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Synthesis and Characterization of Casein/ Carboxymethyl Cellulose Nanocomposite for Enhanced Antibacterial Activity

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Abstract: *In the present work, CMC (Carboxymethyl cellulose) and CAN(Casein) blend nanocomposite hybrid film containing green synthesized Gold nanoparticles were Synthesised. The aqueous bark extract of SyziguimCumini (Java Plum) was used in this study as a stabilizing and reducing agent for synthesizing Au nanoparticles. The efficient incorporation of Au nanoparticles in the polymer matrix has been confirmed by IR and UV–Visible spectroscopy. The morphology of the sample has been studied using Scanning Electron Microscopy. The biological studies of the above nanocomposite were evaluated in vitro by using surface inoculation method. The result indicate that antibacterial activity of CAN/CMC blend has been enhanced by the incorporation of nanoparticles in the polymer matrix and it can be used for the application of food packaging..*

Keywords: Casein, Carboxymethyl Cellulose, Gold nanoparticles, Antibacterial study

I. INTRODUCTION

The investigation on novel polymers has become essential and indispensable in various fields of life such as clothing, food, exterior coatings, biotechnology, medicine, electronics, conductive materials and environmentally remediate materials.[1,2] Polymers make up many of the materials in living organisms, including, proteins, cellulose, and nucleic acids.[3,4]Polymers have been considered as host matrices for composite materials. Many advanced polymer composites have been prepared with a large variety of inclusions like metals, semiconductors, carbon nanotubes and nanoparticles. Fascinating properties of polymers like Nano corrosiveness, light weight, mechanical strength and electrical tunability can be used along with magnetic, optical and biomedical properties of nanoparticles to synthesise multifunctional materials. Nanocomposites are used as an alternative to overcome the limitations like design, uniqueness and property combinations that are not found in conventional composites.

Polymer blends can be defined as the mixture of at least two macromolecular compounds, polymers, or copolymers.[5,6] A polymer blend or mixture is analogous to metal alloys, in which at least two polymers are combined to create a new material with different physical properties. [7,8,9,10] CAN is a nutritive milk protein that is highly biocompatible, biodegradable, and abundant. As a result of these properties, casein is an excellent bio-polymer for the development of pharmaceutical materials.[11] Generally, acid precipitation is used to extract milk protein, which contains approximately 80wt% of milk protein[12]. It has strong intermolecular forces of interaction like H-bonding and shows film forming and thermoplastic properties. Carboxymethylcellulose is an anionic, water-soluble cellulose derivative. The solubility of CMC depends upon the degree of substitution and the uniformity of the substitution distribution.[13] Water solubility of CMC increased with carboxymethyl substitution and its uniformity CMC has wide applications in drug delivery. The pH- dependent swelling characteristics of CMC hydrogels make them capable of releasing entrapped drug at the right pH present in the tissue and shows great potential as a wound dressing material. [14]

Nanotechnology is one of the emerging field of science due to the fact that it focuses on the monodispersity of size and selectivity of shape, which in turn are the two key issues that are being focused in the field of study[15,16] Nanoparticles shape and size strongly depend upon extrinsic magnetic properties. Nanoparticles are typically synthesized from a top-down or bottom-up approach. A bottom-up approach relies on nucleating atomic-sized materials into the eventual nanoparticles. [17,18]

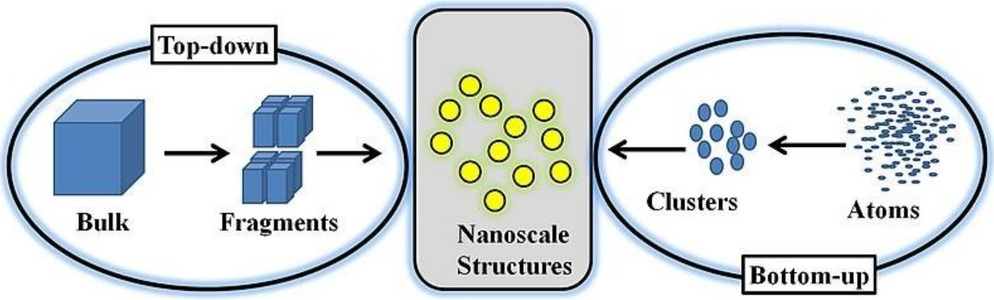

Figure 1: Schematic representation of top-down and bottom-up approach for making nanoparticles.

