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FABRICATION, CHARACTERIZATION AND ANTIBACTERIAL PROPERTIES OF GELATIN/NA-ALGINATE COMPOSITE FILM CONTAINING CURCUMIN/IRON NANOPARTICLES

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Keywords:

Sodium Alginate, Gelatin, Curcumin, Glutaraldehyde, Iron oxide nanoparticles, Antibacterial activity

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ABSTRACT: In this work, gel/na-alginate films containing curcumin were made using the straightforward procedure of mixing gel/na-alg solutions, followed by linking with glutaraldehyde solution. Glutaraldehyde was injected into the film solutions made at various proportions (A: G=25:75, 50:50, 75:25 and 10:90), and the resulting film's mechanical, thermal, and swelling characteristics were evaluated. Gelatin and Sodium alginate were combined in a biocompatible wound dressing material that was effectively used in the biomedical areas. Several biochemical and molecular waterfalls, such as swelling, tissue growth, and the remodeling stage, identify the wound healing procedure. The natural bioactive ingredient curcumin, an anti-inflammatory, antioxidant, and tissue regeneration factor, was introduced in the film. Also, iron oxide nanoparticles are very appealing due to their non-poisonous properties in biomedical applications of biological systems. Composite film loaded with curcumin and iron nanoparticles using biopolymers of gel/na-alg with the help of a crosslinker (glutaraldehyde). The synthesized films are characterized by numerous techniques, including XRD, FTIR, SEM, and Thermo gravimetric analysis (TGA). The chemical studies also carried out were the welling study. Curcumin extract and iron nanoparticles are characterized by ultraviolet (UV)-visible spectroscopy. The morphological investigation confirmed the genesis and creation of the film. Escherichia coli bacteria were used to investigate the efficacy of GEL/Na-Alg films loaded with curcumin extract and iron nanoparticles as antibacterial agents (*E. coli* bacteria). As a result, an all-natural alginate-gelatin-curcumin-iron nanoparticles composite may be investigated as a foundation for effective and affordable wound healing.

INTRODUCTION: When introduced to a traumatic injury with a multiplex, the tissue regeneration involves the disruption or absence of certain cellular structures called wound healing. The invasive pathogens must be removed from the area of damage in order to intervene and heal correctly.

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To control infection and speed up the complicated biological, chemical, and mechanical process of wound healing, the wound site is treated with a clean agent. It is a natural antibiotic that is utilized for its ability to fight bacteria and additionally functions as an antioxidant, anti-inflammatory, anti-tumor, anti-microbial, anti-HIV, and anti-carcinogenic agent [2, 3, 4].

Gelatin: A biodegradable, biocompatible, bioactive, and mechanically highly durable polymer is gelatin. Because of its benefits, which include biological origin, biodegradability, hydroxyl ability, and commercial availability at a reasonable price, it is widely employed in the

biomedical area. Gelatin is a water-soluble polymer that reacts with a chemical crosslinker insoluble film.

Sodium Alginate: Sodium Alginate is a very often used natural polymer for biomedical and drug delivery applications. It is a low-cost, widely accessible, biocidal, bio-degradable, and non-poisonous polysaccharide with countless uses in the food and pharmaceutical industries. Alginate has been employed in producing numerous components for tissue regeneration to speed up wound healing.

Curcumin: The bioactive substance is known as curcumin, curcumin (diferuloylmethane) ($C_{21}H_{20}O_6$), a yellow-colored chemical from the rhizome *Curcuma longa*. Curcumin is water-insoluble but dissolved in ethanol, acetone, and many organic solvents. It is also known as Turmeric. Turmeric (*Curcuma longa*) comes from the ginger family with well-known medical benefits. It shows a broad range of narcotic properties consisting of anti-inflammatory, bacterial, cancer-fighting, and fungal characteristics. In Asia, curcumin has been used medicinally for more than 2500 years. Curcumin has relatively poor physical and chemical stability while having a unique therapeutic potential and being an effective wound-healing agent [5]. As a result, curcumin-specific carriers have been created to boost its stability and bioavailability. Both Gram-positive and Gram-negative bacteria cannot grow when curcumin is present.

