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**FABRICATION OF CARBOXYMETHYL CELLULOSE/GELATIN
POLYMERIC COMPOSITE FILM INCORPORATED WITH CALCIUM
NANOPARTICLES AND *CATHARANTHUS ROSEUS***

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ABSTRACT

This work demonstrates the preparation of polymeric composite of carboxymethyl cellulose and gelatin incorporated with nanoparticles of calcium and *Catharanthus roseus*. Carboxymethyl cellulose and gelatin were crosslinked using glutaraldehyde. Calcium nanoparticles have been proved to contain intrinsic antibacterial activity and thus it can also be used in wound healing composites. *Catharanthus roseus* is an important medicinal plant possessing phytochemicals holding antioxidant, antibacterial, antifungal, antidiabetic and anticancer properties. Several important characterizations such as Scanning electron microscopy (SEM), X-ray diffraction analysis (XRD), Fourier transform Infrared spectroscopy (FTIR analysis) and UV-Visible Spectroscopy (UV-Vis) were also performed. Along with this, chemical analysis i.e. Swelling study was also determined.

Keywords: Carboxymethyl cellulose, gelatin, calcium nanoparticles, *Catharanthus roseus*

INTRODUCTION:

Polymers are defined as long chain monomers synthesized from a process in which monomers are cross-linked called

polymerization. Polymers attain huge range of applications in biomedical, mechanical and packaging field. Polymers are mainly of two

types: Natural and Synthetic. Some examples of polymers are Polyvinyl alcohol, Polylactic acid, polyvinyl pyrrolidone, carboxymethyl cellulose, etc. Sodium salt of carboxymethyl cellulose is found to be a prominent polymer in biomedical field. It is a water-soluble cellulose-derivative which is anhydro-glucose linear polysaccharide [1]. Gelatin is also a polymer with wide range of applications and properties. It has attention of scientists because of the biodegradability it holds as well as mechanical properties and great film forming [2]. Gelatin is obtained from partial destruction of collagen. Gelatin has antimicrobial and antioxidant properties. Nanoparticles is vastly used in biomedical field due to their physical and chemical properties altering the normal biological activity comparing to materials in bulk [3]. The application of nanotechnology in medicinal field which is regarded as Nano medicine which is related to concerning of usage of precisely engineered materials. Metallic nanoparticles contain significant amount of antibacterial properties and hence finds a wide range of uses in medicinal applications too. India has a biodiversity which has not been explored to its extent. *Catharanthus roseus* has many medicinal actions such as antimicrobial, antidiabetic, antioxidant, antidiabetic effect, etc. [4]. It

belongs to the family apocynaceae used to treat many fatal diseases. This plant is widely known as Madagascar periwinkle.

MATERIALS AND METHODS:

MATERIALS:

All chemicals used were of high analytical grade. Sodium salt of carboxymethyl cellulose (CMC), Gelatin (Gel), Glutaraldehyde (25%), Sodium hydroxide pellets (NaOH), Calcium Nitrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$) were all purchased from HiMedia Laboratories Pvt. Ltd. Mumbai (India). Papaya leaves were collected from local botanical garden. Distilled water was used in all experiments.

METHODS:

1. Preparation of CMC/Gelatin composite film:

All films were prepared using solvent casting method. 2% solution of Gelatin and CMC were prepared by weighing 2.0 g of each compound in 100 ml distilled water. The solution was then stirred continuously for 30 min at 55 C with addition of cross-linker glutaraldehyde (1 ml). The solution was left to rest for about 30 min to remove bubbles formed during stirring. The solution was then poured onto the center of petri-dish and spread uniformly. The film were dried in oven for overnight at 35-40 C and then peeled off. In similar way, blend films of different CMC:Gel ratios were prepared (75:25, 50:50, 25:75) [5].

2. Preparation of Calcium nanoparticles (Green synthesis):

Papaya extract was prepared from 100 g of washed papaya leaves. It was then boiled in 200 ml distilled water for half hour, filtered using Whatmann filter paper 1 and the extract obtained was cooled and used for synthesis of calcium nanoparticles.