There are different methods for the preparation of nanoparticles. The use of green methods for making metallic nanoparticles is inexpensive, easily scaled up and environmentally benign. It is especially suited for producing nanoparticles that are free of toxic contaminants as required in therapeutic applications.[20] Gold nanoparticles have high potential towards antimicrobial and antifungal activity. The antibacterial efficacy of gold nanoparticles increases because of their larger total surface area per unit volume. The AuNPs have attracted a significant interest because of their convenient surface bioconjugation, remarkable plasmon-resonant optical properties, chemical stability, and non-toxicity. Studies have also shown that the AuNPs are useful to improve the efficacy, delivery, target specificity, and biodistribution of the drugs which enhance the antibacterial activity against bacteria [21,22]. However, the use of complicated non-bio/non-ecofriendly chemical synthesis processes and dependence on external sources (such as laser pulses) for the synthesis and/or the activation of AuNPs limits their environmental/biocompatibility. Gold nanoparticles have wide range of applications in nano-scale devices and technologies due to its chemical inertness and resistance to surface oxidation[23,24]. Gold nanoparticles also have potential activity against microbial pathogens and it mainly depends on the size and shape of the particles[25]. Polymer nanocomposites, filled polymers in which at one at least one dimension of the filler is smaller than 100nm have been a great spotlight in recent times.[27] When a nanoparticle is embedded in composite because nanoparticle has high surface to volume ratio, it creates large interfacial areas, giving composite unique properties compared to their micro or macro meter scale counterpart. [28]

The present work explores the synthesis of CAN/CMC blend by solvent evaporation method. Prepared blend functionalized by using Au nanoparticles by the aim to develop a smart material with enhanced antibacterial activity.

II. EXPERIMENTAL SECTION AND CHARACTERIZATION TECHNIQUES

2.1 Materials

Commonly available Java Plum has been used throughout this work. Chlauroric acid, Sodium Hydroxide (NaOH),Carboxy Methyl Cellulose (CMC),Casein and distilled water.

A. Preparation of Aqueous Bark Extract from Syziguim Cumini (Java Plum)

Syzigium Cumini bark was collected from the campus of St. Joseph's College, Irinjalakuda, Thrissur, Kerala, India. The bark was washed with distilled water a numerous time to eradicate impurities if any. The 10g bark was chopped, dried and later crushed to powder. The powdered sample was boiled in 100ml water taken in beaker for 15 minutes. The aqueous filtrate was filtered using Whatman grade no.1 filter paper. The filtrate was centrifuged at 7500 rpm for 5 minutes. The collected supernatant solution was kept under 30^0c for further analysis.

B. Green Synthesis of Gold Nanoparticles

1ml of *gold sol* was added into 26 ml of water at room temperature.The reaction mixture was subjected to heat at 70^o c for 15 minutes.Three ml of aqueous Java Plum bark extract was added to the reaction mixture immediately. The photograph of Java plant and prepared nanoparticle was given in the Fig.2

C. Preparation of Polymer Nanocomposite

Nanocomposite film of the polymer blend of Casein/CMC in the weight ratio 50:70 was prepared by solution casting method using gold nanoparticles. Casein/CMC were blended using 10 ml of gold nanoparticle solution by dispersing it into the above solution and it was stirred well. This solution of nanocomposites was then poured into a petri dish and

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the solvent was removed by keeping it in a temperature controlled hot air oven at 40℃.After complete vaporization of solvent for four hours, the films were peeled out from the petri dish.