Iron Nanoparticles: Numerous fields might greatly advantage from nanotechnology. Through the creation of fresh remedies for environmental issues, nanomaterials have the potential to enhance the environment. A new technology called nanotechnology has been hailed as having the potential to overcome the chemical and physical limits of microparticle materials [6]. Recent years have seen a significant increase in interest in nanotechnology in the fields of biomedical research, including cytotoxicity [7, 8], drug discovery, magnetic resonance imaging [9], catalyst supporters [10], and biomedical applications, which include magnetic carriers for bioseparation [11], enzyme and protein isolation [12], and contrast improving content. This research used iron chloride as a co-precipitation catalyst to create ferric oxide nanocomposites. Systemic surface morphological characteristics are discussed by UV-vis analysis.

MATERIALS AND METHODS:

MATERIALS: HI Media Laboratories Pvt Ltd-Mumbai (INDIA) is the source of gelatin, and the same company also provides sodium alginate (India). From the market, freshly prepared curcumin was bought. Ammonium hydroxide, acetone solution, and glutaraldehyde (25% aqueous solution) were acquired from Loba Chemie Pvt Ltd Mumbai (INDIA), and ferric chloride hexahydrate was purchased from HI Media Laboratories Pvt Ltd. (INDIA). Chemicals of analytical grade were used throughout.

Preparation of Gelatin and Sodium Alginate Composite Film: The film was made using the solution casting method. In this, 5.0 g of gelatin and 5.0 g Na-alg were dissolved in 100 ml pure water on a magnetic stirrer at 50°to 60°C and stirred at 480 to 500 RPM. Adding 1 ml Glutaraldehyde solution as a crosslinker till a homogenous solution is obtained. Lastly, the prepared blend solution was poured onto a simple glass Petri dish to dry in air for 48 hours or dry in an oven for 5 hours at 60° C; after that, composite films were removed from the Petri plate and kept for additional analysis. Gel and na-alg solutions were made in the weight ratios shown in **Table 1** [13].

TABLE 1: COMPOSITION IN DIFFERENT RATIO FILMS

Ratios	Gelatin	Na-alginate	Glutaraldehyde
75:25	7.5 g	2.5 g	1 ml
50:50	5.0 g	5.0 g	1 ml
90:10	9.0 g	1.0 g	1 ml

Preparation of Curcumin Extract from Turmeric: The extraction was done as follows: Freshly collected turmeric rhizome was cleaned, washed with distilled water, and dried it up to6 days also dried again at 50°C in an oven for 5 hours and crushed separately in mortal pastel till after the small-scale cutting of dry rhizomes homogenous powder was formed. After that, 0.5 g samples were taken into a cleaned conical flask, and acetone (50 ml) was added to it and allowed for 4 hours on normal stirring. After time duration, the extract was filtered by Whatman filter paper (grade: 1)and after filtration, extracts were stored for further analysis.

Synthesis of Iron Nanoparticles: Iron oxide nanoparticles were created using a basic co-precipitation process. The first step was to dissolve 5 g of $FeCl_3*_6H_2O$ in 75 ml of clean water at room temperature while stirring. Then at room temperature, dropwise addition of 2 ml NH_4OH solution (drop rate = 1 ml min^{-1}). Throughout the synthesis, pH=1 was maintained. A brown powder was produced after the final black mixture was heated to 80°C for 2 hours and mixed continuously at room temperature for an hour. The product was dried and cooled at room temperature. Nanoparticles were kept for later study after drying [14].

Preparation of Curcumin and Fe NPS Loaded Gel/Na-Alg Composite Films: 7.5 g of gelatin and 2.5 g of na-alginate were dissipated in 100 ml of purified water with constant stirring at 60°C and 500 to 520 rpm on a magnetic stirrer for 30 min. after that dissolved Fe Nanoparticles in distilled water to make NPs solution. In a mix of gelatin and na-alginate solution, add 1 ml of Glutaraldehyde solution as a crosslinker, Curcumin, and Fe Nanoparticles solution till a homogenous solution is obtained. The prepared solution was put onto a straightforward glass petri plate to dry in air for 2 days or in an oven for 5 hours at 50°C. The loaded film was removed from the Petri plate and kept for antibacterial testing.

Characterization:

FTIR: Equipment: "Perkin Elmer"- Spectrum two or equivalent triturate about 1 to 2 mg of the sample under test with about 300mg of potassium bromide (suitable for IR analysis) and compress with 13 mm die at a pressure of about 10 tons. Use this pellet to obtain infrared absorption spectra between 400 to 4000 cm^{-1}.