10 ml of calcium nitrate solution was added to 10 ml of papaya extract. Then the mixture was stirred in a magnetic stirrer for half hour. NaOH was added drop wise while stirring till white precipitate of Calcium hydroxide was obtained. The precipitate was filtered and dried in an oven for 1 hour. Then it was washed using distilled water to remove basicity. Then calcination was done in muffle furnace at 500 C for 3 hours [6].

3. Preparation of *Catharanthus roseus* extract:

The *Catharanthus roseus* extract was obtained by boiling the plant with 200 ml water. The resulting solution was filtered using whatmann filter paper 1. This extract was used for further experiment.

4. Preparation of *Catharanthus roseus* and Calcium nanoparticles loaded CMC/Gelatin composite film:

2% solution of CMC and Gelatin was prepared by taking 2 g of each material in 100 ml distilled water. Both solution were mixed

and stirred for 30 min at 55 C with addition of 1ml glutaraldehyde as cross-linker. During this, 20 ml Calcium nanoparticles solution and 20 ml *Catharanthus roseus* extract were added. The solution was then rested for 30 min at room temperature to remove air bubbles. This solution was then poured onto petridish, dried overnight and the resulting film was peeled off to obtain the required loaded polymeric composite.

CHARACTERIZATION:

FTIR: FTIR spectroscopy was utilised to evaluate the polymeric composite of CMC/Gelatin at Chemistry Research for Development, Parul University, Vadodara, utilising an FTIR spectrum of 1000 cm⁻¹. The range of this scan was 3500 to 1000 cm⁻¹, with a resolution having 4 cm⁻¹.

XRD: At MS University, Vadodara, X-ray diffraction investigation was done. The 2θ ranged from 5 to 60 degrees. The scan step time was 8.2550 s, and the step size was 0.0080° 2θ. The wavelength of Copper K-α radiation was 1.54060 Å.

SEM: The Scanning electron microscopy analysis was performed at PNP Analytical Solutions, Vadodara to determine the morphological properties of the composite film. The accelerating voltage of electron beam (HV) 5.00 kV was used and a working distance (WD) of 6.6 mm was maintained.

UV-Visible of *Catharanthus roseus* extract:

The Ultraviolet Visible spectroscopy of *Catharanthus roseus* extract was performed at Chemistry Research for Development, Parul University, Vadodara. The wavelength was set between 200-800 nm.

UV-Visible of Calcium nanoparticles: The UV-Visible analysis performed for the identification of Calcium Nano-particles at Chemistry Research for Development, Parul University, Vadodara. The wavelength for this analysis was set between 200-800 nm.

Swelling Study: Using physiological fluid (PF), the swelling of composite films was studied. 8.307 g of sodium chloride (NaCl) and 0.367 g of calcium chloride (CaCl₂) were dissolved in one litre of distilled water to create this fluid. Both were then combined to create the PF solution. Now, discretely 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 and at 24 hours [5].

RESULTS AND DISCUSSION:

FTIR: The FTIR spectra was recorded using FTIR spectrophotometer with KBr. The samples were run in wavelength range of 3500-1000 cm⁻¹. The results are shown below:

1. FTIR analysis of 25:75 CMC:Gelatin composite: (Figure 1)

The absorbance at 1632 cm⁻¹ and 1554 cm⁻¹ refers to C=O and C-N vibrations of amide I and amide II representing collagen secondary structure. The peaks at 2934 cm⁻¹ and 1409 cm⁻¹ shows stretching vibrations of aliphatic C-H and carboxylate group respectively. The peaks at 1244 cm⁻¹ refers to C-O-C stretching vibrations the glucosidic units and 1058 cm⁻¹ band shows presence of ββ β-(1-4) glucosidic linkage. The band at 2934 cm⁻¹ refers to C-H aliphatic group. The peaks between 3000-3900 cm⁻¹ corresponds to OH groups of secondary alcohols of CMC, absorbed water and H-bonding.

2. FTIR analysis of 50:50 CMC:Gelatin composite: (Figure 2)

The C=O and C-N vibrations from amide I and amide II, which reflect the structure of collagen, are thought to be responsible for the absorption at 1917 cm⁻¹ and 1539 cm⁻¹. Stretching vibrations of the carboxylate group (COO⁻) and aliphatic C-H are visible in the bands at 2520 cm⁻¹ and 1409 cm⁻¹, respectively. The peak at 1323 cm⁻¹ is related to the glucosidic units' C-O-C stretching vibrations, while the band at 1025 cm⁻¹ confirms the presence of the (1-4) glucosidic linkage.

