

Production and characterization of antimicrobial nanocomposite film based on Plantago major seeds gum/carboxymethyl cellulose reinforced by Cerium oxide nanoparticles and fenugreek essential oil

Seyyed Mohammad Ehsan Asadi

Urmia University

Sina Ardebilchi Marand

Urmia University

Saber Amiri (✉ sa.amiri@urmia.ac.ir)

Urmia University

Manuchehr Fazeli

Urmia University

Zhiyar Heidari

Urmia University

Seyyed Moein Nabavi

Urmia University

Ayla Elmi Kashtiban

Urmia University

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Abstract

This study aimed to produce *Plantago major* seeds gum (PMSG) and carboxymethyl cellulose (CMC) antimicrobial nanocomposite film by adding Cerium oxide nanoparticles (CeO₂ NPs) (2.5 and 5%W/W) as reinforcing additive and fenugreek seeds essential oil (FSEO) (4 and 8%W/W) as a bioactive agent. The nanocomposite films were prepared by casting method, and moisture content, moisture absorption, opacity, water vapor permeability (WVP), antimicrobial activity, and antioxidant properties of them were examined. FTIR, FESEM, XRD, and TGA tests were carried out to study the morphology, crystalline structure, and thermal stability of films, respectively. Moreover, mechanical properties, for instance, tensile strength (TS), elongation at break (EB), and thickness of the films, were measured. The results indicated that adding CeO₂ NPs improved the mechanical properties and formed new crystalline structures on the nanocomposite films. The inclusion of 5% CeO₂ NPs and 8% FSEO had a significant reduction in moisture content and moisture absorption ($p < 0.05$). Besides, FSEO addition did not have a significant effect on the WVP amount ($p > 0.05$). However, adding CeO₂ NPs and FSEO caused a significant effect on antimicrobial and antioxidant properties, and the highest antimicrobial and antioxidant activities were obtained by the film containing 2.5% CeO₂ NPs and 8% FSEO. The results showed that the TS was decreased by adding FSEO, and it increased by adding CeO₂ NPs, especially at higher concentrations. EB was significantly affected by the addition of CeO₂ NPs and FSEO, EB was enhanced by the addition of FSEO, and was reduced by the addition of CeO₂ NPs. Instrumental analysis FTIR, FESEM, XRD, and TGA tests showed high potential of PMSG/CMC-based nanocomposite reinforced by CeO₂ NPs and FSEO for food packaging technology.

Introduction

Appropriate packaging is essential to preserve food quality for a long time. Nowadays, there is an increasing interest in replacing petroleum-based plastic films with bio-based natural films in the food industry because of the consumption of food waste and environmental issues [1, 2]. Biopolymers can be a good substitute for synthetic polymers because of their inexhaustibility, non-polluting, harmlessness, and endurance. Several biopolymers, for instance, polysaccharides, proteins, and lipids, have been utilized to make environmentally friendly materials especially in food packaging [3–6]. Among these biopolymers, polysaccharides are evaluated as interesting sources due to their high ability to form films and good mechanical and barrier characteristics [7]. Carboxymethylcellulose (CMC) is a basic and carbohydrate derivative of cellulose and is amply accessible. CMC has high hydrophilic properties due to the high amounts of hydroxyl groups. Therefore, it has poor physical and mechanical characteristics for film formation [8]. Various nanofillers are used to make composites and enhance mechanical, antibacterial, thermal, and anti-water vapor permeability characteristics and control these obstacles. Nanofillers are classified into organic and inorganic ones. Organic nanofillers include clay, chitosan (biopolymer, and Nissin (antibacterial agent). Inorganic nanofiller consist of metal oxides like ZnO, TiO₂, MgO, AgO, and CeO₂ [9].

