Synthesis and Characterization of Gum acacia Stabilized Zinc Oxide / Ferrous Oxide Nanoparticles loaded with Zonisamide as a Drug Delivery carrier for In-vitro Drug Release Testing

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***Abstract*: A novel Gum acacia (GA) stabilized– Magnetic Nanocomposite (MNC) was achieved by spontaneous synthesis of Gum acacia a natural polysaccharide used as a template for the fabrication of metal oxides.A layer of Gum acacia which exhibits stabilizing and size controlling property was coated on the surface of metal oxides such as Zinc Oxide modified Ferrous oxide nanoparticles.The resultant nanocatalyst shows a crystal growth formation andwell Characterized in terms of X-ray diffraction studies (XRD), Scanning electron microscopy (SEM), Ultra violet-visible–diffused reflectance spectroscopy (UV-VIS-DRS), Differential scanning Calorimetry (DSC), Thermogravimetric analysis (TGA), Differential thermal analysis (DTA) and Fourier transform infrared spectroscopy (FTIR). In addition In-vitro drug release activity of synthesized GA-MNC was tested using in-vitro dissolution testing models which shows good pharmacokinetic properties.**

***Keywords*: Nanoparticles, Gum acacia, Zinc oxide, Zerrous oxide, Zonisamide, Nanocomposite, In-vitro drug release.**

INTRODUCTION

Nanoparticles are hollow structures with cavities or pores that entrap small molecules. These cavities are exploited for capturing signaling molecules and functionalized for specific targeting towards the analyte of interest[1]. It has a characteristic dimension of 1-100nm. They are classified into various types based on the size, morphology, chemical and physical properties. Carbon, ceramic, metal, gold, silver, polymeric and lipid based nanoparticles are some of them[2]. Free nanoparticles are formed through either the breaking down of larger particles or by controlled assembly processes[13]. Plant based Nanoparticles are used in pharmaceuticals, biomedicine, therapeutics, sustainable energy and other health care aspects1[7]. They can be used in neuro sciencefor studying the interactions of nanoparticles with neurological systems[3]. It is widely used in multidisciplinary fields like nano biotechnology and nano toxicology[12].

Gum acacia (GA)is abranched, neutral or slightly acidic in nature. It is a polysaccharide which constitutes a mixture of calcium, magnesium and potassium salts[5]. The main chain constitutes 1→3-linked β -D-galactopyranosyl units.Two to five 1→3-linked β -D-galactopyranosyl units are joined to the central chain by 1→6-linkages which form a side chain[8]. A-L-arabinofuranosyl, α-L-rhamnopyranosyl, β-D glucuronopyranosyl and 4-O-methyl –β-D-glucuronopyranosyl units are also present both in main and side chain[1]. Gum acacia is acquired from the trunk and branches of Acacia senegal, and acacia seyal, because of its branched polysaccharide nature it exhibit unique physical, structural and chemical properties. The OH present in arabinose and rhamnose and the COOH in glucuronic acid performs key character in the synthesis of nanoparticles[2]. Gum Arabic gives very pale yellow to orange brown colour when dissolved in water with a pH of ~ 4.5.

Ferous oxide (Fe_3O_4) also known as *magnetite*, this can also acts as both an n-and p- type semiconductor[7], and has a lowest resistivity among other iron oxides due to its small band gap (0.1eV)[6]. Fe_3O_4 exhibits certain unique magnetic properties and due to its low cost synthesis and low toxicity it has wide applications.

Zinc Oxide (ZnO) is an in-organic material, which is of low cost and important industrial material, which is thoroughly investigated with good potential applications. ZnO can be successfully synthesized by certain techniques such as chemical vapour deposition, wet chemical method, sol-gel method thermal decomposition, pyrolysis, and various other physical and chemical methods[9,10]. Among these precipitation technique has been found to be simple, precise, and in economical range. Accordingly they can also be developed into a nano range material with various other structural, morphological growth, size and properties[4] by subjecting them to different experimental conditions such as concentration, variation in time, pH, temperature and pressure.

MATERIALS AND METHODS

Gum acacia is obtained from AVRA laboratories. Ferrous chloride tetra hydrate ($FeCl_2.4H_2O$), zinc acetate dihydrate [$Zn(CH_3COO)_2.2H_2O$], Sodium hydroxide pellets were all purchased from SD fine chemicals, India. All the solutions were prepared with De-ionised water which is acquired from Sigma Aldrich. Zonisamide (API) was bought from Sigma Aldrich.

SYNTHESIS OF GUM ACACIA –MAGNETIC NANOPARTICLES (GA-MNP)

Gum acacia magnetic nanoparticles (GAMNP) was prepared by co-precipitation method. In a typical experiment, different concentrations of homogenized Gum acacia (GA) (1.0%, 1.5%, and 2.0%) solutions were prepared. Uniformly mix the above solution with ($FeCl_2.4H_2O$) (1mmol)under stirring, accordingly add Zn $(CH_3COO)_2.2H_2O$ (1mmol) to the above GAMNP solution. The solution mixture was refluxed for 24hrs in oil bath. 0.1M NaOH is added drop wise to the above solution and the mixture was again refluxed in oil bath, allowed to cool at room temperature. The resulting solution was subjected to centrifugation, washed thrice with ethanol and distilled water, and dried in hot air oven at 80°C[11]. The final product was taken for further characterizations.

$$Zn(CH_3COO)_2.2H_2O + NaOH \rightarrow ZnO + Na(CH_3COO)_2 + 2H_2O$$

SYNTHESIS OF ZONISAMIDE-LOADED GA-MNC

Zonisamide an Active Pharmaceutical Ingredient (API) loaded GA-MNP was prepared by following the similar method. Briefly Weigh Zonisamide (API) and dissolve it in deionised water. Simultaneously, dissolve Gum acacia (1.5%) and add both the solutions. Continue the further process in a similar manner as that of GA-MNP to obtain zonisamide loaded GA-Magnetic nanocomposite. The final composite was taken and characterized for its morphological, structural, chemical, absorption and release properties.

