

SOLID-STATE CHARACTERIZATION OF FERROUS ASCORBATE

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ABSTRACT

The study involved characterization of solid state form of ferrous ascorbate, a haematinic drug used in iron deficiency. Synthesis of ferrous ascorbate involved use of ferrous sulphate as precursor molecules. The solid state form was characterized by UV-spectrometry, Differential scanning calorimetry (DSC), Powder X-ray diffractometry (pXRD), Thermogravimetry analysis (TGA), Fourier transform infrared spectroscopy (FTIR), Nuclear magnetic resonance (NMR), Scanning electron microscopy (SEM). Elemental analysis was done by using Energy-dispersive X-ray spectroscopy (EDX). Stability, solubility of solid ferrous ascorbate and physical property related to flow behavior were also studied.

Keywords: Ferrous ascorbate, Amorphous, Solubility, Bulk density, Carr's Index.

INTRODUCTION

Most drugs are not taken as pure chemicals but are formulated into pharmaceutical dosage form such as compressed tablets, sustained released products, solutions and injections. Physicochemical property of a drug is an important factor which could interfere with its bioavailability and final dosage form. The solid form of a compound affects the solid state properties including particle size, density, flow, wettability, surface area, solubility and hygroscopicity. It also has impact on drug product manufacturability and clinical performance. Wettability, surface area, solubility can affect potential impact on drug product dissolution rate without changing equilibrium solubility^[1-2]. Hence preformulation is required so that stable and efficacious product can be made^[3].

Ferrous ascorbate, L-(+)-Ascorbic acid iron (II) salt shown in Fig.1. Ferrous sulphate used as starting material for preparation of ferrous ascorbate. It is indicated in the treatment of iron deficiency anemia. It is hygroscopic, fine dark violet powder, which is odorless and has no characteristic taste. Iron is most readily absorbed in the ferrous state. But most of the dietary iron is in the ferric form. Not more than a trace amount of iron is absorbed in the stomach. But the gastric secretion (HCl) dissolves the iron and permits it to form soluble complexes with ascorbic acid. Vitamin C and other substances aid its reduction into ferrous form. This is why the patients with partial gastrectomy usually have iron deficiency anemia. Most of the iron is absorbed in the upper part of the small intestine that is duodenum and upper part of the jejunum. The mucosal cell contains an intracellular iron carrier. Some iron is supplied to mitochondria by the carrier, but the remainder is partitioned between apoferritin in the mucosal cells and transferrin, which is the iron transporting polypeptide in the plasma. Apoferritin, which is also found in many other tissues combines with iron to form Ferritin. Ascorbic acid plays an important role in movement of plasma iron to storage depots in tissues. There is also evidence that ascorbic acid improves iron utilization, by its reducing action and it may have direct effect on erythropoiesis.^[4-9]

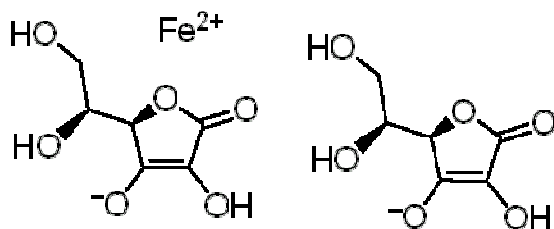


Fig. 1: Chemical structure of ferrous ascorbate

MATERIALS AND METHODS

Ferrous ascorbate was supplied by Bajaj Healthcare Ltd., Mumbai, India.

Characterization

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.

$$\text{Hausner's ratio} = \text{Tap density/Bulk density}$$

$$\text{Carr's index (or) \% compressibility} = (\text{Tap density-Bulk density}) \times 100/\text{Tap density}$$

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.

Ultraviolet spectrum

10 ppm solution of ferrous ascorbate in distilled water and 0.1 N HCl were scanned in the range of 200- 800nm on Shimadzu UV- 1650 PC UV-visible spectrometer and the λ_{max} of the drug was determined.