Polysaccharide polymers with functional properties as a beneficial source of commercial hydrocolloids are obtained from most seeds [10]. Mucilage is a kind of high molecular weight long chain hydrocolloid that is used extensively as food additive, for instance thickening agent and gelling agent [11]. *Plantago major* is a kind of medicinal plant in which leaves and seeds are utilized as an anti-inflammatory, painkiller, antioxidant, anti-infective, immune-moderating, anti-cancer, and also as wound healer [12]. Literature reveals that *P. major* has spread to various regions, particularly Europe, Asia, and America, since 4000 B.C. In addition, it has been used in ancient Iran in various locations due to its widespread availability [13]. *P. major* obtains a large quantity of seeds, which have been used for a long time as an antioxidant, analgesic, anti-inflammatory, anti-ulcerogenic, anti-microbial, anti-cancer, and immune-modulating agent. They have also been used to treat pain [12]. Maceration is the common extraction method of extracting mucilage from seeds. The mucilage with high viscosity is formed by absorbing water, including carbohydrate polymers like xylose, arabinose, glucose, galactose galacturonic acid, glucuronic acid, and rhamnose [10]. The seeds have a slight bitter taste and are small with an elliptical shape (0.4–0.8 · 0.8–1.5 mm). According to Qadry (1963), the swelling of the polysaccharides in the seed coat causes the capsules, which contain eight to sixteen seeds, to become gummy in hot weather [14]. SobhaniPoor et al. conducted a study. on the cytotoxic effects of *P. major* seed extract on MCF-7 cancer cells and how it is used in green AgNPs synthesis [15].

Metallic nanoparticles (NPs) have been extensively studied as antimicrobials. Due to the reinforcement phase interacts with dislocations, nanoparticle reinforcement enhances specific stiffness, strength, creep resistance, and thermal conductivity [16]. The current interest in utilizing novel materials like metal oxide nanoparticles and natural polymers may improve some desired characteristics, such as electrical conductivity, antimicrobial capacity, and magnetic conductivity [17]. Cerium oxide nanoparticles (CeO_2 NPs) have antioxidant (oxygen scavenger ability) or pro-oxidative characteristics depending on the environment pH, so they can be used in active packaging [18, 19]. CeO_2 -NPs has antibacterial, autocatalical, biocompatible properties [20].

The annual legume *Trigonella foenum-graecum*, also known as Fenugreek, is a member of the Fabaceae family. Fenugreek seeds essential oil (FSEO) has various components, for instance, phenolics, flavonoids, alkaloids, tannins, anthracene glycosides, and saponins which are impressive antimicrobial agents [21]. FSEO has possible protection impacts as opposed to diverse food-borne cause bacteria strains. Seyedi et al. (2014) reported that FSEO has an antimicrobial effect on Gram-negative and Gram-positive bacteria consisting of *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* [22]. Furthermore, due to Alwhibi et al., Fenugreek oil was found to be effective against both gram-positive and gram-negative bacterial isolates, including *Staphylococcus aureus*, *Salmonella Typhi*, *E. coli*, and *Pseudomonas aeruginosa* [23].

To our knowledge, there is not specific research about preparing biodegradable active films utilizing both CeO_2 NPs and FSEO. In this manuscript, the production of active nanocomposite films based on *Plantago major* seeds gum (PMSG) and CMC containing CeO_2 NPs and FSEO and their physicochemical, biochemical, inhibitory, mechanical, structural, and morphological properties were studied.

Materials And Methods

Materials

Plantago major seeds and fenugreek seeds were bought from the local market in Urmia, Iran. 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), CeO₂ NPs, CMC powder, and glycerol were purchased from Sigma Aldrich, USA. Distilled water and other chemical and reagents were analytical grades purchased from representatives of reputable companies in Tehran, Iran.

Preparation Of Nanocomposite Films

The casting method was used to prepare PMSG-CMC nanocomposite films containing FSEO and CeO₂ NPs. First, 0.5 grams of *Plantago major* powder was dissolved in 50 mL of distilled water at a temperature of 80°C. Then, 1 gram of carboxymethyl cellulose was dissolved separately in 50 mL of distilled water at room temperature. The resulting solutions were combined and stirred for 1 hour. Then cerium oxide nanoparticles were added and mixing was done again for 30 minutes. After the temperature of the solution dropped to 35 degrees, fenugreek plant essential oil and finally glycerol were added to the solution. Finally, 50 mL of film solutions were poured into 8 cm plates and dried at room temperature. The control film was made using only carboxymethyl cellulose and *Plantago major* in the same conditions. Subsequent to drying, the film was taken out from the plate surface and assessed.

Characterization And Properties Of The Film

Moisture content

The moisture content of the nanocomposite films was evaluated by the oven-drying method. The weight of the nanocomposite films was calculated before and after drying in a conventional oven. The samples remained at 110 °C for 24 h. They were determined in triplicate, and the results were reported in percentage [24].

