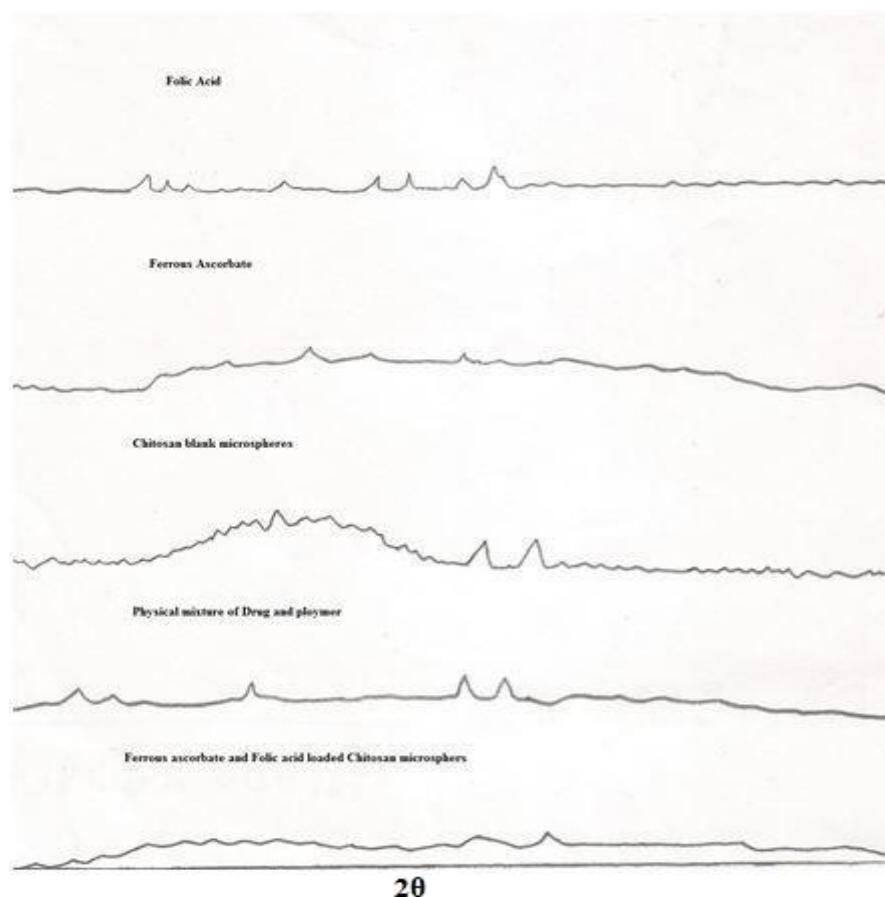


Fig. 7: PXRD of Ferrous Ascorbate

Preparation and optimization of microspheres

Sustained release formulation of Ferrous ascorbate and folic acid was prepared by using the w/o emulsion technique. Chitosan was used as the matrix forming polymer, light liquid paraffin and heavy liquid paraffin as the solvent and organic phase. Light liquid paraffin was used as it yields discrete and spherical microspheres. For optimization of microspheres, different formulations (F1-F9) were prepared using the various quantities of chitosan, rotation speed. Formulation with maximum entrapment efficiency and optimum floating time considered as optimized formulation. For optimization, trial batches were formulated by keeping the drug amount constant and varying chitosan concentration. Formulation with 3% chitosan concentration i.e. F3 was found to possess good entrapment efficiency, % yield and more controlled release. Further batches were prepared by varying the parameter of rotation speed. For this purpose rotation speed was varied in between the range 1000-4000. At 2000 rpm the microspheres were

found to possess good entrapment efficiency, % yield and good release rate. Microspheres formulated with chitosan in 3% concentration, 2000 rpm have maximum controlled drug release, maximum floating time, good entrapment efficiency and % yield. Chitosan is a matrix forming polymer, so the microsphere yield increased with increase in chitosan concentration. Increase in rotation speed increases the rate of evaporation. So, the organic phase gets more evaporated, when the rotation speed was increased. This results in decrease in particle size which further leads to decrease in drug entrapment efficiency.

Drug Entrapment

Increase in rotation speed leads to decrease in particle size, which directly leads to decrease in entrapment efficiency. Drug entrapment increased with increase in chitosan concentration entrapped. Effect of polymer and rotation speed on drug entrapment shown in table.

Table 6: %age Drug Entrapment

Formulation Code	% Yield	%Drug Entrapment
F1	9.98	6.5
F2	18.18	38.91
F3	73.72	94.75
F4	54.45	76.10
F5	57.68	66.68

F6	54.60	87.7
F7	64.55	71.42
F8	74.56	80.95
F9	54.90	78.64

Scanning Electron Microscopy

The microspheres were spherical and having a smooth surface. SEM of microspheres taken before dispersion in 0.1 N HCl showing no pores on the surface but SEM

taken after dispersion in 0.1 N HCl because of solubilization of microspheres when they are dispersed in acidic media.