The C-H aliphatic group is represented by the band at 2932 cm⁻¹. The bands from 3500 and 3900 cm⁻¹ are related to hydrogen bonding, secondary alcohols (CMC), and OH groups of absorbed water. FTIR analysis of 75:25 CMC:Gelatin composite:

3. FTIR spectra of 75:25 CMC:Gelatin composite (Figure 3)

The C=O and C-N vibrations from amide I and amide II representing the structure of collagen, are responsible for the absorbance at 1633 cm⁻¹ and 2000 cm⁻¹, respectively. Stretching vibrations of the aliphatic C-H and carboxylate group (COO⁻) are visible in the peaks at 1550 cm⁻¹ and 1409 cm⁻¹, respectively. The glucosidic units' peaks at 1403 cm⁻¹ indicate C-O-C stretching vibrations, and the 1062 cm⁻¹ band indicates the presence of -(1-4) glucosidic linkage. The OH groups of secondary alcohols present in CMC, and hydrogen bonding as well as absorbed water are shown by the peaks between 3500 and 3900 cm⁻¹.

4. **XRD:** XRD analysis is a material science method for determining the material's crystallographic structure. This method shows irradiating the sample with X-ray and measurement of intensities and scattering of X-rays exiting sample.

Figure below shows XRD spectrum of CMC/Gelatin composite. As seen in the spectrum, peaks are observed referring to crystalline structure of the film (**Table 1, Figure 4**).

5. **SEM:** Scanning electron microscopy is an analytical technique which examines the sample with the help of an electronic beam giving us enlarged image for further analysis. Figure shows the surface morphology images of CMC/Gelatin composite film produced by SEM. The size of particles were seen in range of 350-550 nm. In the surface morphology images, it can be clearly seen that the film has irregular surfaces (**Figure 5**).

6. **UV-Visible of *Catharanthus roseus* extract:** The UV-Visible analysis of this sample was taken with wavelength ranging from 200-800 nm. The absorbance was determined and peak of this sample was found at 250 nm (**Figure 6**).

7. **UV-Visible of Calcium Nanoparticles:** The Absorbance of Nanoparticles of calcium was determined using UV-Visible spectrophotometer. Ca-NPs were dissolved in a suitable solvent and UV analysis was conducted. The Absorption peak recorded around 290 nm wavelength (**Figure 7**).

8. Swelling Study: Swelling can be defined as increase in volume of solid or gel with the uptake of liquid or gas. The swelling study was carried out according to the process mentioned above in Characterization section of this paper

(Table 2, Figure 8). The Ratio of Swelling denoted by SR was determined using formula:

$$SR = (Mt-Mo)/Mo \text{ g/g} \quad \text{where } Mo = \text{mass at initial and } Mt = \text{mass at interval } t.$$