Moisture Absorption

Moisture absorption of the nanocomposite films was evaluated based on the Sadeghnezhad et al. method [25]. Initially, pieces of the dried nanocomposite films (20×20mm) were kept in an oven at 50°C for 24h and weighed. Then, they were put in the desiccator comprising K₂SO₄ solution with RH 97% at 20–25°C. Then, the weight of the samples was measured at proper time intervals until it reached a constant weight. Ultimately, the moisture absorption was measured by the following equation:

$$Moisture\ absorption = \frac{W_f - W_0}{W_0} \times 100$$

1

Where W_f is the weight of the final weight of the sample, and W_0 is the initial weight of the sample.

Water Vapor Permeability (WVP)

To calculate the WVP of the samples, the water vapor transmission rate (WVTR) should be determined first. WVTR was measured by the standard cup method ASTM (1995) with some modifications. At first, 8 g silica gel was filled into the special aluminum cups, then slightly larger pieces (than the diameter of the cups) of films were sealed in the cup. Then 3 g water-free CaSO_4 was added to each cup. Using water-free CaSO_4 led to zero relative humidity in the cups. The cups were put in the desiccator with K_2SO_4 saturated solution. K_2SO_4 produces a constant relative humidity of 97%. The desiccator was kept inside the oven at 25°C for the whole research period. The weight of the samples was measured every 1 hour for 5 days. The WVTR was calculated by the following equation where the slope was the slope of the resulting line of the weight gain curve over time determining the linear regression, A (cm^2) was the area exposed in the cup:

$$WVTR \left(\text{gday}^{-1} \text{mm}^{-2} \right) = \frac{\text{slope}}{A}$$

2

Afterward, the WVP of the films was calculated by the following equation:

$$WVP = (WVTR \times L) / \Delta p$$

3

Where L = the thickness of the film (m), Δp = water vapor pressure difference through the film [26, 27].

Opacity

The nanocomposite films were cut into rectangular pieces ($2 \times 2 \text{ mm}$) and placed on the top of the cuvette. The absorbance of the empty cuvette and the samples were recorded at 600 nm by applying a UV-visible spectrophotometer (Model 80-2088-64, Pharmacia LKB Biochrom, Cambridge, UK). The opacity content of the films was calculated by the following equation [28, 29]:

$$Opacity = \frac{Abc_{600}}{X}$$

4

where Abc_{600} is the absorbance value at 600 nm, and X represents the film thickness (mm).

Antioxidant Activity

According to Amiri et al. [30] 0.25 g of each film sample was added to the 4 mL pure water and vibrated for 5 min, later the mixture was added to 1 mL DPPH solution and incubated at room temperature for 30 min and calculated the absorbance at 517 nm. Free radicals scavenging activity (FRSA) was calculated as follows:

$$FRSA = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

5

Antimicrobial Test

Amiri et al. [31] method was used to assess the antibacterial activity of the films. Disk diffusion method was utilized, and *Escherichia coli* PTCC1763 (*E. coli*) and *Staphylococcus aureus* PTCC 1431 (*S. aureus*) were recognized. 20 mL tryptic soy broth was used to inoculate these bacterial strains. They were incubated for 37 h at 37 °C. Next, cultured microorganisms were put in the centrifuge for 10 min. Then, a ten-fold dilution of uncultivated tryptic soy broth was performed. $2 \times 2\text{cm}^2$ pieces of film samples were prepared and put on a particular plate of Muller Hinton Agar which was previously inoculated with 0.1 mL of $10^5\text{-}10^6$ CFU/mL pathogens. Afterward, the plates were incubated for 24 h at 37 °C, and the inhibition zone areas around the films were measured by a digital caliper.

Mechanical Properties

The thickness of the biofilms was determined by applying a digital micrometer. Measurements were performed in five different places for each film and reported their average value [32]. Tensile Strength (TS) and elongation at break (EB) were determined using an Instron Universal Testing Machine (TA. XT plus texture analyzer, Stable Microsystems, UK) according to the standard (ASTM19 D882). The procedure was performed with 100 mm/min crosshead speed and 20 mm initial grip separation. For measurement of the mechanical properties, the samples were cut into rectangular strips ($0.5 \times 0.5\text{cm}$). The TS and EB were calculated by the following equations:

$$TS (MPa) = \frac{F_{max}}{A}$$

6

$$EB (\%) = \frac{\Delta L}{l_0} \times 100$$

7

Fourier-transform Infrared (FTIR) Spectroscopy

FTIR is a molecular oscillation spectroscopy to evaluate molecular structure and composite composition. The prepared films were utilized directly for FTIR analysis. They were recorded with (Spectrum two, Perkin Elmer, USA) at the $400\text{--}4000\text{ cm}^{-1}$ wavenumber with the resolution of 4 cm^{-1} [33].