Fourier transmission Infrared (FT-IR) spectrum

An Infrared spectrum of ferrous ascorbate was recorded on Perkin Elmer-FTIR, Spectrum RXI spectrometer LM 500 model by conventional KBr (potassium bromide) pellet method. Pellet was prepared by mixing 1-4% sample with potassium bromide in a mortar and compressed at 15000 psi.

Nuclear magnetic resonance (NMR) Spectrum

¹H (NMR) was recorded on Jeol 60 MHz FT-NMR spectrometer using tetramethylsilane (TMS) as internal standard and dimethyl sulphoxide-D₆ (DMSO D₆) as a solvent.

Differential scanning calorimetry (DSC)

DSC thermograms were recorded using Perkin Elmer Pyris 6 DSC. Both temperature axis and cell constant were calibrated using indium. Four milligrams of the samples were weighed and analysed in pin holed aluminium pans. Ferrous ascorbate samples were exposed to heating rates of 10°C/min over a temperature range of 35°C-300°C under continuous nitrogen purging (20 ml/min).

Thermogravimetric analysis (TGA)

TGA was performed using SDT Q 600 V8, 2 Build 100. The loss of solvent/water was determined by heating the sample in aluminium crucibles at the rate of 10°C/min from 25°C-600°C under continuous nitrogen purge (20 ml/min).

Powder X-ray diffractometry (p XRD)

Powder X-ray diffraction patterns of ferrous ascorbate samples were recorded at room temperature using a Rigaku CuK α radiation Japan. Radiations generated from CuK source and filtered through Ni filters with a wavelength of 0.154 nm at 20mA and 35 kV were used to study the X-ray diffraction patterns. The instrument was operated over the 2θ range of 0° - 80° .

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) of ferrous ascorbate samples were recorded by using a Scanning electron microscope (JEOL JSM-680LA 5KV-Japan).

Elemental analysis

Elemental analysis of ferrous ascorbate samples were recorded by using Energy-dispersive X-ray spectroscopy (EDX). (JEOL JSM-680LA 5KV-Japan).

Solubility

Solubility of ferrous ascorbate was determined in various solvent like water, methanol, ethanol, isopropyl alcohol, chloroform and DMSO qualitatively.

Solid state stability

1g of ferrous ascorbate was kept in closed amber colure vial at 60°C for one week for determining heat stability. For moisture uptake, drug was kept in Petridis at 30°C /65% RH for one week. Drug was exposed to sunlight for 2 week at 30°C .

RESULTS AND DISCUSSION

Density and flow measurement

The bulk density, tap density, Carr's index and Hausner's ratio and flow behavior of solid-state forms of ferrous ascorbate are enlisted in Table 1. Hausner's ratio is related to the interparticle friction and

could be used to predict the flow properties of the powder. Powders with a low interparticle friction such as coarse spheres have ratio of approximately of 1.2 whereas more cohesive, less free flowing powders such as flakes have ratios greater than 1.6. Carr's index of 5-15 indicates free flowing granules while a ratio of 15-21 indicates a fair flow whereas a ratio above 21 indicates a bad flow. Poor flow and rat holing of ferrous ascorbate powder were observed due to amorphous nature small particle size and less density. The density and flow of ferrous ascorbate powder can be improved by adding glidant, granulation and compaction.

Table 1: Density and flow parameter of solid state form of ferrous ascorbate

Parameter	Observation
Bulk density	0.333 g/ml
Tap density	0.555 g/ml
Hausner's ratio	1.666
% Compressibility	40
Angle of repose	43.07°

Ultraviolet spectrum

Absorbance maxima were found in water at λ_{max} of 257nm and in 0.1 N HCl solutions at λ_{max} of 243nm. The λ_{max} of 257nm and 243nm indicates presence of the uncoordinated ascorbate anion in solution. The same band position was found in ascorbic acid. (fig.2)

FTIR spectroscopy

The major band of hydroxyl group (-OH) at 3378.4 cm^{-1} , keto group (C=O) at 1620 cm^{-1} and ether linkage C-O-C at 1114 cm^{-1} was observed in IR spectrum of ferrous ascorbate. IR spectrum of ferrous ascorbate resembles the ascorbic acid and sodium ascorbate spectra (fig.3). FTIR characteristics of ferrous ascorbate, ascorbic acid and sodium ascorbate are shown in Table 2.