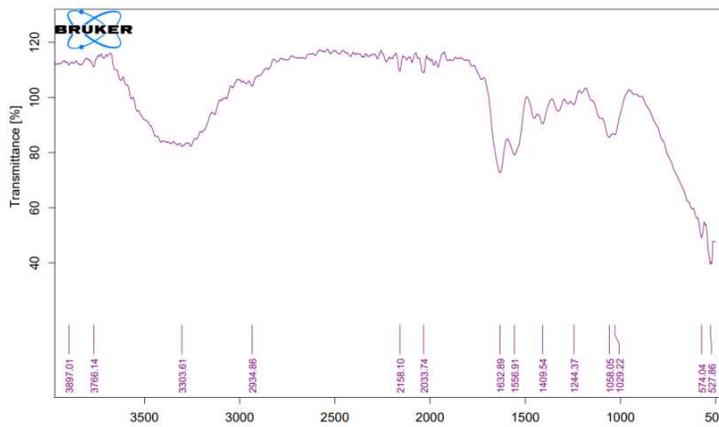


Figure 1: FTIR spectra of 25:75 CMC:Gelatin composite

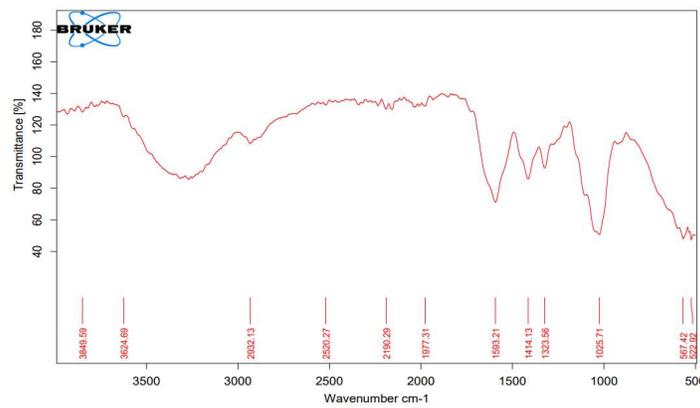


Figure 2: FTIR spectra of 50:50 CMC:Gelatin composite

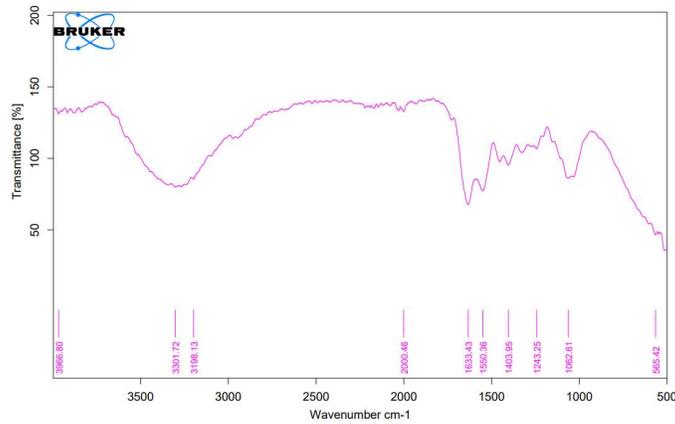


Figure 3: FTIR spectra of 75:25 CMC:Gelatin composite

Table 1: Peak list

Position (2θ)	Height (cts)	FWHM (2θ)	d-spacing (Å)	Area cts*2θ	Relative intensity (%)
19.9836	186.67	0.6927	4.43958	191.34	10.80
27.3606	153.29	0.1574	3.25702	35.71	8.87
31.6606	1264.37	0.0768	2.82379	194.21	73.15
31.7167	1728.52	0.0708	2.81892	181.20	100.00
45.4502	825.50	0.0480	1.99399	79.25	47.76
56.4156	188.51	0.1260	1.62967	35.13	10.91