Thermogravimetric Analysis (TGA)

The thermal stability of the biofilms (10–15 mg) was estimated by the thermo-gravimetric analyzer (Linseis model Sta Pt-1000 Germany) by heating from $25\text{ }^{\circ}\text{C}$ to $700\text{ }^{\circ}\text{C}$ under the nitrogen flow condition ($10\text{ }^{\circ}\text{C}/\text{min}$). The heating rate was set at $60\text{ mL}/\text{min}$ [26].

X-ray Diffraction (XRD) Analysis

The structure of extended crystalline biofilms was determined by an X-ray diffractometer instrument (Siemens, model D 5005, Baden Wurttemberg, Germany). The setting of the generator of the X-rays was 40 kV and 40 mA . XRD patterns were achieved at $2\theta = 10 - 70^{\circ}\text{C}$ with a scan rate of $1\text{ degree per minute}$ [26].

Field Emission Scanning Electron Microscopy (FESEM)

A field emission scanning electron microscope (XL30 ESEM-FEG, Philip, Netherland) was used to observe film surface, broken surface, cross-section, and nanocomposites performing at different magnifications. Initially, the samples were coated with gold nanoparticles before observations [25].

Statistical analysis

Statistical analyses were performed based on a completely randomized design using a one-way analysis of variance (ANOVA) procedure followed by Tukey's test with Minitab software version 20 (Minitab Inc., State College, PA, USA) at first error $\alpha \leq 0.05$ to determine significant differences. The experimental design is shown in Table 1.

Table 1
Samples with different concentrations of CeO₂ NPs and FSEO and abbreviations

Samples	Abbreviation	CeO ₂ NPs (%W/W)	FSEO (%W/W)
1	NPs 0/EO 0	0	0
2	NPs 0/EO 4	0	4
3	NPs 0/EO 8	0	8
4	NPs 2.5/EO 4	2.5	4
5	NPs 2.5/EO 8	2.5	8
6	NPs 5/EO 4	5	4
7	NPs 5/EO 8	5	8
8	NPs 2.5/EO 0	2.5	0
9	NPs 5/EO 0	5	0
CeO ₂ NPs: Cerium oxide nanoparticles and FSEO: Fenugreek seeds essential oil			

Results And Discussion

Moisture content

According to Table 2, moisture content of the films ranged from 17.02 to 26.73. the control sample had the highest amount of moisture content, while the lowest amount was the sample with CeO₂ NPs 5% + FSEO 8%. The inclusion of the CeO₂ NPs and FSEO caused a significant reduction in the moisture content of the film samples ($p < 0.05$). The same results were achieved by Kalaycioglu et al. They reported that adding CeO₂ NPs caused a decrease in the moisture content of the films [34].

Moisture Absorption

Moisture absorption is an important index to evaluate the water barrier properties of the films. As shown in Table 2, moisture absorption varied from 9.31 to 23.31. It decreased with adding FSEO and CeO₂ NPs. The inclusion of the FSEO and CeO₂ NPs was significantly effective ($p < 0.05$). A considerable diminish was gained by CeO₂ NPs 5% + FSEO 8%. It might be due to the strong hydrogen bonds between hydroxyl groups of the CMC [35, 36, 37]. Similar findings have been obtained by [25].

Water Vapor Permeability (WVP)

Water vapor permeability is an important characteristic of food packaging. WVP of many bio-polymeric films is enhanced by the considerable hydrogen bonds. However, hydrophilic plasticizers can induce WVP of biopolymers. While lipid substances can notably amend the WVP of biopolymers, they cause poor mechanical properties, a waxy taste remaining, and a non-transparent appearance of the films. Nanomaterials can boost the moisture, gas barrier, and aroma without making a specific change in taste and opacity and they also improve mechanical properties of the films. The WVP values varied between 3.9 and 6.73×10^{-9} g/m.s.Pa. Adding FSEO wasn't significantly effective in the WVP value ($p > 0.05$), but the inclusion of NPs had a significant effect on the WVP value ($p < 0.05$). Due to Table 2, the WVP of the films was reduced with the inclusion of the CeO₂ NPs. Thus, the barrier property of the films was amended because of the CeO₂ NPs spread in the CMC matrix. NPs fill the spaces and voids among the molecules of the films and lessen the porosity and additionally decrease the WVP of the films. Similar results are achieved by Marandi et al. (2022) and Swaroop et al. (2018) utilizing MgO and CuO NPs, respectively [38, 39].