Xrd (X-Ray Diffraction): In materials research,(XRD) is now a technique for determining a substance's crystalline structure. The gel/na-alg powder was then analyzed using the X-ray diffraction technique.

Sem (Scanning Electron Microscopy): The surface morphological study of the composite film was determined using scanning electron microscopy. The composite film was analyzed in the granular form obtained by grinding it. A working distance (WD) of 6.6 mm was kept while an electron beam with an accelerating voltage of 5.00 kV was utilized.

TGA (Thermogravimetry Analysis): TGA technique evaluates the mass of such an experiment as a variable of time or temperature under control heating. Temp and wt. loss are seen during the progressive volatilization of the material's component components over time. TGA testing is a valuable method for assessing polymers because it can detect losing weight at high temperatures. At about 200°C, polymers normally melt before breaking down; however, some can withstand temperatures of 301°C in air and 505°C in inert gases without degrading. These polymers can be examined by TGA as well.

Uv-visible of Curcumin Extract: The UV-Visible spectroscopy technique is advantageous because it offers important facts regarding materials' absorbance, transmittance, and reflectance [15]. The wavelength was set between 200-800 nm.

UV-visible of FE-NPS: UV-Visible absorption spectrum analysis may improve understanding of the material's optical band gap's electrical structure.

SWELLING STUDY: Using physiological fluid (PF), the composite films' swelling was studied. 8.307 g of sodium chloride (NaCl) and 0.367 g of calcium chloride ($CaCl_2$) were dissolved in 1 Litre of distilled water to create this fluid. The PF solution was created by combining the two of them. Now, carefully weighed little sections of each ratio film were cut. The films were submerged in the PF solution, retrieved at periodic intervals, dried on filter paper, and weighed. This procedure was repeated multiple times until 24 hours had passed.

Antibacterial Activity: The antibacterial activity of Curcumin and Iron Np_s loaded with Gel/Na-alg composite film was determined using *E. coli*. Prepare 2.8 gm of nutrient agar in 100ml distilled water in a flask and Petri dish. After sterilization of media allowed to room temperature and spread test organism (*E. coli*) was put into solidify agar solution *via* a spreader.

A piece of the composite film is placed over an agar plate and incubated at 37º C for 24 hours. Next day observe the zone of inhibition.

RESULTS AND DISCUSSION:

FTIR: These FTIR tests were performed with full precision at PNP Analytical Solutions, Baroda, Gujarat (India).

50:50 Gel/Na-alg Composite Film: Typical absorption bands were visible in the IR spectrum at 3325.86 cm^{-1} (OH group) in gelatin and na-alginate. The peak with wave number 2885.03 cm^{-1} arises due to the CH_2 group present in a Gel/Na-alg. Another peak in 1681.02 cm^{-1} is due to the C=O group stretch present in gelatine **Fig. 1.**

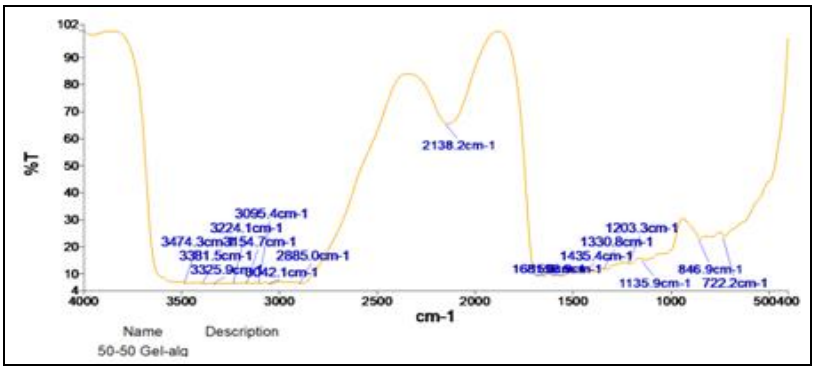

FIG. 1: FTIR SPECTRUM OF 50:50 GEL/NA-ALG COMPOSITE FILM

90:10 Gel/Na-alg Composite Films: The FTIR result of the 90:10 Gel/Na-alg composite film is shown in **Fig. 2**. The peak with wave number 3562.1 cm^{-1} gives large transmittance because of -OH stretching. The peak at 1703.9 cm^{-1} is the presence of C=O stretching. Some peaks are around between 700 to 900 cm^{-1} may be because of C-H bending.

