Synthesis and Characterization of Collagen Copper Oxide Nanocomposites Incorporated with Herb and Their Antibacterial Applications

T.Sobana premlatha[1] , S.Kothai[2]

[1] Department of Chemistry, Anna Adarsh College for women
[2] Department of Chemistry, Ethiraj College for women
Chennai-600008, Tamil Nadu, India
[1]sobanavasudevan@gmail.com, [2]kopran2002@yahoo.com

Abstract — **Bio nanocomposites open an opportunity for the use of new, high performance light weight green nanocomposite making them to replace conventional non-biodegradable synthetic composites. The present study focussed on the synthesis of three samples of collagen copper oxide nanocomposites incorporated with different medicinal herb of each. The synthesized bionanocomposites were characterized by UV, FTIR, SEM analysis techniques. The SEM study showed that the particle size in each composite was found to be less than 100 nm. The antibacterial study of the composites were carried by well diffusion method and they showed excellent activity against various bacterial strains.**

Keywords: Bio nanocomposite, UV, FTIR, SEM

I. INTRODUCTION

Bio nanocomposites are a novel class of nano sized materials derived from natural polymers and inorganic materials. In today's world, bio-nanocomposites are becoming increasingly prevalent due to their extraordinary properties. They are frequently observed in health care and environment friendly studies.[1]. Keeping this into consideration we have started our work with natural polymer collagen with metal and medicinal herbs. The main source of collagen are from skin or scales or bones of fish[2]. Collagen products are so popular as it can make the body function more effective. Collagen production is vital to healthy skin, hair, bones and joints.

The metal chosen for our study is copper in its nanoscale possess more antibacterial properties[3]. The herbs selected for our work are 1. H1-Senna auriculata, 2. H2-Mimosa pudica 3.H3-Andrographis paniculata . The herbs have a number of important medicinal uses in the treatment of upper respiratory infections , ulcerative colitis and rheumatic symptoms[4,5].

II. EXPERIMENTAL DETAILS

Materials and Methods

Chemicals used in the synthesis were of analytical grade and were purchased from HIMEDIA and used as such.

A. Isolation of fish scale collagen

The isolation of collagen from fish scales was performed using a modified method [6]. Fish scales were collected from local market and washed with running water to remove sand and other foreign bodies and later exposed under sunlight. 200gm of dried fish scales were soaked in 10% sulphuric acid solution for 24hours. The fish scales were then minced with an industrial lab blender and the resultant fine paste was subjected to centrifugation (12,000rpm) at 4ºC for 20mins. This supernatant was collected and its pH was adjusted to 7 using calcium hydroxide solution. The supernatant solution was further centrifuged at 10,000 rpm for 15mins to remove calcium sulphate salts. The supernatant solution containing collagen (60% solids) was stored at 4ºC for composite preparation.

B. Synthesis of Copper oxide nanoparticle [7]

Nanocopper oxide was prepared from copper sulphate pentahydrate by simple precipitation method.

C. Collection, Processing and Extraction of Herbs

Three Herbs selected for the study (1. H1-Senna auriculata 2. H2-Mimosa pudica 3. H3-Andrographis paniculata) were collected in and around Chennai. The leaves were shade dried , powdered and sieved. 20 grams of the powder was suspended in 100 ml of ethanol and incubated overnight. The supernatant liquid was filtered with whatmann number 1 filter paper and the filtrate is air dried.

D. Synthesis of fish scale collagen impregnated with copper oxide nanoparticle and Graft copolymerized with GMA (C-Cu-G)

To the beaker containing 5mL of the fish scale collagen solution, added 0.5gm of Copper oxide nanoparticles with continuous stirring by the magnetic stirrer. The process of stirring was carried on for 15min. Subsequently, 5µL of ethylene glycol was added to the contents.
To the above mixture 25mg of potassium per sulphate and 25mg of sodium meta bi-sulphate were added to the contents followed by the subsequent addition of monomer glycidyl methacrylate (GMA) 100µL and stirred for 1hour.