Opacity

The opacity of the film samples is shown in Table 2. The opacity of the control sample and CeO₂ NPs 5% + FSEO 8% was 7.31 and 12.31, respectively. Adding CeO₂ NPs and FSEO caused a significant impact on the opacity of the films ($p < 0.05$). A lower opacity value indicates a higher transmittance of the films. The same results were reported by Dhull et al. (2021). They used FSEO in the production of cross-linked starch films, the opacity of essential oil-added films was higher than the neat film. It might be due to the enhancement in the thickness of the essential oil-added films [40]. Zahedi et al. evaluated that increasing the concentration of the ZnO NPs to 4% leads to a completely opaque film [41].

Antioxidant Activity

Generally, phenolic compounds in herbal extracts can perform the function of reducing agents and convert the cation (Ce⁺) to radicals (Ce[•]). Therefore, they could stabilize the constructed NPs and prevent agglomeration of CeO₂ NPs [42]. *P. major* extract consists of alkaloids, terpenoids, flavonoids, phenolic acids, fatty acids, polysaccharides, glycosides, and vitamins [43]. According to Table 2, the highest antioxidant activity was shown in NPs 2.5%/ FSEO 8% sample. The inclusion of CeO₂ NPs and FSEO had a significant effect on the antioxidant activity of the films ($p < 0.05$). The ions of CeO₂ NPs hamper the interactions of free radicals in the film matrix and enhance the antioxidant activity of the films. Similar results were reported by Marandi et al. They evaluated the effect of adding CuO NPs and *Mentha asiatica* oil on zein biodegradable films. They deduced that the inclusion of CuO NPs and *M. asiatica* oil enhances the antioxidant activity of the films. Likewise, the existence of the phenolic compounds in the FSEO, like α -pinene, γ -terpinene, camphor, and caryophyllene is indicative of the antioxidant activity of the FSEO [38].

Table 2
Physicochemical and biochemical properties of the films

Films	Moisture content (%)	Moisture absorption (%)	WVP ($\times 10^{-9}$ g/m.s.Pa)	Opacity (%)	Antioxidant activity (%)
NPs 0/EO 0	26.73 $\pm$ 0.35 ^a	23.31 $\pm$ 1.07 ^a	6.73 $\pm$ 0.03 ^a	7.31 $\pm$ 0.36 ^e	3.53 $\pm$ 0.03 ^f
NPs 0/EO 4	25.01 $\pm$ 0.49 ^b	19.14 $\pm$ 1.76 ^b	4.96 $\pm$ 0.01 ^d	8.62 $\pm$ 0.54 ^{de}	13.32 $\pm$ 0.01 ^c
NPs 0/EO 8	23.82 $\pm$ 0.33 ^c	16.18 $\pm$ 0.75 ^c	5.80 $\pm$ 0.02 ^b	9.01 $\pm$ 0.82 ^d	19.31 $\pm$ 0.04 ^a
NPs 2.5/EO 4	22.07 $\pm$ 0.12 ^d	15.15 $\pm$ 1.39 ^d	3.90 $\pm$ 0.05 ^g	9.84 $\pm$ 0.17 ^d	12.17 $\pm$ 0.04 ^d
NPs 2.5/EO 8	20.10 $\pm$ 0.64 ^e	13.22 $\pm$ 0.95 ^e	4.12 $\pm$ 0.01 ^f	10.17 $\pm$ 0.51 ^c	18.92 $\pm$ 0.07 ^b
NPs 5/EO 4	20.40 $\pm$ 0.44 ^e	13.01 $\pm$ 1.05 ^e	4.33 $\pm$ 0.06 ^e	11.23 $\pm$ 0.24 ^b	12.01 $\pm$ 0.06 ^d
NPs 5/EO 8	17.02 $\pm$ 0.81 ^f	9.31 $\pm$ 0.97 ^f	5.20 $\pm$ 0.03 ^c	12.31 $\pm$ 0.90 ^a	18.63 $\pm$ 0.09 ^b
NPs 2.5/EO 0	23.42 $\pm$ 0.56 ^c	18.42 $\pm$ 1.10 ^b	4.43 $\pm$ 0.07 ^e	8.93 $\pm$ 0.19 ^{de}	4.32 $\pm$ 0.05 ^e
NPs 5/EO 0	21.31 $\pm$ 0.09 ^e	16.02 $\pm$ 1.13 ^c	4.56 $\pm$ 0.08 ^e	10.82 $\pm$ 0.32 ^c	4.02 $\pm$ 0.02 ^e