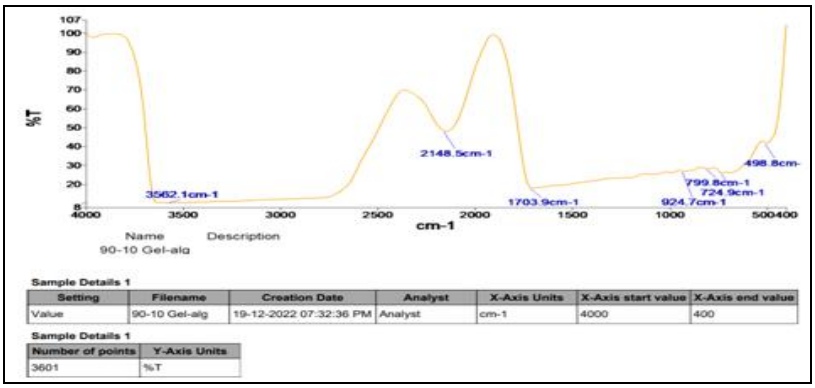

FIG. 2: FTIR SPECTRUM OF 90:10 GEL/NA-ALG COMPOSITE FILM

25:75 Gel/Na-alg Composite Films: The FTIR result of the 25:75 Gel/Na-alg composite film is shown in Figure 3. Some peaks were observed between 3300 to 3550 cm^{-1} may be due to the presence of -OH and -NH stretching. The transmittance at 1204.5cm^{-1} and 1298.3cm^{-1} is equivalent to the C-O stretching. The peak at wave number 1655.0 cm^{-1} for N-H stretching **Fig. 2.**

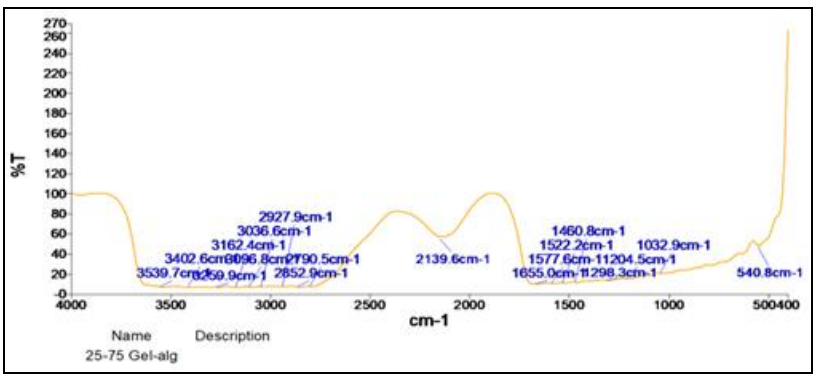

FIG. 3: FTIR SPECTRUM OF 25:75 GEL/NA-ALG COMPOSITE FILM

XRD: X-ray diffraction analysis tests were performed with full precision at PNP Analytical Solutions, Vadodara (Gujarat, India). The X-ray diffraction method is used for the identification of the crystallinity of polymers. The Gel/Na-alg composite film phase was investigated using X-ray diffraction reflections, as seen in the image below. Several crystal peaks were characteristics of a crystalline structure in the 2-thita range 10-35 degrees [16]. It provides well-defined signals with reduced peak width. A hump was observed at 32.50 A° in the spectrum, corresponding to the film's amorphous nature confirmed using XRD **Fig. 4.**