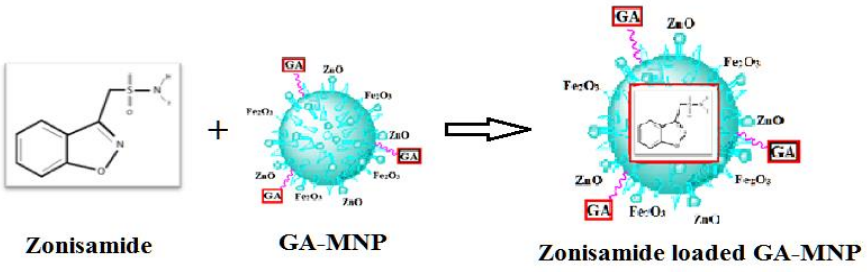


Figure 1 Synthesis of GA-MNP-ZNS Nanoparticles.

CHARACTERIZATION METHODS

X-ray diffraction [XRD] to determine the structural properties using a Rigaku diffractometer (Cu radiation, λ = 0.1546 nm) running at 40 kV and 40 mA [Tokyo, Japan]. The morphology and size assessment determined using Scanning electron microscopy [SEM] ("Field Emission Ion Quanta" 200 "Field Emission Gun" with "Energy Dispersive Spectroscopy"). The cover glass containing nanoparticles was mounted on SEM stub and coated with gold for SEM analysis.

UV-VIS-DRS (Ultraviolet-visible-diffused reflectance spectroscopy) were recorded on Perkin Elmer Lambda 750 spectrophotometer. Differential Scanning Calorimetry (DSC) was carried out using DSC-250 system, at an initial temperature of 25°C with a heating rate of 10°/min to "200.00" °C, containing sample weighing about 2-3mg by using nitrogen as a pure gas for measurement.

Thermo gravimetric analysis (TGA) coupled with blazer mass were carried out on a TGA (SDT Q 600) e systems using nitrogen as an inert gas atmosphere. The Fourier Transform-Infrared spectra (FT-IR) of synthesized nanoparticles were recorded using Thermo Nicolet Nexus 670 Spectrometer (Washington, USA) at a resolution of "4cm^{-1}" by using Deutirated Triglycine sulfate (DTGS) detector and Potassium bromide (Kbr) KBr as a beam splitter.The In-vitro Drug Release studies were carried out by using UV-VIS spectrophotometer [Model- Spectro-2080] with 10mm quartz cuvettes at a λ_{max} of 230nm.

RESULTS AND DISCUSSIONS

I. Structural characterization of composite Nanoparticles

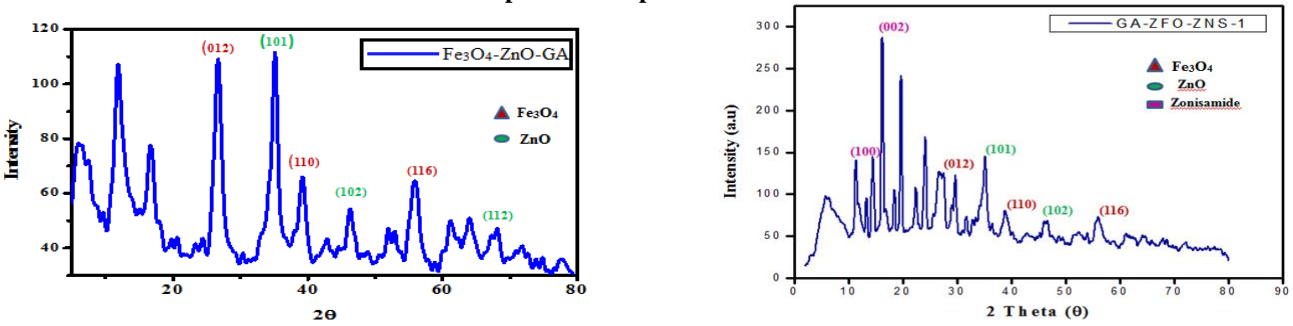


Figure 2 XRD pattern of (a) GA-ZnO- Fe_3O_4 composite Nanoparticles (b) GA-ZFO-ZNS

The composite Nanoparticles was prepared with 1% Gum acacia using oil bath method, to determine the crystallinity and phase of the synthesized product. The complete pattern shows the hexagonal phase with (101) orientation at 2θ= 36.4, along with the other small intensity diffractions at (012), (110), (102), (116), (112) with 2θ= 24.2, 39.9, 47.6, 57.4, 68.4. Figure 2a, shows the presence of ZnO and Fe_3O_4 with corresponding peaks at (101), (102), (112) and (110), (116), (012) assigned with pure hexagonal phase.
The peak intensities originated in figure 2b. (100), (002) indicates the polycrystalline nature of Zonisamide at 2θ= 12.6 and 18.2. The strong and narrow diffraction peaks the synthesized Nanoparticles has good crystallinity.Absence of other irregular peaks indicates no impurity is present in the compound.

II. Morphology of GA-ZnO-Fe_3O_4-Nanocomposite

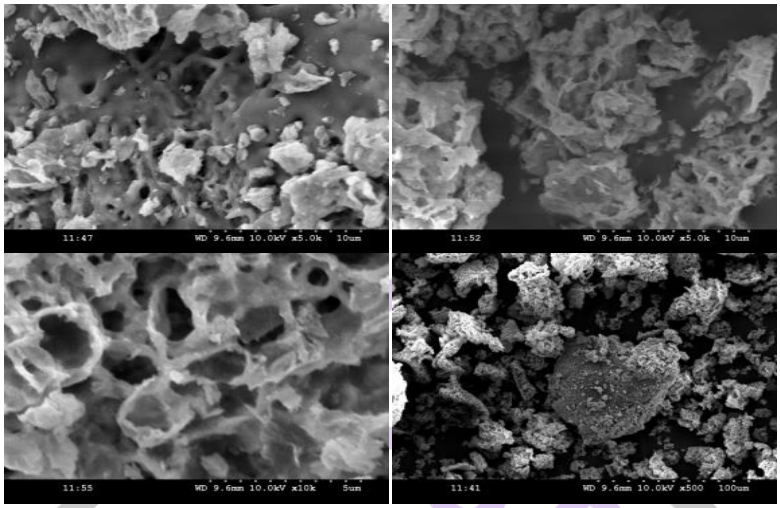


Figure 3 SEM images of GA-ZnO-Fe_3O_4-Nanocomposite.