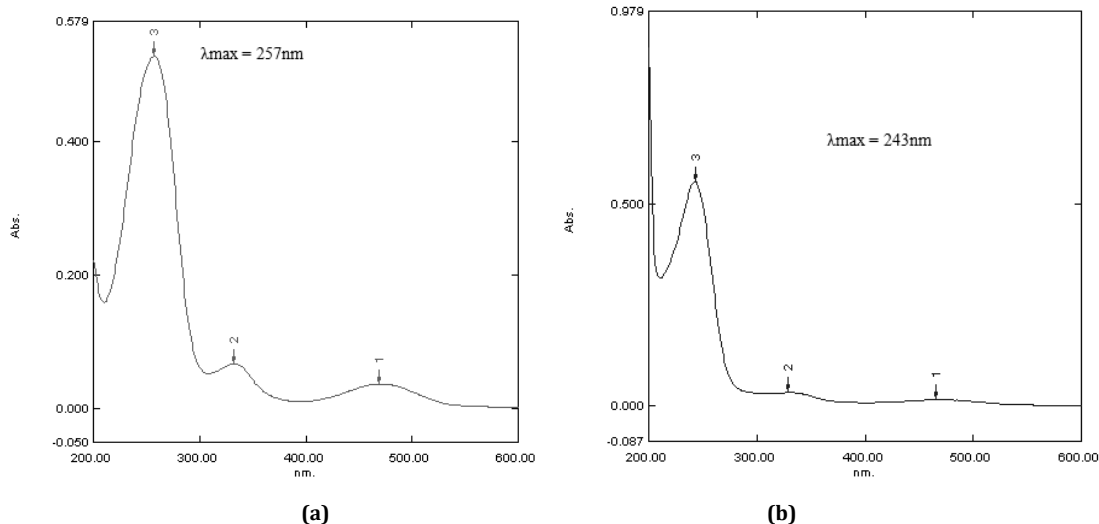


Fig. 2: (a) UV spectrum of ferrous ascorbate in distilled water. (b) UV spectrum of ferrous ascorbate in 0.1 N HCl

Table 2: FTIR Characteristics of Ferrous Ascorbate, Ascorbic acid and Sodium Ascorbate

Ferrous ascorbate Wave Nos. (cm^{-1})	Ascorbic acid Wave Nos. (cm^{-1})	Sodium ascorbate Wave Nos. (cm^{-1})	Assignment
3378.4	3528.3,3414.2,3310.4	3451.5	OH
1720, 1618	1747,1669.3	1710.06	C=O
1114,1061	1119.3,1025.9	1123.6,1070	C-O-C

Nuclear magnetic resonance (NMR) Spectrum

Interpretation of NMR spectrum of ferrous ascorbate is complicated due to overlapping of peaks (fig.4). There was upfield shift due to

ferrous in ferrous ascorbate as compared to NMR spectrum of ascorbic acid. The upfield shift was due to presence of unpaired electron in 3d orbital of Fe showing paramagnetic property which shielded the protons.