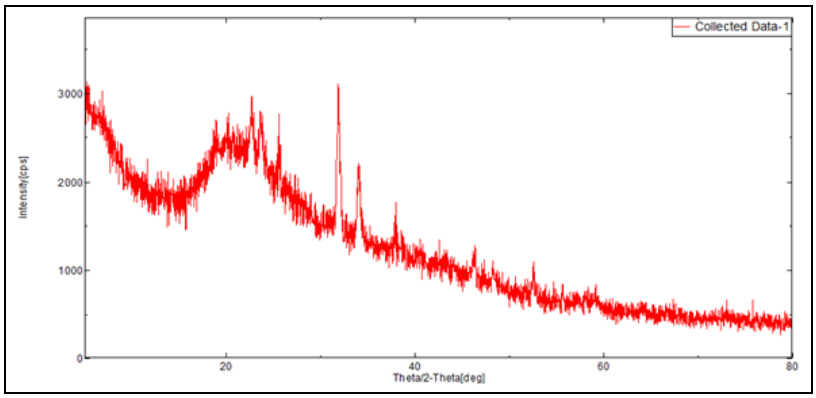

FIG. 4: X-RAY GRAPH OF THE COMPOSITE FILM

SEM (Scanning Electron Microscopy): Semanalysis tests were performed at PNP Analytical Solutions, Vadodara (Gujarat, India). Using an electron beam to scan material, scanning electron microscopy creates a magnified image that can be examined. It provides information about the presence of voids, the homogeneity of the composite, the distribution of the Np_s, and the possible orientation of nanoparticles. The observations were made on the surface of the Gel/Na-alg composite film **Fig. 5.**

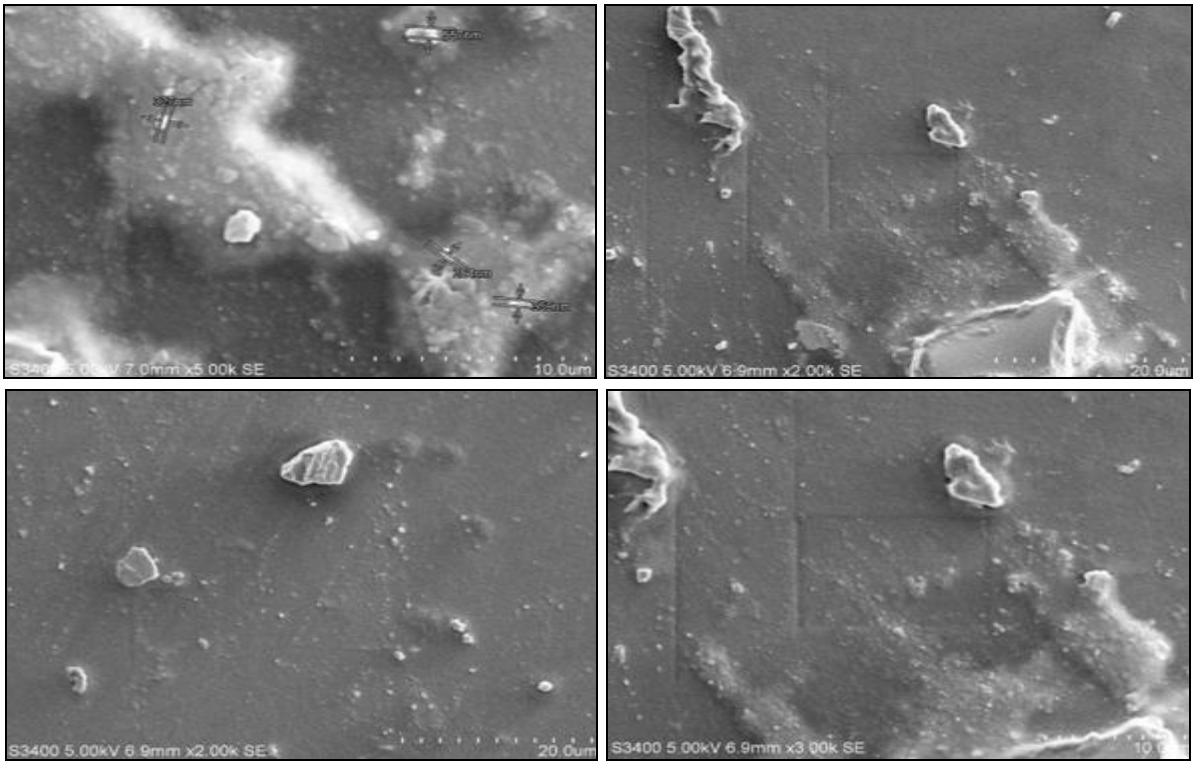

FIG. 5: ELECTRON MICROGRAPH OF THE COMPOSITE FILM

TGA (Thermogravimetric Analysis): The highest temperature at which the composite film can operate may be discovered. It can be stable by looking at the TGA graph and table below.