Antimicrobial Properties

Figure 1 shows the *in vitro* antibacterial properties of the CMC/PMSG-based nanocomposite films. The control film didn't have any inhibitory effect on *E. coli* (Gram-negative bacterium) and *S. aureus* (Gram-positive bacterium). The results indicated that CeO₂ NPs increased the antimicrobial activity thank to the high surface-to-volume ratio. Utilizing CeO₂ NPs in high concentrations can lead to a high antimicrobial property of film samples. CeO₂ NPs decrease reactive oxygen species, and this reduction accelerates cell death. Same results were obtained by Kalantari et al. [18]. The antibacterial properties of FSEO are associated with the phenolic compounds existing in the structure of FSEO. Figure 1 reveals the significant antimicrobial effect of FSEO on gram-negative and gram-positive bacteria. The same findings were

reported by Asdaghi et al. [44] utilizing coconut essential oil. In addition, the inclusion of CeO₂ NPs and FSEO had the most significant impact on both bacteria.

Mechanical Properties

Table 3 presents the mechanical properties of the prepared CMC/PMSG-based nanocomposite films. The thickness of the control sample was 0.052 mm, and it enhanced significantly ($p < 0.05$) by adding CeO₂ NPs and FSEO. The thickness content of samples varied from 0.052 to 0.111 mm. The highest thickness was attained at CeO₂ NPs 5% and FSEO 8%. Tensile strength (TS) and Elongation at break (EB) were significantly affected by adding NPs and FSEO ($p < 0.05$). As shown in Table 3, the value of TS ranged from 7.93 to 12.4 MPa. According to the results, the value of TS was decreased when FSEO was added, and it increased when NPs included, specially at higher concentrations. The highest content of TS was obtained at adding NPs 5% without FSEO. Also, Dao et al. evaluated that CeO₂ NPs inclusion cause an enhancement in the increase of the TS [45]. Similar results achieved by Kalaycioglu et al. They reported that adding CeO₂ NPs causes an increment in TS, indicating that CeO₂ NPs improve the mechanical strength of the films [34]. However, the amount of EB varied from 129.27 to 160.11%. EB was enhanced by the inclusion of FSEO, and reduced by adding NPs. The maximum amount of EB was achieved at FSEO 8% without adding NPs. Dhull et al. [40] reported that adding FSEO caused an increment in the thickness of the film samples. Furthermore, this is similar to Sharma et al. findings They deduced that the enhancement of the thickness resulted from the spread of the FSEO [46]. Similar results were obtained by Dhull et al. showed that the addition of FSEO caused a reduction in TS, and an increment in EB [40]. Souza et al. concluded that it might be because of the essential oil impact on the reduction of intermolecular interaction and lower TS of the film samples [47]. Besides, Li et al. deduced that adding essential oil cause the substitution of the stronger intermolecular interactions with weaker interaction, leading more flexible films [48].

Table 3
Mechanical properties of the prepared films

Films	Thickness (mm)	Tensile strength (MPa)	Elongation at break (%)
NPs 0/EO 0	0.052 ± 0.003^f	8.27 ± 0.20^c	$137.21 \pm 1.121_d$
NPs 0/EO 4	0.061 ± 0.002^e	8.01 ± 0.18^c	$148.17 \pm 1.63_b$
NPs 0/EO 8	0.079 ± 0.001^d	7.93 ± 0.35^c	$160.11 \pm 0.98_a$
NPs 2.5/EO 4	0.081 ± 0.001^d	8.41 ± 0.11^c	$142.81 \pm 0.78_c$
NPs 2.5/EO 8	0.085 ± 0.001^c	8.42 ± 0.10^c	$160.01 \pm 1.00_a$
NPs 5/EO 4	0.107 ± 0.002^b	10.27 ± 0.08^b	$143.01 \pm 1.72_c$
NPs 5/EO 8	0.111 ± 0.001^a	10.07 ± 0.15^b	$159.91 \pm 1.01_a$
NPs 2.5/EO 0	0.103 ± 0.002^b	9.87 ± 0.21^b	$136.01 \pm 1.56_d$
NPs 5/EO 0	0.106 ± 0.002^b	12.36 ± 0.12^a	$129.27 \pm 2.05_e$