Fig.2. Photograph of Java plant and prepared gold nanoparticles

III. RESULTS AND DISCUSSION

3.1 UV-Visible Spectroscopy

A strong broad absorption band is observed in the absorption spectra of Gold nanoparticles at 520 nm range. This occurs due to the coupling of incident electromagnetic radiation into a surface plasmon at the interface between the particle and the medium surrounding the particle. [26]Linear absorption spectra of CAN/CMC and CAN/CMC/Au were recorded and the spectra are given in figure 3.The SPR peaks for CAN/CMC/Au is observed at 510 nmconfirming the presence of Au nanoparticles in the polymer matrix.

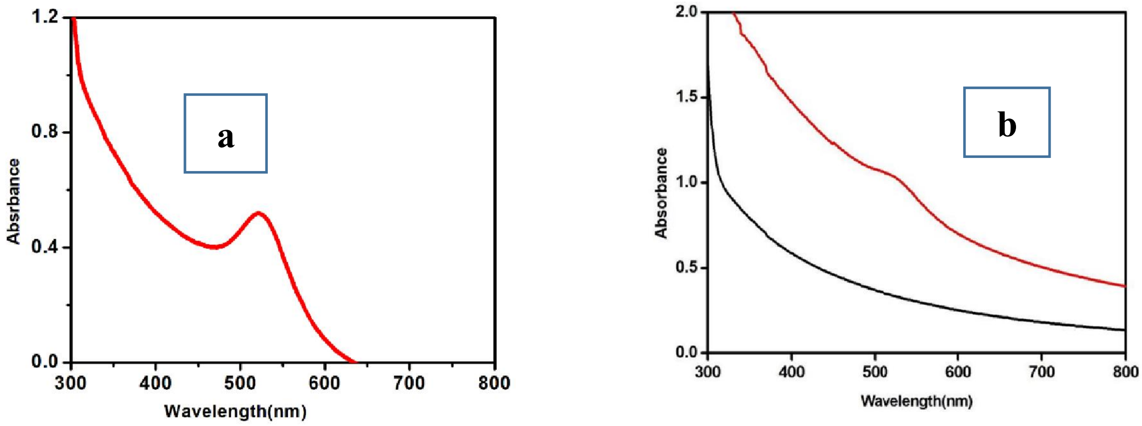

Figure 3: UV-Visible spectra of a) CAN/CMC and b) CAN/CMC/Au

3.2 ATR FTIR Spectroscopy

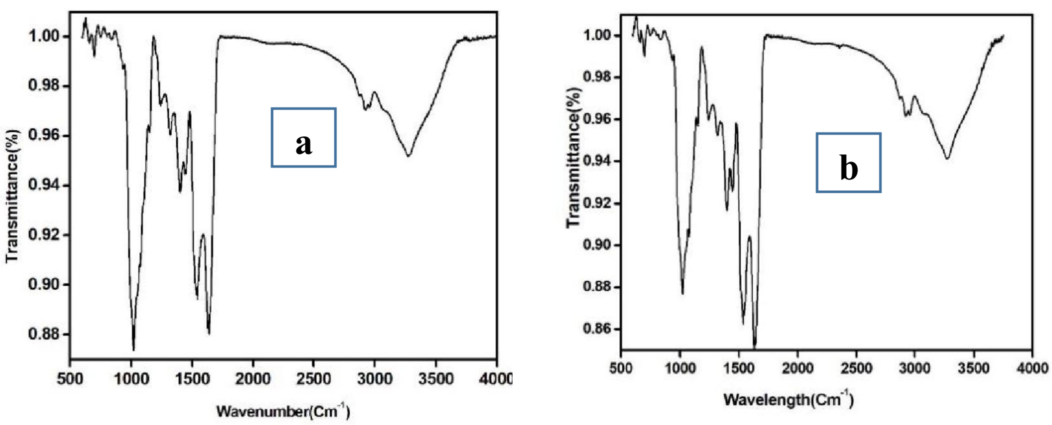

Fig. 4 ATR FTIR spectrum of a) CAN/CMC and b) CAN/CMC/Au

The chemical structure of the CAN/CMC were investigated with this spectral study.