At 784.95°C temperature, only 22.238% of the film weight is still present in the final measurement. **Table 2** summarizes **Fig. 6** in thermal parameters, including temperature and weight **Fig. 6.**

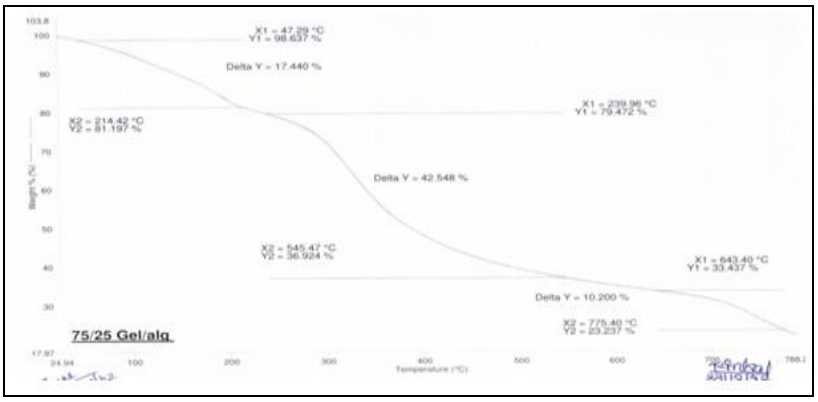

FIG. 6: TGA (THERMOGRAVIMETRIC ANALYSIS)

TABLE 2: TGA DATA

Temperature (°C)	24.95	104. 95	204.95	304.95	404.95	504.95	604.95	704.95	784.95
Weight (%)	99.810	94.193	82.219	70.809	47.224	38.847	34.683	30.931	22.238

UV-Visible of Curcumin Extract: UV-Visible analysis tests were performed at PNP Analytical Solutions, Vadodara (Gujarat, India). The absorbency of curcumin extract was determined by a UV-Visible spectrometer **Table 3.**

TABLE 3: UV DATA

No.	P/V	Wavelength	Abs.
1	↑	421.00	0.343
2	↑	268.00	0.691
3	↓	326.00	0.076
4	↓	221.00	0.284

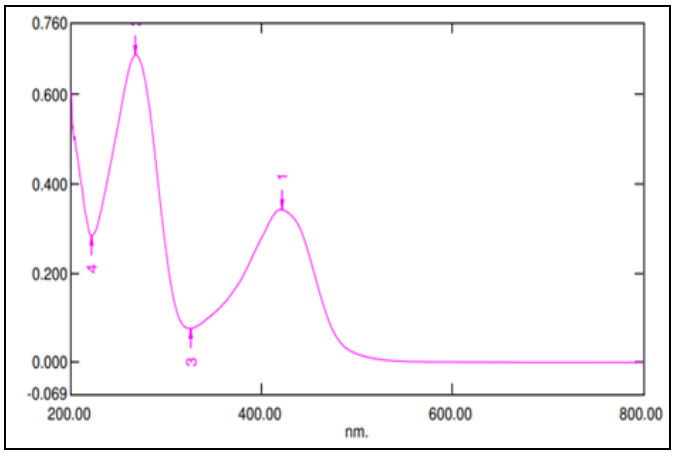

FIG. 7: UV-VISIBLE SPECTRUM OF CURCUMIN EXTRACT

UV-visible of Iron Nanoparticles: The optical density of iron nanoparticles was determined by UV-Visible Spectroscopy. The Absorption peak recorded is at 345 nm wavelength. Fe-Nps were dissolved in a suitable solvent, and UV analysis was performed in **Fig. 8** and **Table 4.**

TABLE 4: WAVELENGTH

No.	P/V	Wavelength	Abs.
1	↑	345.00	0.178
2	↓	264.00	0.254

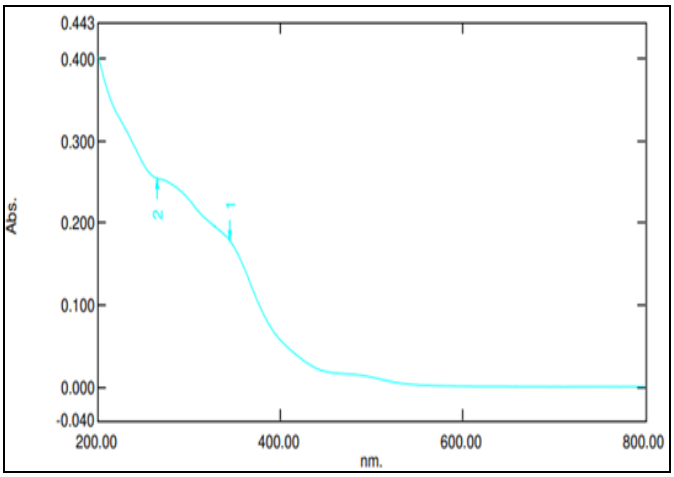

FIG. 8: UV-VISIBLE SPECTRUM OF Fe-NPS

Swelling Study: The swelling test determines how long composite film can be kept wet.