FTIR Analysis Results

Alteration in specific peaks considers the chemical interaction between the film components, specifically CeO₂ NPs and FSEO. Figure 2 represents the FTIR spectrum of control film (CMC/PMSG), run3 (CMC/PMSG + FSEO 8%), run 7 (CMC/PMSG + CeO₂ NPs 5% + FSEO 8%), and run 9 (CMC/PMSG + CeO₂ NPs 5%). Generally, no significant changes were noticed in the FTIR spectrums. The broad peak between 3100–3500 cm⁻¹ correlates with O-H extended of hydroxyls and bound water [49]. The peak at 679 cm⁻¹ in run 3 is related to the C-H bond bending mode. The bond in the range of 3200–3500 cm⁻¹ is attributed to the –OH group of aromatic structures, which authenticates the presence of the phenolic compounds in the *P. major* extract. The peak at 2925 cm⁻¹ is related to carboxylic acids. The peak observed at 1594 cm⁻¹ is also attributed to carboxylic acids. The absorption peak at 1033 cm⁻¹ indicates the existence of alcohols in the *P. major* extract. According to the results, phenolic compounds of the *P. major* extract can perform the function of reducing agent of Ce⁺ ions, thus can stabilize the NPs formation.

TGA Analysis Results

The thermal stability of the films is determined by TGA analysis. Figure 3 shows TGA thermograms. The initial mass reduction occurred at 92-101°C due to the evaporation of moisture. The second and major decrease in weight loss of run 1, run 3, run 7, and run 9 was observed at 244.8, 233.5, 235.7, and 244.3°C,

respectively. $T_{\max}$ of the control sample and the sample with 5% CeO_2 NPs were close. Besides, the $T_{\max}$ of the sample, including FSE08%, and the sample, including FSE08%/5% CeO_2 NPs was approximately the same. It can be concluded that adding NPs can increase thermal stability due to homogenous dispersion. However, the inclusion of FSEO damage the efficiency of CeO_2 NPs and diminish its improvement impact. The same results were reported about the weakening effect of essential oils and the healing ability of NPs on the thermal characteristics of other biofilms [38, 47, 50, 51].

XRD Analysis Results

The crystalline structure of biofilms was studied by XRD. Figure 4 shows the XRD diffractograms of pure CMC films, CMC with 8% FSEO, CMC with 5% CeO_2 NPs, and CMC with 8% FSEO and 5% CeO_2 NPs. According to Fig. 4, adding essential oil did not have a considerable impact on the crystalline behavior of the films. Run1 and run 3 XRD diffractograms had similar sharp peaks at 2θ equal to 32° , 45.5° , and 56.5° . The same results were achieved by Niknam et al. [52]. The inclusion of CeO_2 NPs generated new crystalline structures at 28.5° , 33° , and 47.5° . These new sharp peaks indicate the enhancement of the crystalline structure of the films with CeO_2 NPs.

FESEM Analysis Results

Figure 5 (a-d) shows the electron microscopy of the surface of control film (CMC/PMSG), (CMC/PMSG + FSEO 8%), (CMC/PMSG + CeO_2 NPs 5% + FSEO 8%), and (CMC/PMSG + CeO_2 NPs 5%), respectively under the magnification of 20 KX and 50 KX. FESEM image under 20 KX of the control sample indicates that the control film had a harsh and rugous structure with many pores and ridges, while under 50 KX a smooth and uniform structure was observed. The presence of a large number of cracks and pores causes an increase in WVP amount. Therefore, the control sample has a high amount of WVP. As shown in Fig. 5b and 5d, essential oil addition caused a smoother surface on the film samples. Similar results obtained by were Dhull et al. [40] and Asdaghi et al. [44]. Figure 5c represents the surface morphology of the film consisting of 5% CeO_2 NPs. Adding NPs caused no agglomeration, and CeO_2 NPs were spread uniformly throughout the film. Figure 5d shows the inclusion of the highest amount of CeO_2 NPs and FSEO. In this film, a decrease in the amount of cracks and pores is noticed, and the film was smoother due to the essential oil addition. The same results were achieved by Marandi et al. [38].