E. Incorporation of herbs in collagen copper oxide matrix(C-Cu-GH)

To the above mixture 1mg of Senna auriculata powder was added and stirred for one hour. Finally 10µL of ethylene glycol was added to the solution. The final obtained composite (C-Cu-G-H1) was stored for other potential medical applications. The procedure was followed for other two herbal powders Mimosa pudica and Andrographis paniculata to get composites (C-Cu-G-H2) and (C-Cu-G-H3) respectively.

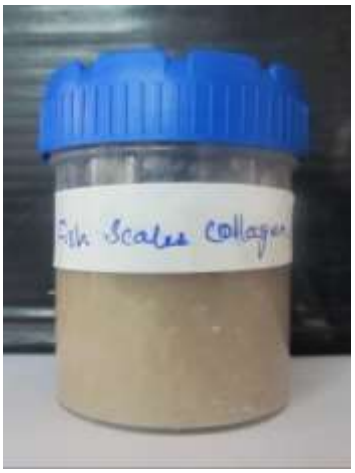

Figure 1. Fish Scale Collagen

Fig-2 **Three Nanocomposites :** C-Collagen, Cu- Copper oxide nanoparticles, G- Glycidyl methacrylate(GMA) an H- Herbal powder (H_1-Senna Auriculata, H_2- Mimosa pudica and H_3-Andrographis paniculata)

F. Characterization

The three different collagen metal oxide incorporated with herb bio nano composites were analysed by UV –visible spectrophotometer of the model SHIMADZU 1650 PC and FT-IR spectroscopy using IR affinity 1 model of SHIMADZU IR 1650 PC. SEM images were obtained by field emission microscope (FESEM) DST-nanoemission model. Antibacterial application studies were carried out by Agarwell diffusion method.

III. RESULTS AND DISCUSSION

A. Ultra violet spectroscopy

The bionanocomposites were analysed using the model of SHIMADZU 1650 PC UV-visible spectrophotometer**.**

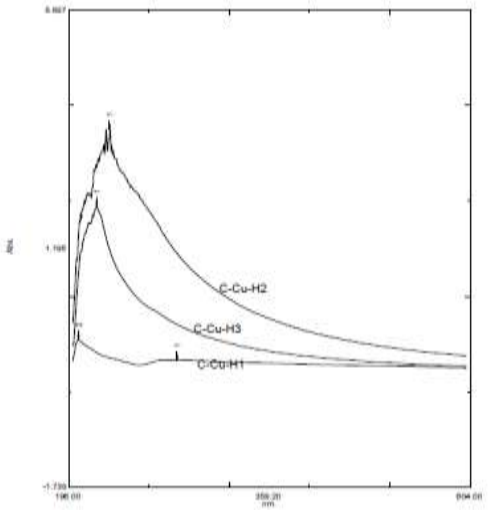

Fig :3 Ultraviolet spectrum of the three composites

The UV spectrum of collagen is transparent in the UV and visible region and its optical properties are hard to characterize by spectroscopic method , whereas the peaks obtained for the bio nanocomposites showed weak electronic transition and there is shift in the absorption peaks due to composite formation.