The Magnetic Nanoparticles (MNP) synthesized using Gum acacia are large, non spherical with rough surface and closely arranged , though it can be said that a framework of network has been formed between zinc oxide, ferrous oxide and Gum acacia. The images indicate that the GA-MNP is in well aligned with each other and also agglomeration has been taken place. Some globular structures are also visible due to the presence of Zinc Oxide (ZnO).

III. Morphology of GA-ZnO-Fe_3O_4-Nanocomposite Loaded with Zonisamide (API)

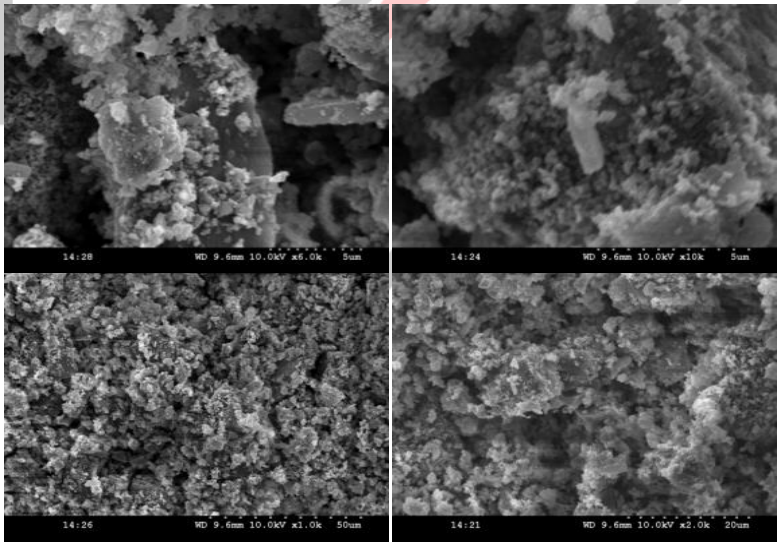


Figure 4 SEM images of GA-ZnO-Fe_3O_4-Nanocomposite Loaded with Zonisamide

The fig.4 shows the homogeneous, spherical shaped crystalline structures aligned with thick and continuous sphere shaped particles. Initially magnetic nanoparticles interact with functional groups of Gum acacia results in nucleation and crystal growth occurs. Then the magnetic Nanocomposite self assemble to form large spheres. Hence it can be said that the natural biopolymer such as Gum acacia can be used as a template for the fabrication of metal oxides as a drug delivery carriers.

IV. UV-VIS-DRS Spectral studies

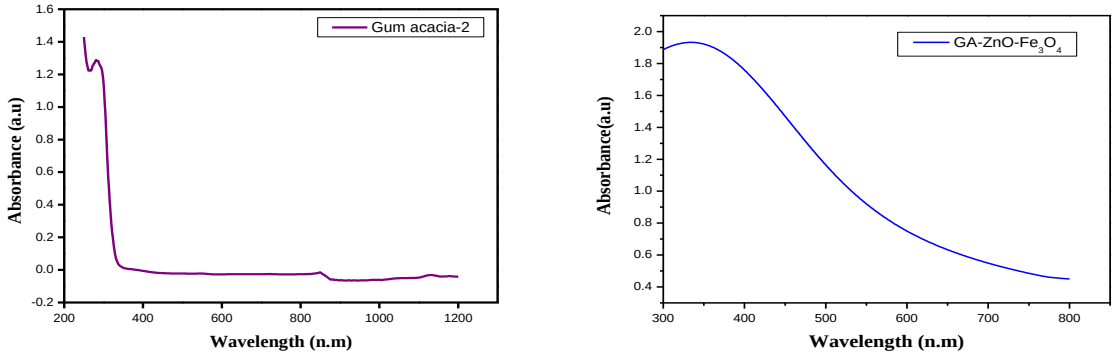


Figure 5 UV- VIS- DRS Spectra of (a) Gum acacia (b) GA-ZnO-Fe_30_4 Nanocomposite

The ultraviolet-visible emission of Gum acacia at 300 nm with a strong absorption at 1.29 a.u. This demonstrates that absorption coincides with the wavelength, as the concentration increases the absorption was found to be increased and exhibits good optical properties.Due to the intrinsic band gap electron transitions of ZnO [43, 44] a strong absorption can be observed at about 400nm. Subsequently Ferrous Oxide (Fe_30_4) lies between 250-350 nm as characteristic peak of absorption can be observed at about 320nm. Gum acacia shows the absorption peak at 300nm. Hence in the present study the observed absorption values are compared with the commercial values which have complied with each other this demonstrates that the GA-ZnO-Fe_3O_4 nanocomposite are synthesized purely and has good optical properties.