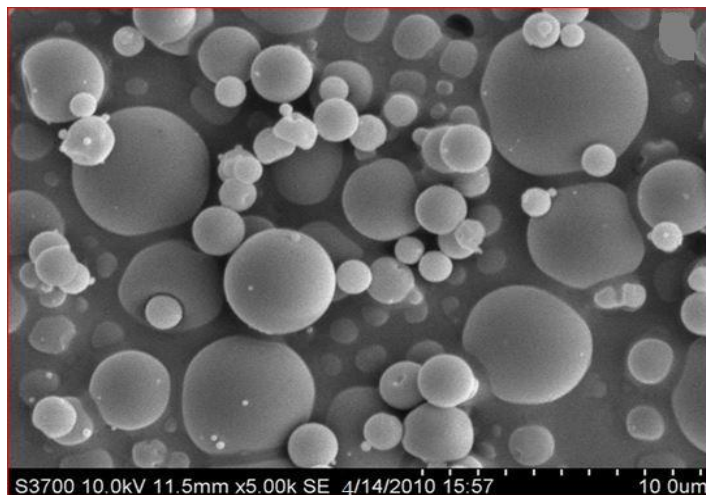


Fig 8:- Scanning Electron Microscopy of microspheres

In vitro Release Study

□□ *In vitro* drug release of iron in simulated gastric fluid pH 1.4

The formulation optimized using 3% chitosan and 2000 rpm shows good release. In simulated gastric fluid of pH 1.4 release was noted at interval of 1hr. Release was found to be 4.66% and 8.45% at 30 min. and 1hr

respectively. The release was 14.75% and 20.82% at 2hr and 3hr respectively. Release was greater as it was formulated with more rotation speed this results in decreased particle size and quick drug release. The release was then checked in simulated intestinal fluid. Because the drug remain in stomach maximum for 3 hours.

Table 7: *In vitro* drug release of iron in simulated gastric fluid pH 1.4:

Time(Hr.)	%age Release
0.5	5.78
1	10.49
2	15.77
3	17.32

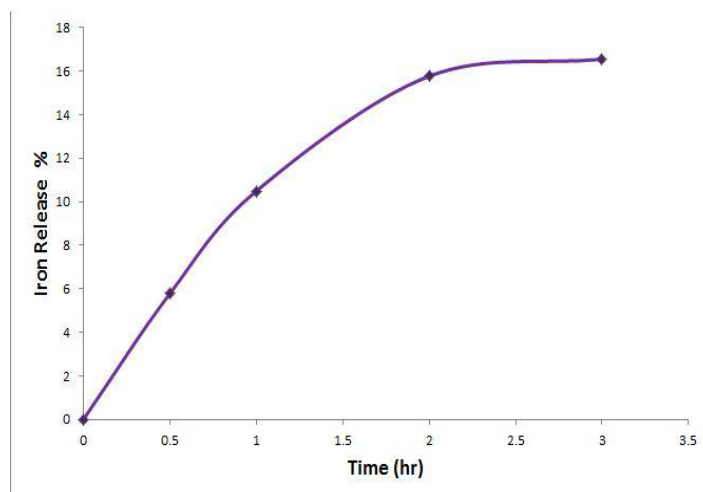


Fig: 9 *In-vitro* release of iron in simulated gastric fluid

□□ *In vitro* drug release of iron in simulated intestinal fluid pH 6.8

The formulation optimized using 3% chitosan and 2000 rpm shows good release. In simulated intestinal fluid of pH 6.8 release was noted at interval of 2hrs. Release was found to be 15.36% and 32.68% at 2hr. and 4 hr respectively. The release was 56.29% and 78.37% at 6hr and 8 hr respectively. And the release was about 96% after 24 hours of dissolution study. Release was greater as it was formulated with more rotation speed this results in decreased particle size and quick drug release. Therelease was then checked in simulated intestinal fluid. The maximum part of iron absorbed in the intestine.^[16-20]

Table 8: *In vitro* drug release of iron in simulated intestinal fluid pH 6.8

Time(Hr.)	%age Release
2	14.32
4	32.68
6	56.29
8	78.37
24	96.20

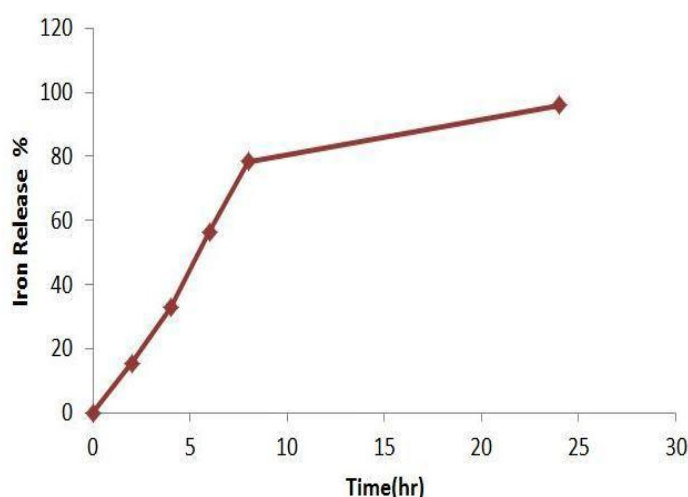


Fig: 10 In-vitro release of iron in simulated Intestinal pH.

CONCLUSION

The purpose of research was to design a sustained release dosage form of model drug to enhance patient compliance and to reduce the dose frequency. From this work it was concluded that the iron supplement can be given in combination with the ascorbic acid and folic acid.

The formulation have modified following properties:

- Due to sustained release iron release slowly, hence side effects are minimized.
- Absorption of iron & folic acid increased & hence their bioavailability is also enhanced.
- Ascorbic acid reduces ferric iron to ferrous iron which is soluble even at neutral pH.
- The iron will absorb slowly within 24 hours so toxicity is reduced.

Drug release retard due to rate controlling polymer. Drug release kinetic study was performed, based on r^2 value & it was concluded that the release of drug from the microspheres complies zero order kinetics i.e. the release is concentration independent. So, this is a controlled release formulation. From this we concluded that controlled release capsule for oral delivery will be an

alternate dosage form of model drug to enhance the patient compliance and clinical benefits.

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