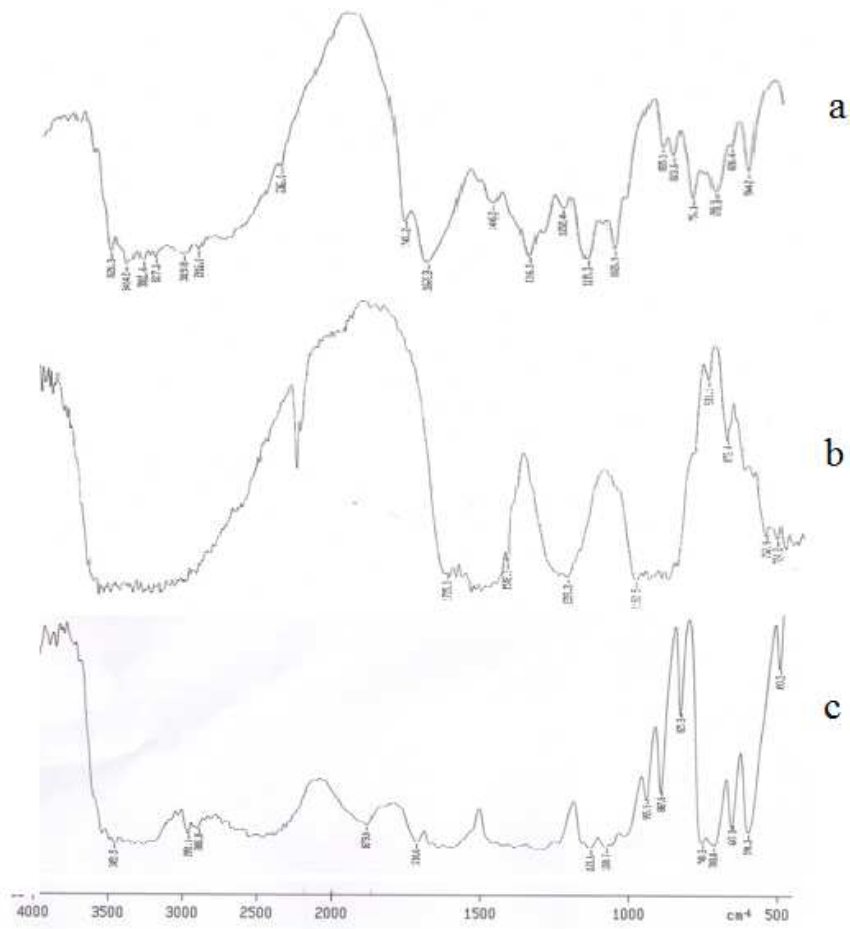


Fig. 3: FTIR spectrum of (a) Ascorbic acid (b) Ferrous ascorbate and (c) Sodium ascorbate