Figure clearly shows peak around 2950-2850 cm^{-1} (2925cm^{-1}) corresponding to C-H stretching, broad peak around characteristic peaks at about 3290-3300 cm^{-1} corresponding to the -N-H stretching and out-of-plane bending of amide groups –NH-C=O on the polymer backbone are observed. Peak around 1300-1400 cm^{-1} (1365cm^{-1}) corresponds to –CH_2 bending, peak around 1550-1650 cm^{-1} (1644cm^{-1}) corresponds to N-H stretching. The absorption peaks for –C=C and –C=O can be clearly found at 1533 cm^{-1} and 1641 cm^{-1} respectively. The spectra of proteins generally have characteristic absorption peaks associated with the peptide bonds (-CONH-).In the case of Au nanocomposites (figure 4) there is very slight shift in the absorption peaks of carbonyl group of amide due to the physical interaction of Au NPs into the polymer matrix.

3.3 Scanning Electron Microscopy (SEM)

The morphology and compatibility between the components of the polymer nanocomposites were studied using scanning electron microscopy. Figure 5 shows the SEM images of polymer and polymer nanocomposites.

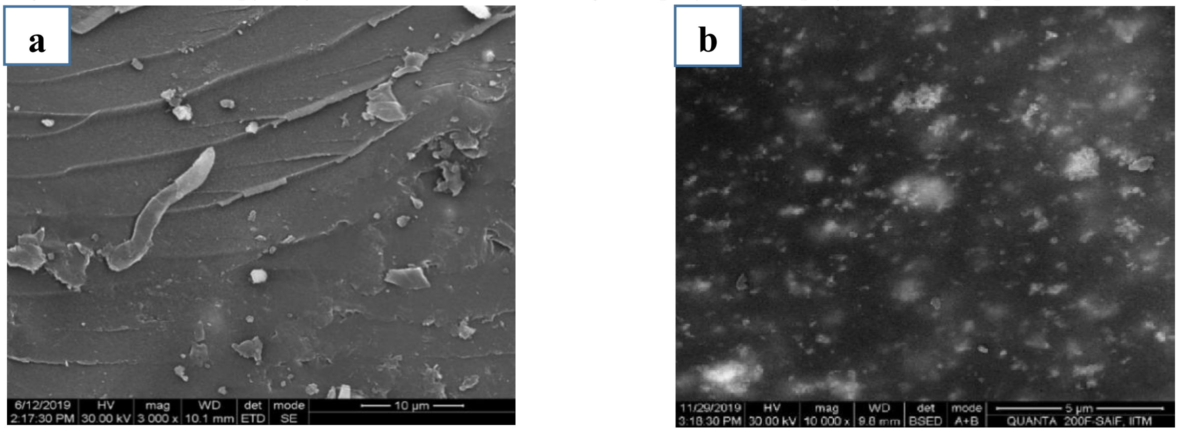

Fig.5 SEM image of CAN/CMC (a) and CAN/CMC/Au (b)

SEM images show a significant change in the surface structure of nanocomposites due to the incorporation of Au nanoparticles. The modified polymer has a smooth surface and on addition of Au nanoparticles into the polymer matrix, white spots are visualised on the surface of the polymer matrix. The images show effective incorporation of the nanoparticles and no significant cluster formation is observed.[30]

3.4 Antibacterial Studies

The antibacterial activity of polymer and polymer nanocomposite were assayed by agar diffusion method. This was studied by using *Staphylococcus aureus*as test organisms. The sensitivity test is mainly based on the size of zone of inhibition. Diameter of the zone of inhibition was measured for sample film.