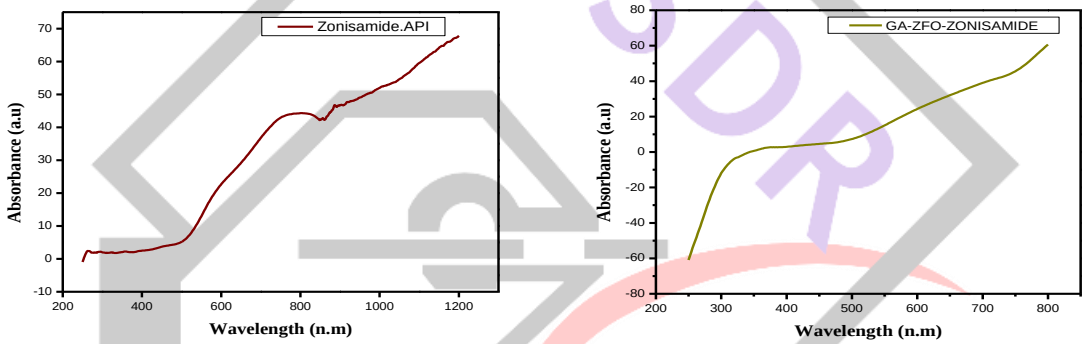
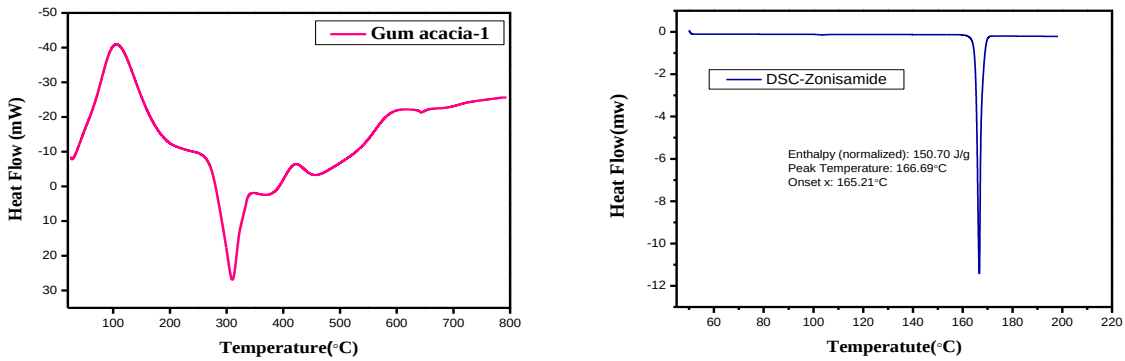


Figure 6 UV-VIS-DRS Spectra of (a.) Zonisamide API (b.) GA-ZFO-ZNS

Typically zonisamide shows an absorption peak at a wavelength of about 700-800nm. As displayed in the fig.6(a) a characteristic absorption peak was observed at 780nm respectively. Absorption peak was observedin fig.6(b) 300, 320, 400, and 700nm which are the characteristic absorption peaks of particular elements present in the composites, this indicates that all the metal oxides are present in the composite along with Gum acacia incorporated with zonisamide as a nanoparticles.

V. Differential Scanning Calorimetry [DSC]Analysis



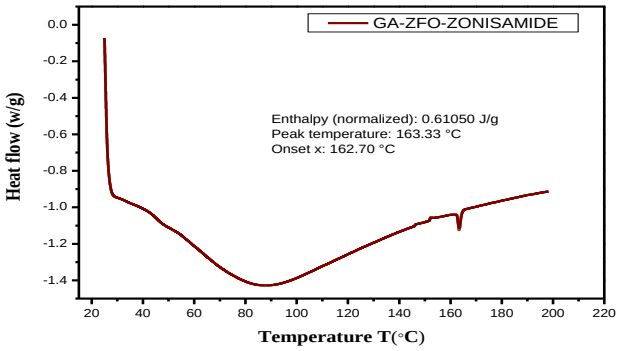


Figure 7 DSC thermogram of (a) Gum acacia (b) Zonisamide (C)GA-ZFO-ZNS

Endothermic peak at a temperature range from 250°C to 350°C due to loss of water content in Gum acacia and large energy flow. Exothermic peak from 100°C to 150°C decomposition of Gum acacialoss of water of crystallization indicating the uniformity of crystallization.

Fig.7(b). The thermogram of Zonisamide is characterized by a sharp endothermic peak appears at a temperature range of 166.69°C, which indicates the melting point [% weight loss] of the drug with a temperature onset at about "165.21"°C is recorded. The enthalpy is normalized at "150.79" j/g.

Fig.7(c).The sample under goes a phase transition at a temperature between 60°C to 150°C. It can be seen clearly that a broad peak is observed at "163.33"°C which may consider as a melting point of Zonisamide [API] which is present in the total nanocomposite at a peak temperature "162.70"°C. An enthalpy can be observed at a range of about "0.61050" j/g.

VI. Thermo Gravimetric Analysis [TGA]

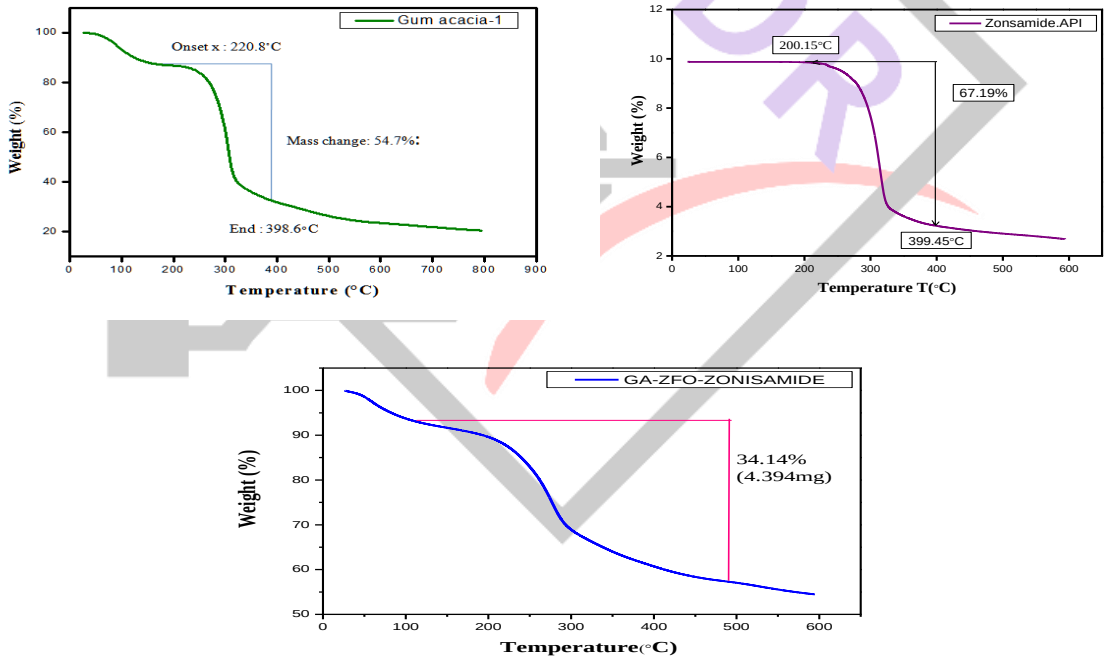


Figure 8 TGA thermogram of (a) Gum acacia (b) Zonisamide API (C) GA-ZFO-ZNS

The thermogram shows the thermal stability and thermal behaviour of Gum acacia. Heating of Gum acacia sample from 10 to 900°C, at 10°C per minute results in "54.7"% mass loss due to polysaccharide decomposition at an onset temperature of about 220.8°C is recorded.