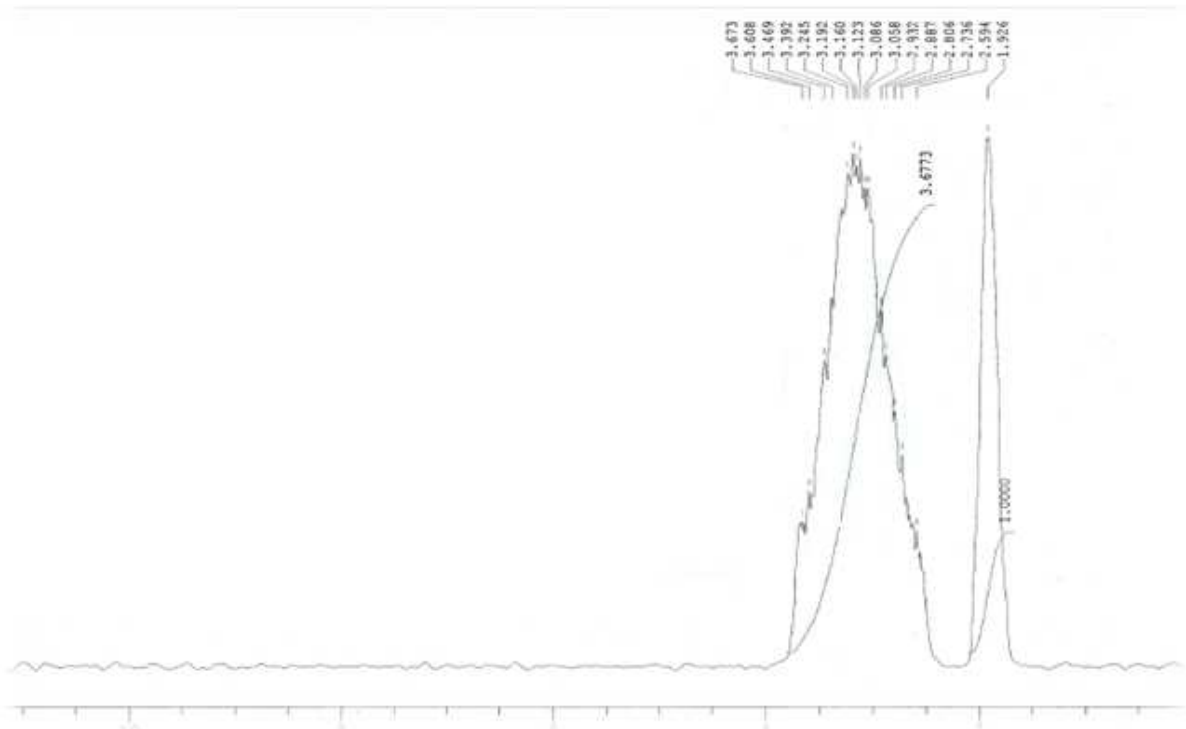


Fig. 4: NMR spectra of ferrous ascorbate

Differential scanning calorimeter (DSC)

The glass transition temperature of ferrous ascorbate was found to be 84.94°C because the material was amorphous which became crystalline at 242.4°C showing crystallization temperature. The DSC of ferrous ascorbate was compared with sodium ascorbate and calcium ascorbate; it was found that sodium ascorbate decomposed at 233.45°C. It might be due to oxidation of ascorbic acid into dehydroascorbic acid, whereas calcium ascorbate showed melting endothermic peak at 153°C and gets decomposed at 214.34°C. (See fig.5 and fig.6)

Thermogravimetric analysis (TGA)

As ferrous ascorbate was subjected to heat at 46.56°C, 99.18% of ferrous ascorbate was found to be stable. As temperature increased to 191.89°C, decrease in weight observed due to loss of moisture and solvent, which was used for synthesis of ferrous ascorbate. After that, degradation

started and it continued till 238.81°C and beyond that there was no significant loss in weight due to charred material. (See fig.7)

Powder X-ray diffractometry (p XRD)

The PXRD pattern of ferrous ascorbate showed no sharp peaks at 2θ scattered angles 0° to 80° (Fig.8), indicating the amorphous state of ferrous ascorbate. The total crystallinity was found to be 1.904, which indicated that material was amorphous.

Scanning electron microscopy

The SEM clearly indicated that particle size of ferrous ascorbate was very fine having size 14-73 μm . Shape of ferrous ascorbate was found to be spherical and the material was found to be porous in nature. The irregularities in particle size might be responsible for the poor flow properties. Due to porous nature and small size, density is very low, which was supported by density measurements.