Table 1: Zone of inhibition of CAN/CMC and CAN/CMC/Au

Sample	Zone of Inhibition (mm)			Antibiotic(1mg/ml)
	Concentration((µg /ml)			
	125	62.5	31.2	
CAN/CMC	10	8	-	16
CAN/CMC/Au	14	11	8	18

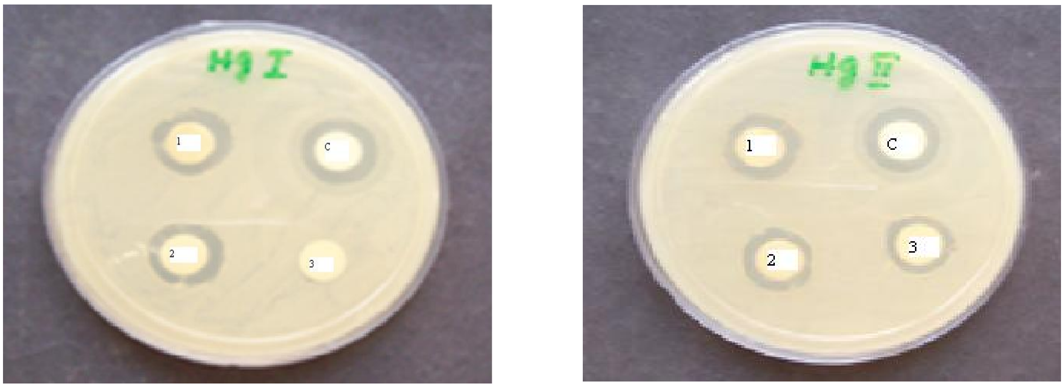

Fig. 6 Antifungal activity of CAN/CMC and CAN/CMC/Au

IV. SUMMARY AND CONCLUSION

CMC and CAN blend nanocomposite hybrid film containing green synthesized Au nanoparticles were developed and properties were studied. In this study, *Syziguim Cumini* (Java Plum) aqueous bark extract was used as a stabilizing and reducing agent to synthesize Au nano particles.IR and UV-Visible spectroscopy have confirmed that Au nanoparticles are effectively incorporated into the polymer matrix. Using Scanning Electron Microscopy, the morphology of the sample was examined. The antimicrobial characteristics was studied by using *Staphylococcus aureus*as test organisms. Due to the presence of Au nanoparticles CAN/CMC/Au shows good activity.

REFERENCES

[1]. Schmitt, Christophe; Sanchez, Christian; Desobry-Banon, Sylvie; Hardy, Joël (1998). Structure and Technofunctional Properties of Protein-Polysaccharide Complexes: A Review. Critical Reviews in Food Science and Nutrition, 38(8), 689–753.

[2]. Mariana Pereda; Mirta I. Aranguren; Norma E. Marcovich (2008). Characterization of chitosan/caseinate films. , 107(2), 1080–1090.

[3]. Perkel, J. Flow Cytometry On-a-Chip. Sci. 3–5 (2015).

[4]. Senthilkumar S, Kashinath L, Ashok M, Rajendran A (2017) Antibacterial Properties and Mechanism of Gold Nanoparticles Obtained from PergulariaDaemia Leaf Extract. J Nanomed Res 6(1)

[5]. Vishakha, K., Kishor, B. & Sudha, R. Natural Polymers – A Comprehensive Review. Int. J. Res. Pharm. Biomed. Sci.**3,** 1597–1613 (2012).

[6]. Shanmugam, S. &Manabalan, R. Natural polymers and their applications. Nat. Prod. Radiance**4,** 478–481 (2005).

[7]. Journal, A., Sciences, P., Prafulkumar, D., Vidya, S. & Trust, B. Gums and mucilages : Versatile excipients for pharmaceutical formulations Gums and mucilages : versatile excipients for pharmaceutical formulations. **4,** 308–322 (2014).

[8]. Sam Coveney, Fundamentals of Phase Separation in Polymer Blend Thin Films. PhD Dissertation, University of Sheffield, 2014.

[9]. Wanchoo RK; Sharma P.K, Viscometric study on the compatibility of some water soluble polymer-polymer mixtures. Euro. Polym. J. 2003, 39,1481-82.