Fig.8(b) Heating the sample at a controlled heating condition from 0°C to 600°C, at an interval of 10°C/min shows the 67.19% mass loss due to Decomposition of zonisamide at an onset temperature of 200.15°C and end point at 399.45°.

Fig.8(c) To determine the behaviour and thermal decomposition of sample. The thermogram results the onset decomposition temperature from "125.7" °C upto "487.4" °C with a percentage weight loss of "34.14" % which is approximately "4.394"mg.

VII. DTG of Study of Gum acacia

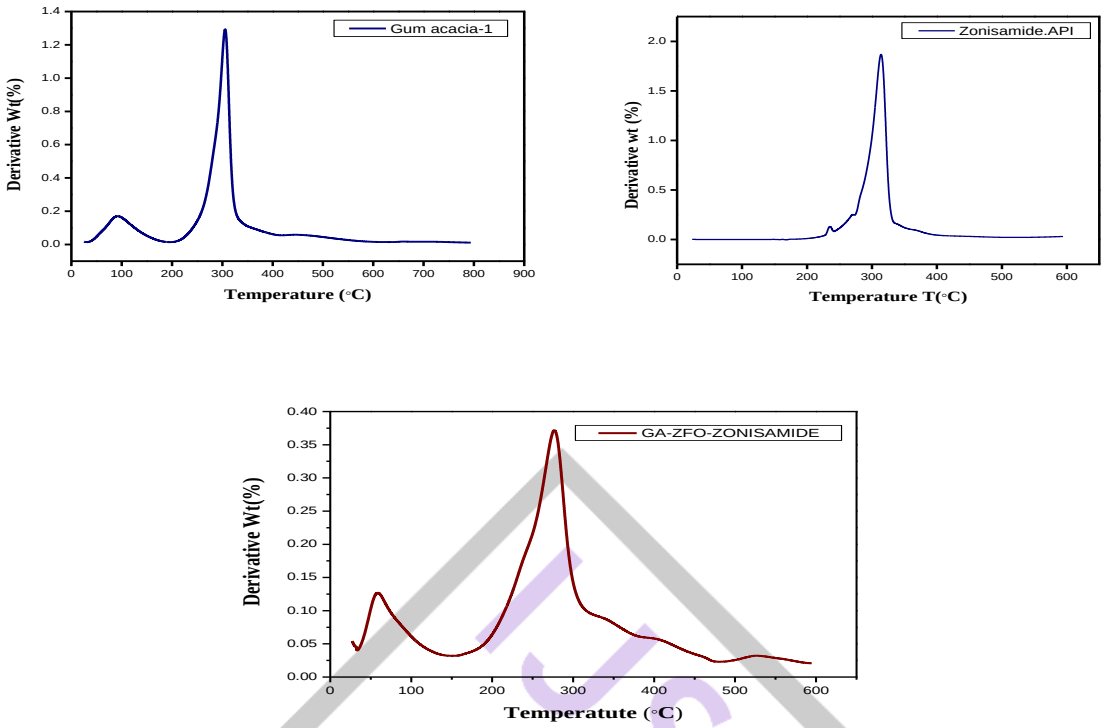
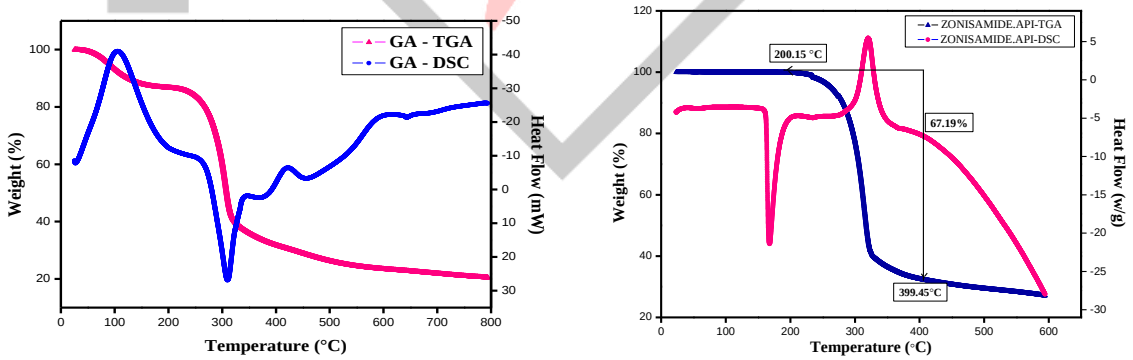


Figure 9 DTG thermogram of (a.) Gum acacia (b.) Zonisamide API (C.) GA-ZFO-ZNS

Gum acacia is subjected to thermogravimetric analysis at a temperature ranging from 0°C to 900°C under nitrogen atmosphere at 10°C. A sharp exothermic peak at 300°C which conform the weight loss of biopolymer.
The Zonisamide thermogram displays the two endothermic peaks at "247.45"°C and a sharp peak at 329°C which confirms a two step weight loss of Zonisamide drug.Zonisamide was subjected for heating at a temperature of about 0°C to 600°C under nitrogen gas atmosphere at an interval range of 10°C.
The DTG curve in the Fig.9 (c) shows two stages of decomposition. The first decomposition occurs slightly at 50 to 100°C, considered as evaporation of moisture. The second step of maximum weight loss occurs at 200 to 300°C which is due to the heat absorbed by the sample.