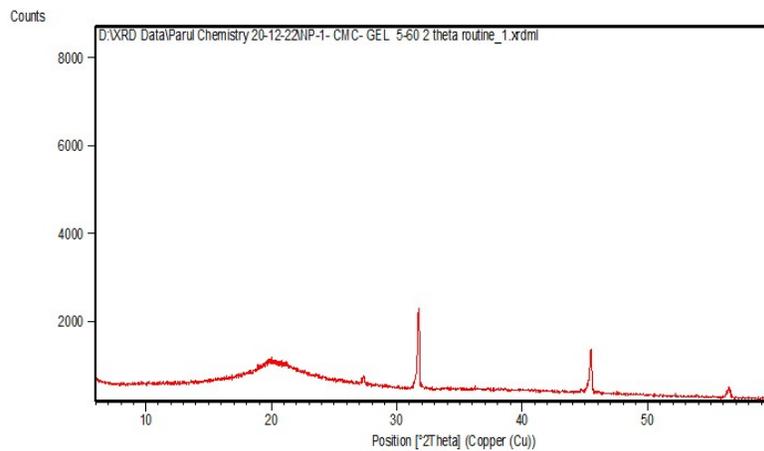


Figure 4: XRD spectra of CMC/Gelatin composite

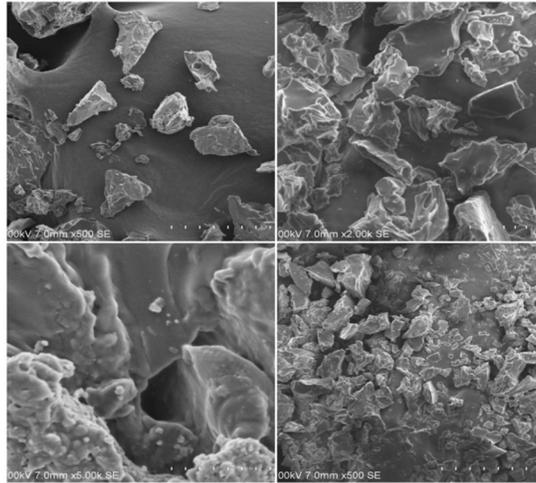


Figure 5: Electron micrographs of CMC/Gelatin composite

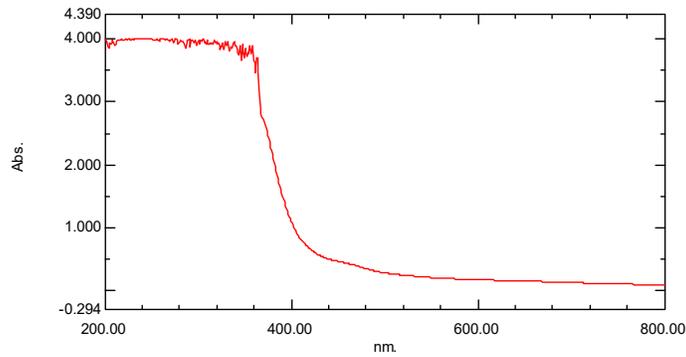


Figure 6: UV-Visible spectra of *Catharanthus roseus* extract

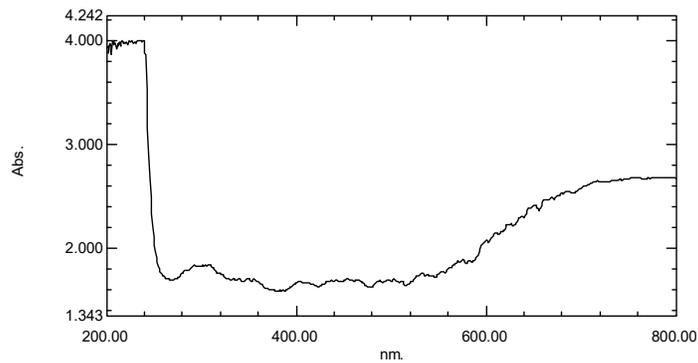


Figure 7: UV-Visible spectra of Calcium nanoparticles

Table 2: Swelling Study of all ratio films

Time	Swelling Ratio (g/g)		
	CMC:St (25:75)	CMC:St (50:50)	CMC:St (75:25)
0 min	0	0	0
30 min	1.69	2.47	1.18
60 min	3.18	3.71	1.68
120 min	3.55	3.74	3.875
180 min	3.67	4.19	4.11
240 min	5.122	4.46	4.78
24 hours	7.025	6.77	6.87

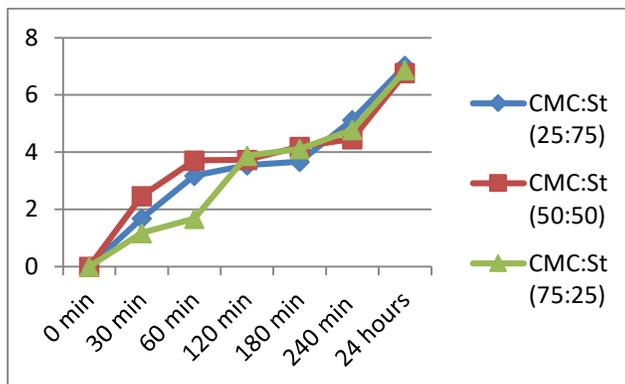


Figure 8: Swelling graph of CMC/Gelatin composite films

CONCLUSION:

This paper covers the fabrication of CMC/Gelatin composite film using solvent casting method. This polymeric composite was found to have absorption properties as per shown by swelling study. In addition to this, characterizations such as XRD, UV-visible spectroscopy, FTIR were done to determine structure, shape and functional groups in the sample as well as SEM analysis was carried out to determine surface morphology. Moreover, Preparation of *Catharanthus roseus* and calcium nanoparticles incorporated CMC/Gelatin composite film is also mentioned in this paper.

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