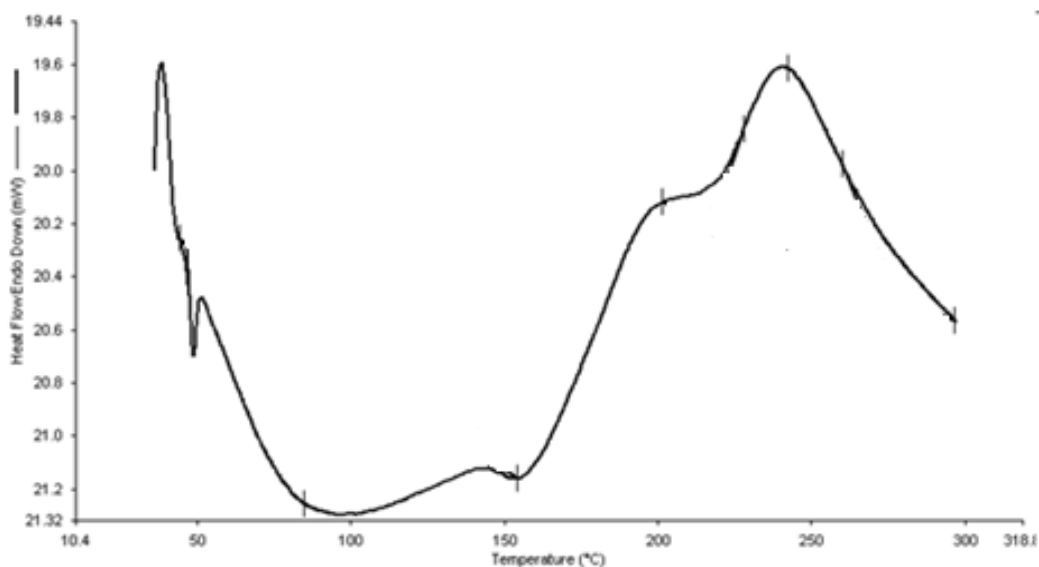


Fig. 5: DSC of ferrous ascorbate

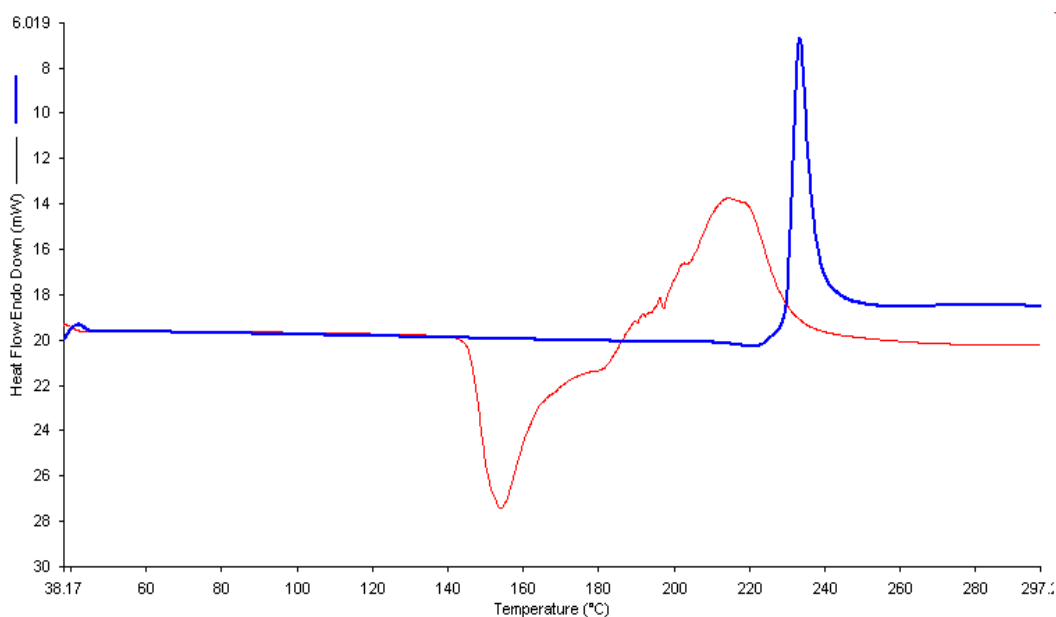


Fig. 6: DSC of (a) Red: Calcium ascorbate and (b) Blue: Sodium ascorbate

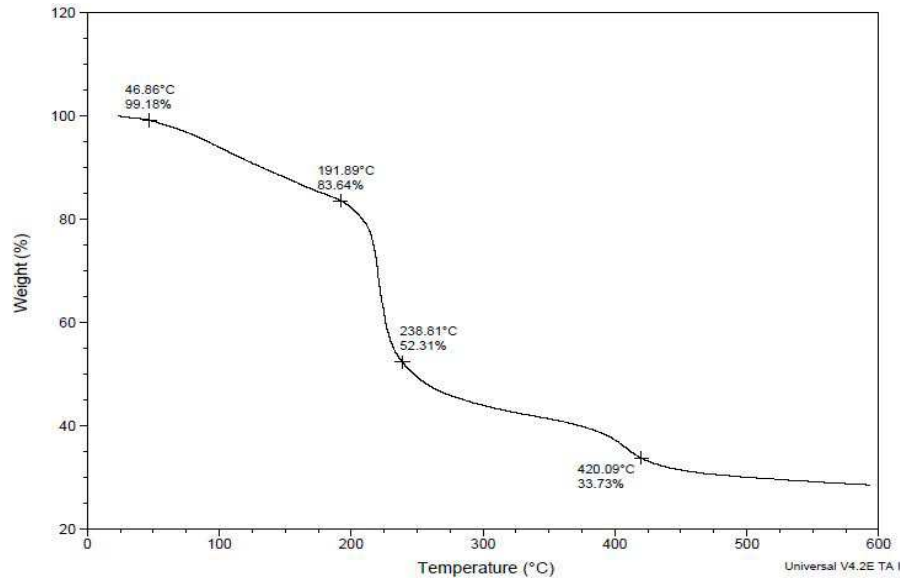


Fig. 7: TGA of Ferrous ascorbate

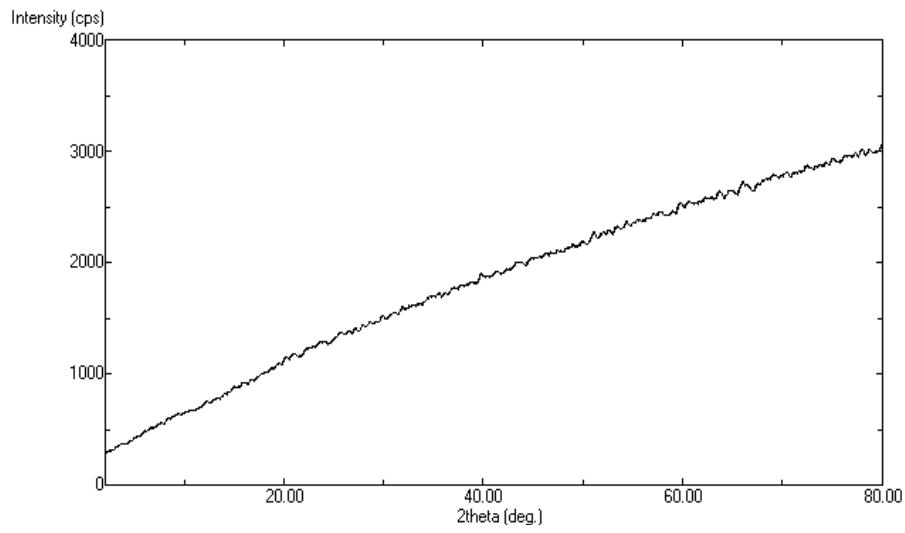
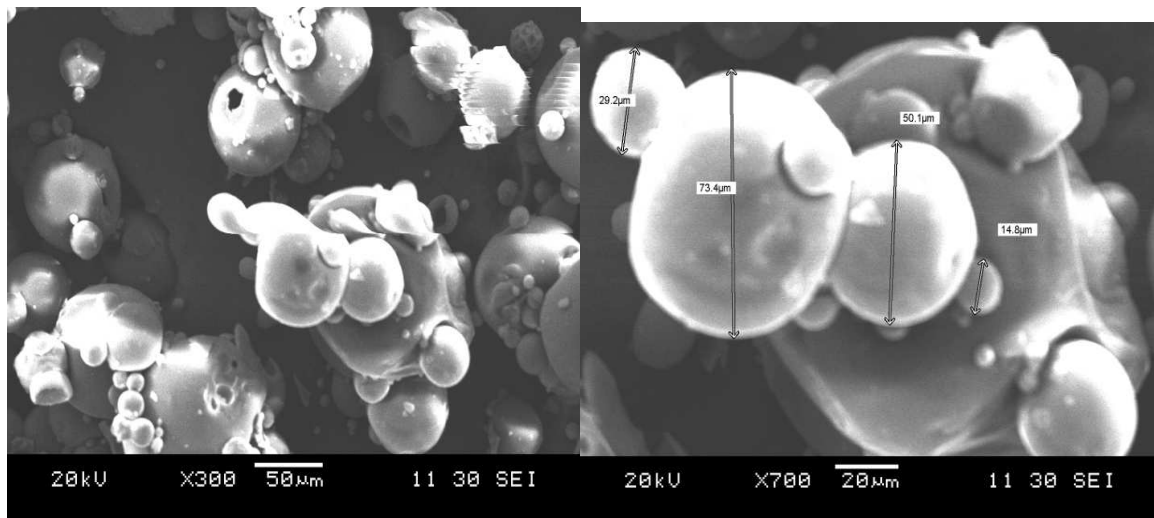


Fig. 8: pXRD of Ferrous ascorbate



(a)

(b)

Fig. 9: SEM of Ferrous ascorbate

Elemental analysis

Energy dispersive X-ray spectroscopy is an analytical technique used for elemental analysis or chemical characterization of a sample. Sample is determined through interaction between radio magnetic interaction and matter, analyzing x-ray emitted by the matter in response to being hit with charged particles. The fundamental

principle that each element has a unique atomic structure allowing X-rays, that is characteristic of an element's atomic structure to be identified uniquely from one another^[10].