B. Fourier Transform Infrared Spectroscopy (FTIR)

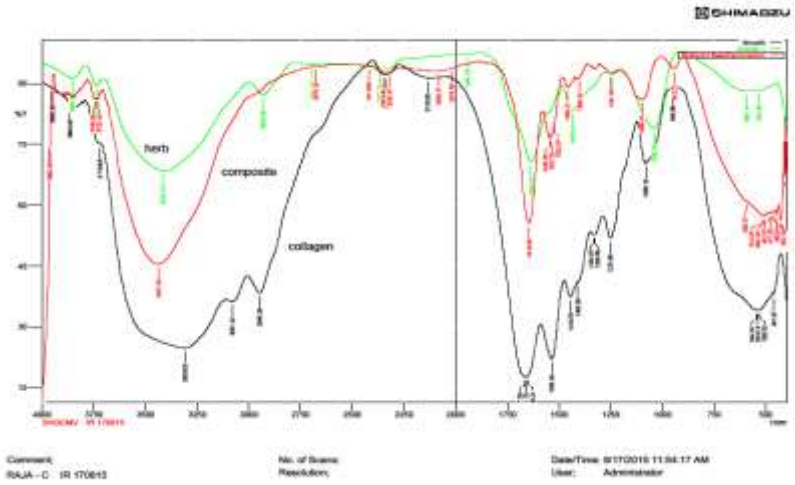

Fig 4: IR spectrum of collagen, herb (H3) and composite C-Cu-H3

The FT-IR spectrum of bio nanocomposite showed a mixture of characteristic absorptions similar to that of pure collagen . The intensity of the absorption peaks for the nanocomposite found to be lower than that of collagen , because of the result of formation of intermolecular hydrogen bonding between collagen-herb.

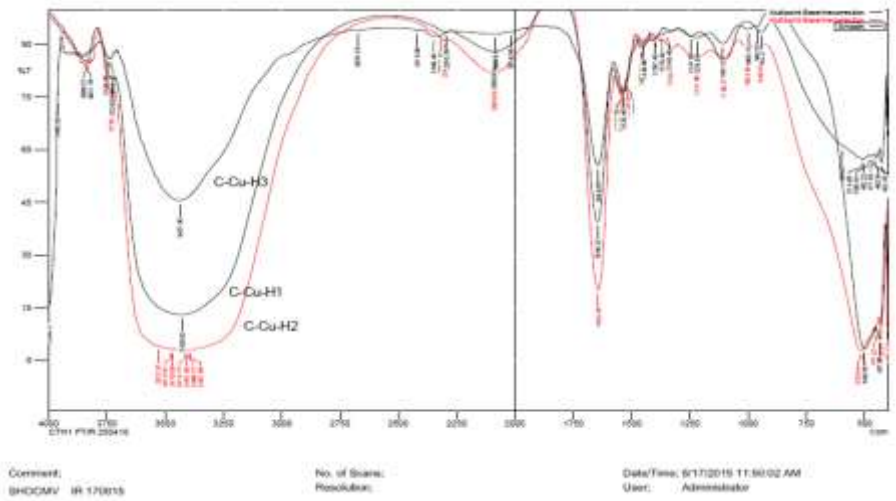

Fig :5 FTIR Overlay Spectrum of the three composites

The FTIR spectra of the three composites are given in Fig- 5 . The spectrum shows the characteristic amide absorption bands of 1646cm-1,1535cm^{-1},1219cm^{-1} for composite-I, 1642cm^{-1},1546cm^{-1},1519cm-1for composite-I1,644cm^{-1},1545cm^{-1},1537cm^{-1} for composite- III representing amide I ,amide II and amide III of collagen respectively. Also the bands observed between 1109cm^{-1} to 1221cm^{-1} are assigned as hydroxyl groups present in collagen . However collagen in presence of copper nano particles shows a shift in the absorption peak of amides. The bands 850cm^{-1},949cm^{-1} represent the oxirane rings of glycidyl group of GMA. This confirms the grafting of GMA on to C-Cu. The peaks at 3428cm^{-1},3421cm^{-1}and 3437cm^{-1} shows the formation of hydrogen bonding since the COO^- group might have reacted with the NH_2 group available on the collagen.

C. SEM Analysis

An assessment on the morphological aspects of the three synthesized bio nanocomposite were carried out in (FESEM) DST- Nano Emission model. The Fig 6–shows the SEM image of the composites and it was observed that the size of the particles are less than 100 nm.