VIII. DSC and TGA Combinational Studies



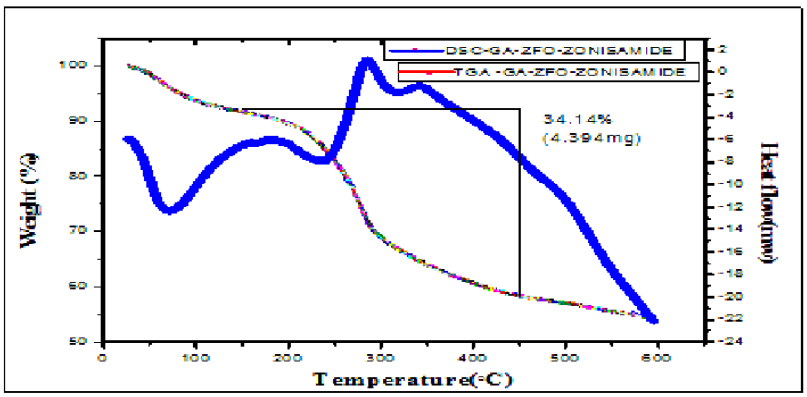


Figure 10 DSC & TGA thermograms (a) Gum Acacia (b) Zonisamide API (c) GA-ZFO-ZNS

DSC and TGA monitors the physicochemical changes in the sample that occur during the thermal variations. The combined thermogram Fig.10 (a) Gum acacia represents the weight loss process which can be seen clearly from the brooder endothermic peaks with a percentage weight loss of about "54.7"%.
Fig.10(b) Zonisamide compound was stable upto 150 °C. onset temperature of "165.21" ◦ C, indicates the melting point. An exothermic curve is also visible at "340.3"°C at an onset temperature from "332.2"°C, followed by an endothermic process at temperature of "200.5"°C with corresponding loss of mass of "67.19"% due to the decomposition of Zonisamide [API].
A steep curvein Fig (c.) indicates endothermic phase transition with a mass loss of "34.1"% [4.394mg] from the sample at a temperature from 10°C to 600°C. Lower temperature melting point indicates nanoparticles which all melts at lower temperature shown in the figure 10 "163.33"°C.

IX. Mass Spectroscopic studies & Interpretation of Mass Spectra

Mass spectroscopic studies were carried out for the synthesized GA-MNP loaded nanoparticles to determine the decomposition process and to know the evolved gas fragments present in the nanocomposite as a function of temperature or time.

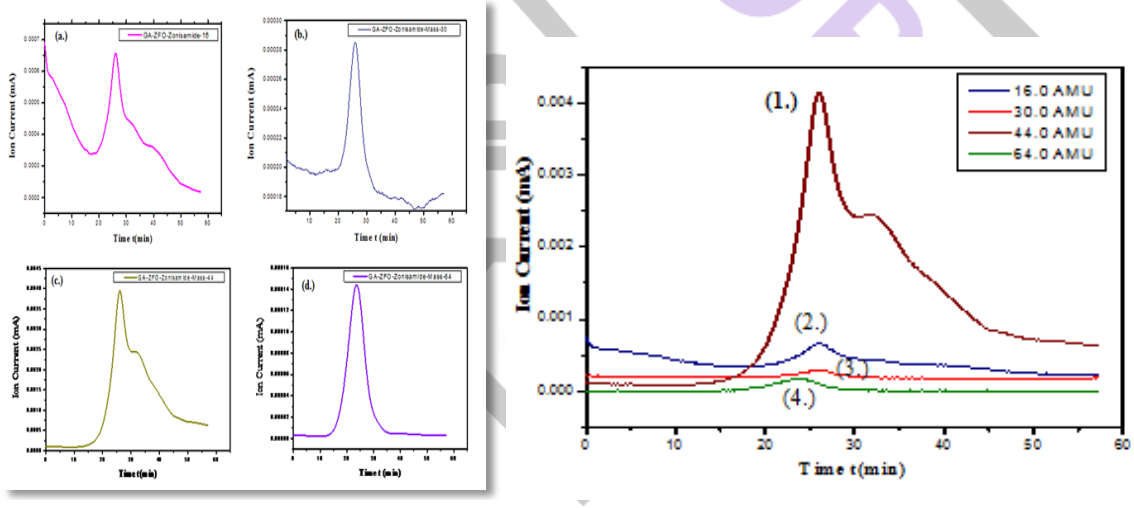


Figure 11 (a) GA-ZFO-Zonisamide-16 (b) GA-ZFO-Zonisamide-30 (C) GA-ZFO-Zonisamide-44 (d) GA-ZFO-Zonisamide-64

Fig.11 (a) shows a characteristic decomposition of $-NH_2$ functional group present in Zonisamide. 20°C to 40°C Fig.11 (b) shows the single step decompositionof [NO] functional group present in the Zonisamide drug, at 23°to 34°C Fig.11 (c) shows the percentage weight loss 20° to 45°C with a mass fragment 44 indicates the decomposition of CO_2 from COOH functional group in Gum acacia. Figure (d) shows the broad exothermic decomposition pattern from 15°C to 35°C which measure the percentage weight loss of SO_2 functional group present in the nanocomposite.
All the above elemental fragments indicate the presence of Gum acacia and Zonisamide in the synthesized nanocomposite without any degradation in various thermal conditions. This serves as an advantage for further characterization of synthesized GA-MNP loaded zonisamide as drug delivery carriers.