EDX showed that sample contained 8.51mol % sulphur in the form of sulphur trioxide and 91.49 mol % of ferrous in the form of ferrous oxide.

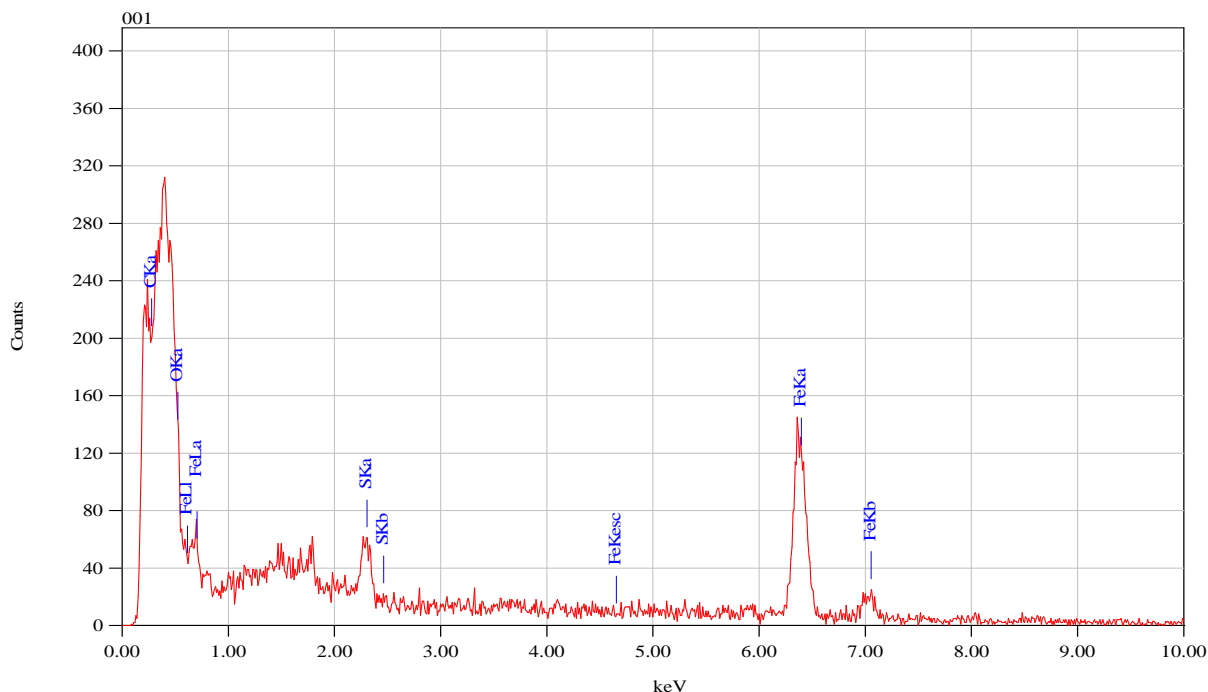


Fig. 10: EDX of Ferrous ascorbate

Solubility

Ferrous ascorbate is very soluble in water and soluble in DMSO. pH of 1% ferrous ascorbate in water was found to be 6.73. It is insoluble in organic solvent like methanol, ethanol, isopropyl alcohol, chloroform.

Solid state stability

Ferrous ascorbate was found to be heat stable when it was kept at 60°C for a week. Ferrous ascorbate took up moisture and form lumps after one week of exposure to 65% relative humidity at 30°C and after two weeks of exposure to sunlight, it turned to dark brown color, which indicates that the drug is sensitive to both moisture and light.

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