The Gel/Na-alg composite film was dissolved in pf solution of NaCl and $CaCl_2$ solution and was kept for 30 minutes.

The swelling ratio (SR) has been calculated using the following equation **Fig. 9** and **Table 5.**

$$SR = (Mt - Mo) / Mo \text{ g/g}$$

Where Mo = initial mass and Mt = mass at different time intervals.

TABLE 5: SWELLING DATA

Time	Gel: Na-alg (75:25)	Gel: Na-alg (50:50)	Gel: Na-alg (90:10)
0 min	0.6	0.5	0.4
30 min	0.8	0.7	0.6
60 min	1.0	0.89	0.8
90 min	1.3	1.2	1.1
120 min	1.5	1.3	1.4
150 min	1.7	1.6	1.5
180 min	1.7	1.6	1.5

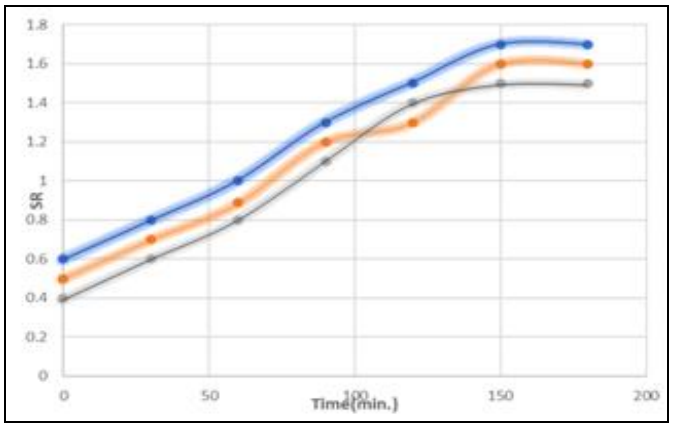

FIG. 9: SWELLING RATIO GRAPH

Antibacterial Activity: The antibacterial effectiveness of a film comprising iron nanoparticles and curcumin extract was tested using *E. coli*. One portion of the Gel/na-alg film loaded with curcumin extract and iron nanoparticles was placed in a petri-dish containing [16] an *E. coli*. The zone inhibition in the composite film containing curcumin extract and iron nanoparticles measured 3.2 cm in diameter **Fig. 10.**

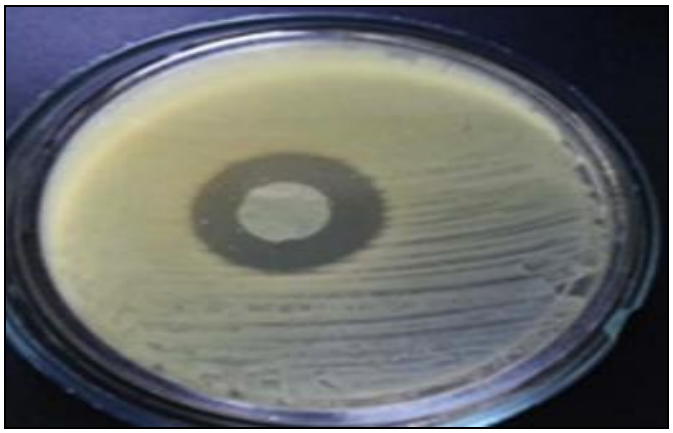

FIG. 10: ANTIBACTERIAL ACTIVITY AGAINST *E. COLI*

CONCLUSION: In this work, Gel/Na-alg composite film was created using the solvent casting procedure. The composite film was discovered to have absorption qualities supported by swelling studies, and the hydrophilic and swelling properties of gel/na-alg film were used to explain the high water content in produced sponges [17]. Furthermore, XRD, SEM, FTIR, Thermogravimetric analysis, and UV-Visible spectroscopy were used to determine functional group, structure, and shape features. A Gel/Na-alg composite film loaded with curcumin extract and iron nanoparticles was also made, and *E. coli* bacteria were used to investigate its antimicrobial effects. It was discovered that the loaded film had a wider zone of inhibition than the unloaded film.

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CONFLICTS OF INTEREST: Nil

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