X. Fourier Transform-Infrared (FT-IR) Analytical studies

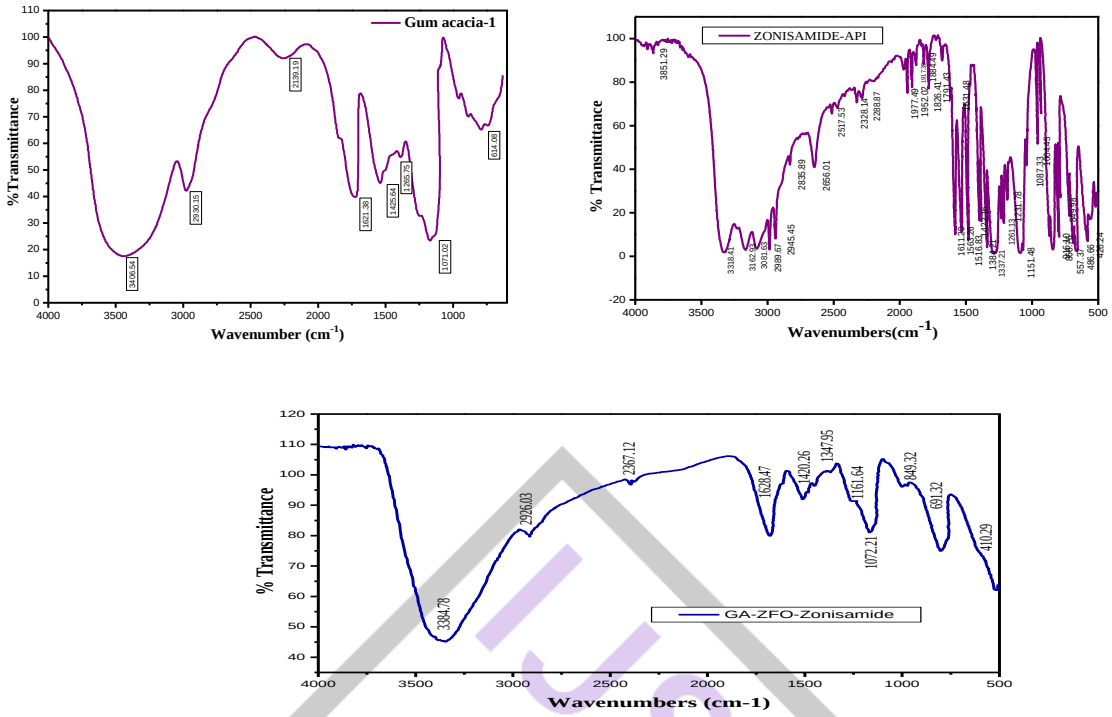


Figure 12 FTIR Spectra of (a.) Gum acacia (b.) Zonisamide API (c.) GA-ZFO-ZNS

Fig.12 (a) Gum acacia has subjected FT-IR studies to observe the interaction of functional groups and to identify organic constituents in the sample. Gum acacia has exhibited the characteristic peaks at "3406.54", "2930.15", "2139.19", "1621.38", "1425.64", "1265.75", "1071.02", and "614.08" typically representing molecular fingerprint of Gum acacia from 1000Cm^{-1} to 400Cm^{-1}. GA exhibits a band at "3406.54" cm^{-1} and "2930.15"$^{-1}$cm, represents the presence of –OH and –CH_2,-CH_3 aliphatic grou indicates the presence of sugars, galactose, arabinose, and rhamnose. The band at 1621.38 cm^{-1} is due to symmetric and asymmetric stretching of COO^- group. The band at "1425.64"cm^{-1} is indicates –OH group bending. The bands at "1265.75" represents alkane CH_3 bending, alcohol and ether C-O-C stretch, carboxylic acid CO stretch , amines C-N stretch, and alkyl stretch due to the presence of sugar backbone.The band at "1071.02" represents alkene C-H bend of polysaccharide of Gum acacia sample. The peak at 614.08 is observed due to the variation in shape and contributes to the reduction process.

Table 1 FT-IR Specral observation of Zonisamide API

S r.No.	Peak	Interpretation
1	3318.41 cm^{-1}	Aliphatic amine: N-H stretching.
2	2989.67 cm^{-1}	Aromatic ring : C-H
3	1611.20-1631.48 cm^{-1}	Aromatic ring : C=C stretching
4	1384.21 cm^{-1}	Tertiary Aromatic amine :C-N stretching
5	1260.13 cm^{-1}	S = O stretching

Fig.12 (c) In the entire GA-MNP stabilized Zonisamide Nanocomposite sample, "410.29" cm^{-1} indicates the characteristics of ZnO, and the peak observed at "691.32" cm^{-1} confirms the presence of ZnO due to Zn-O stretching. The strongest peak arises at "3384.87" cm^{-1} is originated from carboxylic group of GA molecule along with the N-H stretching of Zonisamide. The FTIR shows weak absorption at "1420.26" cm^{-1} which is due to COO- symmetric stretching of GA. The appearance of well defined peak at "691.32" cm^{-1} is due to the presence of iron-oxygen [Fe-O] which states that the synthesized Nanocomposite contains iron oxide. The peak observed at "1628.47" cm^{-1} are due to bending vibrations of hydroxyl and O-H stretching of magnetic nano particle [iron oxide]. The absorption peak at "3384.78" cm^{-1} is due to the presence of aliphatic amine of zonisamide.

XI. Interpretation of FT-IR spectra

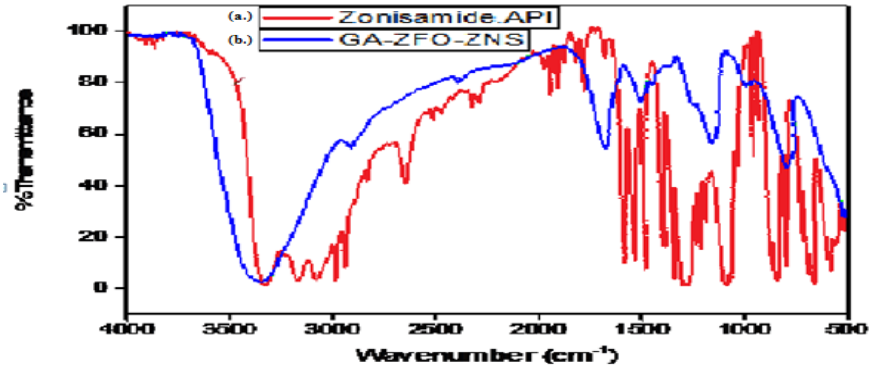


Figure 12 (a.) FT-IR of Zonisamide API. **(b.)** FT-IR of [GA-ZFO-ZNS].