[10]. Pereda, M,;Aranguren, M. Characterization of Chitosan/Caseinate Films. J. Appl. Polym. Sci.**2008**, 107 , 1080–1090. Szyk-Warszyńska, L.; Kilan, K.; Socha, R. P. Characterization of Casein and Poly-l-Arginine Multilayer Films. J. Colloid Interface Sci.**2014**, 423, 76–84

[11]. Ponce, A.; Roura, S. I.; Moreira, M. R. Casein and Chitosan Polymers: Use in Antimicrobial Packaging. Antimicrob. Food Packag.**2016**, 455–466

[12]. Thomas Heinze,Katy Pfeiffer ;Studies on the synthesis and characterization of carboxymethyl cellulose

[13]. Water Soluble Carboxymethyl cellulose Fibers Derived from Alkalization-etherification of Viscose Fibers Hongbo Gu1,2, Jinmei He1 ,Yudong Huang1, and Zhanhu Guo

[14]. Bai, Xue; Wang, Yueying; Song, Zhiyun; Feng, Yanmin; Chen, Yuanyuan; Zhang, Deyuan; Feng, Lin (2020). The Basic Properties of Gold Nanoparticles and their Applications in Tumor Diagnosis and Treatment. International Journal of Molecular Sciences, 21(7), 2480–. doi:10.3390/ijms21072480

[15]. R. Nagarajan, "Nanoparticles: Building Blocks for Nanotechnology," in Nanoparticles: Synthesis, Stabilization, Passivation, and Functionalization, American Chemical Society, 2008, 2-14.

[16]. W. J. Stark, P. R. Stoessel, W. Wohlleben and A. Hafner, "Industrial applications of nanoparticles," Chemical Society Reviews, vol. 44, no. 16, pp. 5793-5805, 2015.

[17]. B. F. G. Johnson, "Nanoparticles in Catalysis," ChemInform, vol. 35, no. 12, pp. 147-159, 2004.

[18]. M. Bengisu, Engineering Ceramics © Springer-Verlag Berlin Heidelberg2001

[19]. Steen Mørup, CathrineFrandsen, and Mikkel F. Hansen Volume 2: Materials: Structures, Properties and Characterization Techniques Oxford Hand book of Nanoscience and Technology ;July 2017

[20]. Raj Chhabra and Madivala G. Basavaraj ; Coulson And Richardson's Chemical Engineering, Vol.2a;Particulate system and Particle Technology ; 2019

[21]. Jagpreet Singh, TanushreDutta ; Green synthesis of metals and their oxide nanoparticles Appilcation for Environmental remediation ; Journal of Nanobiotechnology Article number: 84 (2018)

[22]. LuigiNicolais, Gianfranco Carotenuto ; Metal polymer Nanocomposites ; A John Wiley Sons, INC., Publication, 2005

[23]. EnisaOmanovic-milklicanin, AlmirBadnjevic, AneraKazlagic ;Nanocomposite; a briefReview ; Health And Technology ; 08 Nov 2019

[24]. PendroHenriqueCury Camargo; KesturGundappaSatyanarayanaNanocompositessynthesis, structure, properties and new application opportunities. Mat.Res. (12)1

[25]. J. Turkevich, P. C. Stevenson and J. Hillier, "A study of the nucleation and growth processes in the synthesis of colloidal gold," Discussions of The Faraday Society, 11, 55-75, 1951.

[26]. M. Rai, A. P. Ingle, S. Birla, A. Yadav and C. Alves Dos Santos, "Strategic role of selected noble metal nanoparticles in medicine," Critical Reviews in Microbiology, vol. 42, no. 5696-719, 2016.

[27]. WolfgangHaiss, Nguyen T. K. Thanh, Jenny Aveyard, and David G. Fernig "Determination of Size and Concentration of Gold Nanoparticles from UV-Vis Spectra"J Nanomed Res 2017, 6(1): 00146

[28]. M.Zimbone a , L. Calcagno a,, G. Messina b , P. Baeri a , G. Compagnini"Dynamic light scattering and UV–vis spectroscopy of gold nanoparticles solution"

[29]. GracielaGoizueta, Tsuneo Chiba and Takashi Inoue "Phase morphology of polymer blends" scanning electron microscope observation by backscattering from a microtomed and stained surface"