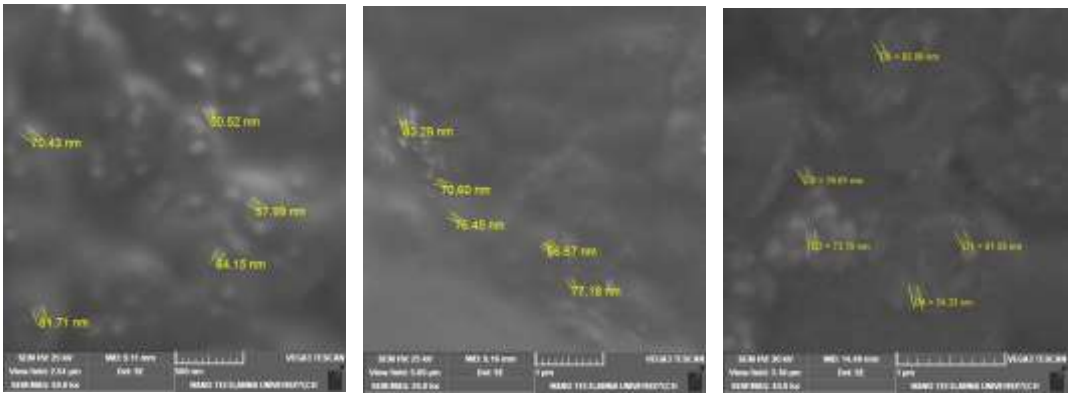

Fig 6 :**SEM image of Three bio nanocomposites :** C-Cu-G-H1 ,C-Cu-G-H2 and C-Cu-G-H3

D. Antibacterial Activity

The antibacterial activity of the three composites were evaluated by Agar well diffusion method . Sterile Nutrient agar plates were prepared and wells of 6mm were punctured using a well borer, 0.1 ml inoculum of Staphylococcus aureus and Escherichia coli were swabbed uniformly over the surface of the agar. About 100ul of each sample was loaded into the well and the plates were kept for incubation at 370° C for 24 hours. The antibacterial activity was evaluated in terms of inhibition in millimeters.

TABLE 1 : Antibacterial activity values of the three Composites

S.No	Composite	Zone of inhibition (mm)	
		E.coli	***S.aureus***
1	C-Cu-G-H_1	16	20
2	C-Cu-G-H_2	18	22
3	C-Cu-G-H_3	19	24

Figure-7: Antibacterial activity of the three Composites by well diffusion method

IV. CONCLUSION

The herb incorporated collagen copper oxide bio nanocomposites were successfully synthesized. The UV and IR spectral studies show that the composites were formed. SEM result shows that the size of nanoparticles in nanocomposite were in the range of 59 to 84 nm. The synthesized bio nanocomposites showed excellent activity against S.Aurus & E.Coli bacterial strains. The synthesized bio nanocomposite with herb Andrographis paniculata showed higher antibacterial activity than the other two nanocomposites with herbs Senna auriculata and mimosa pudica .

ACKNOWLEDGEMENT

Authors are thankful to the management of Ethiraj College for Women(autonomous), Chennai-8 and Anna Adarsh College for Women,Chennai-40 for their continuous support in completing the study.
A special thanks to Nanotechnology Centre of Anna University, Chennai and Instrumentation Centre of Ethiraj College for Women, Chennai for providing instrumentation lab facilities.

REFERENCES

[1] Progress in polymer science volume 38,Issues 10-11,October-november2013 pages1629-1652 Progress in Bio nanocomposites: from green plastics to biomedical applications.

[2] Bin Wang, Yu-Meiwang, Cheng-Feng Chi, Hong-Yu Luo, Shang-Gui Deing and Jian-Yin Ma. Marine Drugs 2013,11 (11), 4641-4661. Isolation and characterization of collagen and Antioxidant collagen peptides from scales of croceine (pseudosciaene crocea)

[3] T.Sobana Premlatha, S.Kothai, Jeyavathana Samuel, P.Shanthi, "Synthesis characterization and antibacterial studies of copper oxide nanoparticles" ,published in proceedings of the National conference on recent advances in applied sciences, in CTTE college ,Chennai, India. ISBN: 978-81-923944-2-3.

[4] "Andrographis".Memorial sloan –Kettering Cancer Center.13 February 2013.Retrieved August 2013.

[5] "3"-Mayo Clinic Book of Alternative medicine (second ed.) 2010.

[6] Sankar,S., Sekar,S.,Mohan,R.,Sundaraseelan,J.,Sastry,T.P.,Preparation and partial characterization of collagen sheets from fish", International Journal of Biological Macromolecules,Vol,42,pp,6-9,2008.

[7] M.Kooti and L.Matouri.,Fabrication of nanosized cuprous oxide using Felhing's solution.Transaction F.Nanotechnology,vol.17,No.1.pp73-78©Sharif university of technology,June2010.