The overlapping FT-IR of GA-ZnO-Fe_3O_4 nanocomposite loaded with zonisamide [solid blue line] along with zonisamide API [solid red line]. Gum acacia shows a broad and strong absorption bands from a range of 3000 to 600 cm^{-1}, where as ZnO exhibits absorption peaks in the range 1000-500 cm^{-1}. Iron oxide possesses the absorption peaks from 3300 to 650 cm^{-1} and Zonisamide shows the absorption peaks from 3500 to 750 cm^{-1}. From the above figure we can conclude that the absorption peaks observed from the synthesized GA-MNP loaded zonisamide nanocomposite complies with the standard values of all the components present in the Nanocomposite. Hence it is proved that the prepared nanoparticles is pure and has good electrical and optical properties.

XII. In-vitro drug release studies

The ability of the synthesized zonisamide loaded GA-MNP nanoparticles to regulate drug release, are performed using a dissolution medium such as phosphate buffer. The prepared nanoparticles of sufficient quantity is suspended in a buffer solution for a particular time period and the drug molecules dissolved (dispersed) into the medium is frequently with drawn to determine the amount of drug release using UV-VIS spectro-photometric methods.

Suitable volume of aliquots in a series such as (2ml, 4ml, 6ml, 8ml, 1.0ml, 1.2ml) respectively is withdrawn by using micro pipette from the above standard stock solution, and each aliquot is transferred into 10ml volumetric flask. The remaining volume is adjusted upto the mark with phosphate buffer solution (7.5) to produce the concentration range of 2 to 1.2µg/ml. The absorbance of prepared solutions was recorded against phosphate buffer solution as a blank reference standard. Calibration curve was plotted by taking absorbance on Y-axis and concentrations on X- axis. A linear regression equation was obtained from the calibration curve.

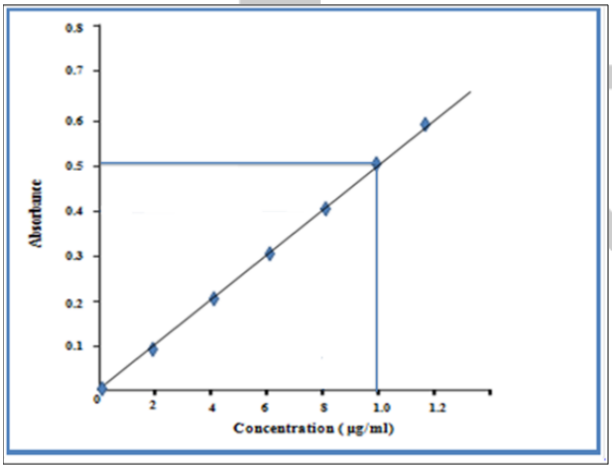


Figure 13 Calibration curve of GA-MNP-ZNS

Table 2 Linearity data of GA-MNP-ZNS

S.No	Concentration (µg/ml)	Absorbance
1.	2	0.118
2.	4	0.201
3.	6	0.312
4.	8	0.454
5.	1.0	0.525
6.	1.2	0.605

XIII. Percentage (%) Drug release data of Gum acacia-Magnetic nanoparticles

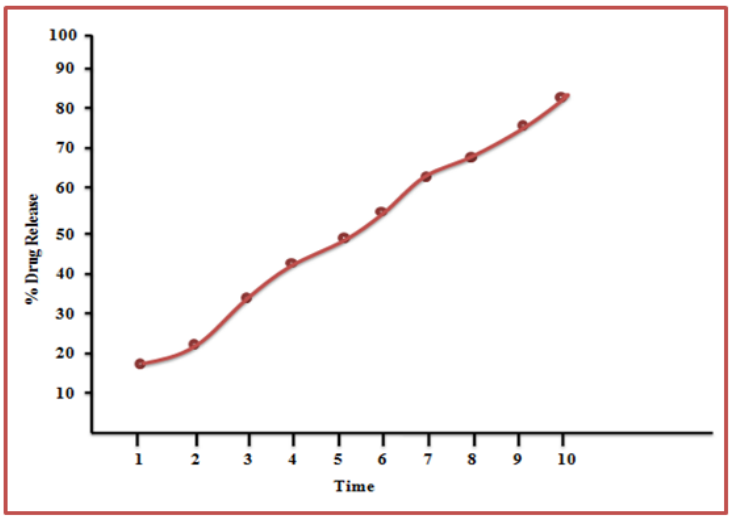


Figure 14 Percentage Drug release pattern of GA-MNP loaded with Zonisamide.

Table 3 Drug release pattern of GA-MNP-ZNS nanoparticles

S.No.	Time	Absorbance	Concentration (µg/ml)	Drug release	% drug release
1.	5 min	0.182	2.02	18.18	18.1%
2.	15 min	0.244	2.71	24.39	24.3%
3.	30min	0.352	3.91	35.19	35.1%
4.	1 hr	0.411	4.56	41.04	41%
5.	1.5 hrs	0.498	5.53	49.77	49.7%
6.	2 hrs	0.570	6.33	56.97	56.9%
7.	4 hrs	0.632	7.02	63.18	63.1%
8.	6 hrs	0.689	7.65	68.85	68.8%
9.	8 hrs	0.762	8.46	76.14	76.1%
10.	10 hrs	0.867	9.63	86.67	86.6%

CONCLUSION

In summary a unique synthesis of ZnO/Fe_3O_4 nanoparticles loaded with ZNS (API) by chemical reduction method followed by controlled growth of nanoparticle along with green chemistry using (Gum acacia) was successfully synthesized . This study illustrate that integration of Gum acacia into the Zn/Fe Oxides composite stabilizes the nanoparticle with control in size of nanoparticle. The lesser the concentration of Zn/Fe the higher will be the stabilization along with magnetization of nanoparticles. This study also shows higher the concentration of Zn/Fe higher will be the antibacterial activity. Hence composite nanoparticles may be used as antibacterial agents.Size and shape of nanoparticles can be changed depending upon the concentration of Gum used. The biological drug release activity of synthesized GA-MNP-ZNS nanoparticles has also been reported. The study rate profile of the drug exhibits sustained drug release for a prolong period of 10hrs with increase in bioavailability and decrease in the dose interval. Hence the above formulation minimizes the dose related adverse effects, cost and improve patient related compliance and drug efficiency.

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