Conclusion

In the present paper, CMC comprising CeO_2 NPs and FSEO was employed to produce biofilms. The biofilms containing 5% CeO_2 NPs had maximum TS. However, the biofilms containing 8% FSEO had major thickness and EB among other film samples. The addition of CeO_2 NPs and FSEO diminishes moisture content and moisture absorption, increase opacity, and antimicrobial and antioxidant activity of biofilms. The inclusion of FSEO did not have a significant effect on the WVP amount. However, the

addition of CeO₂ NPs reduced WVP. Due to FESEM results, FSEO inclusion caused a smoother surface, and the inclusion of CeO₂ NPs reduced the cracks and pores of the biofilms. XRD test analysis indicated a new crystalline structure generated by adding CeO₂ NPs, but FSEO did not have a significant effect on the formation of crystalline structure. Moreover, the results of the TGA test showed that CeO₂ NPs increment thermal stability of the biofilms, besides FSEO diminished this enhancement.

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Figures

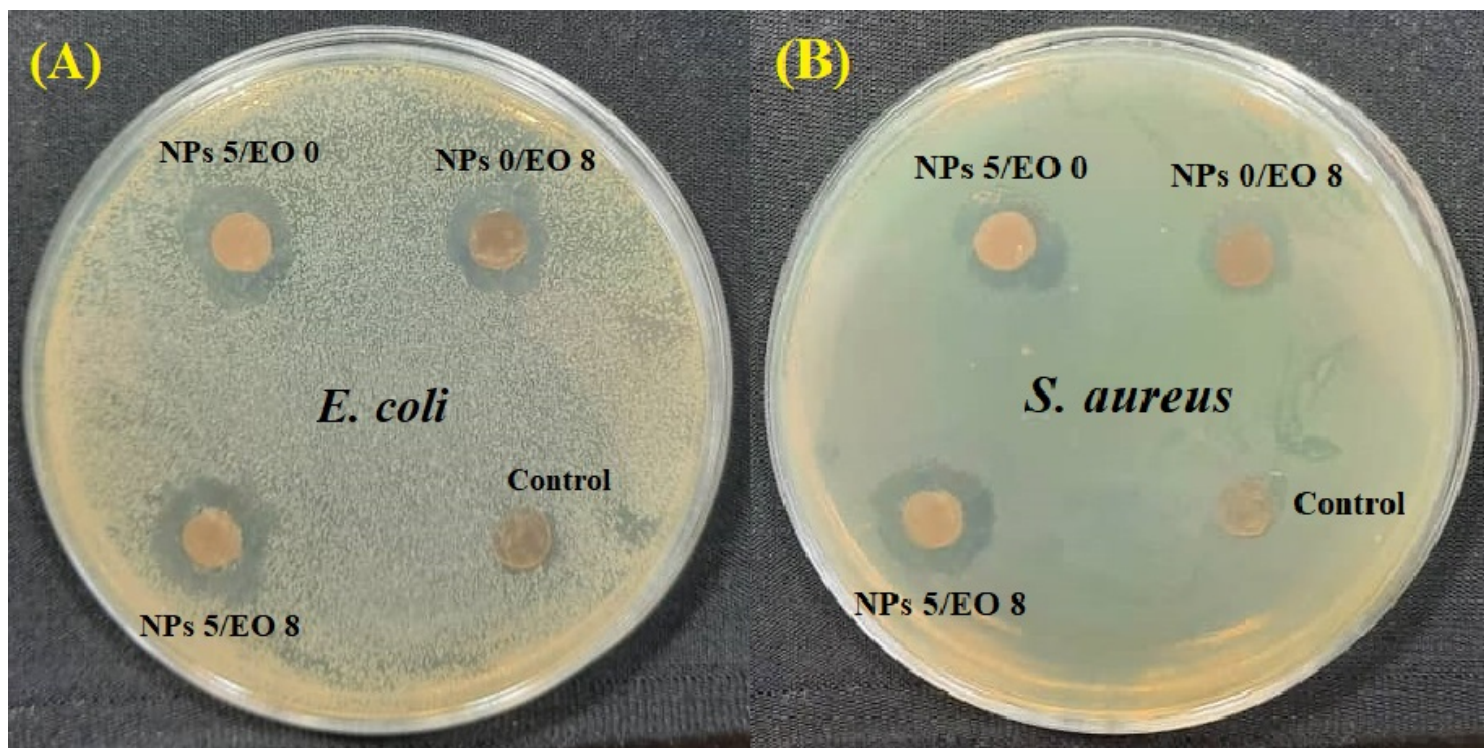


Figure 1

the images of antimicrobial properties of films: CMC/PMSG, CMC/PMSG + FSEO 8, CMC/PMSG + CeO₂ NP_s 5%, CMC/PMSG + CeO₂ NP_s 5% + FSEO 8

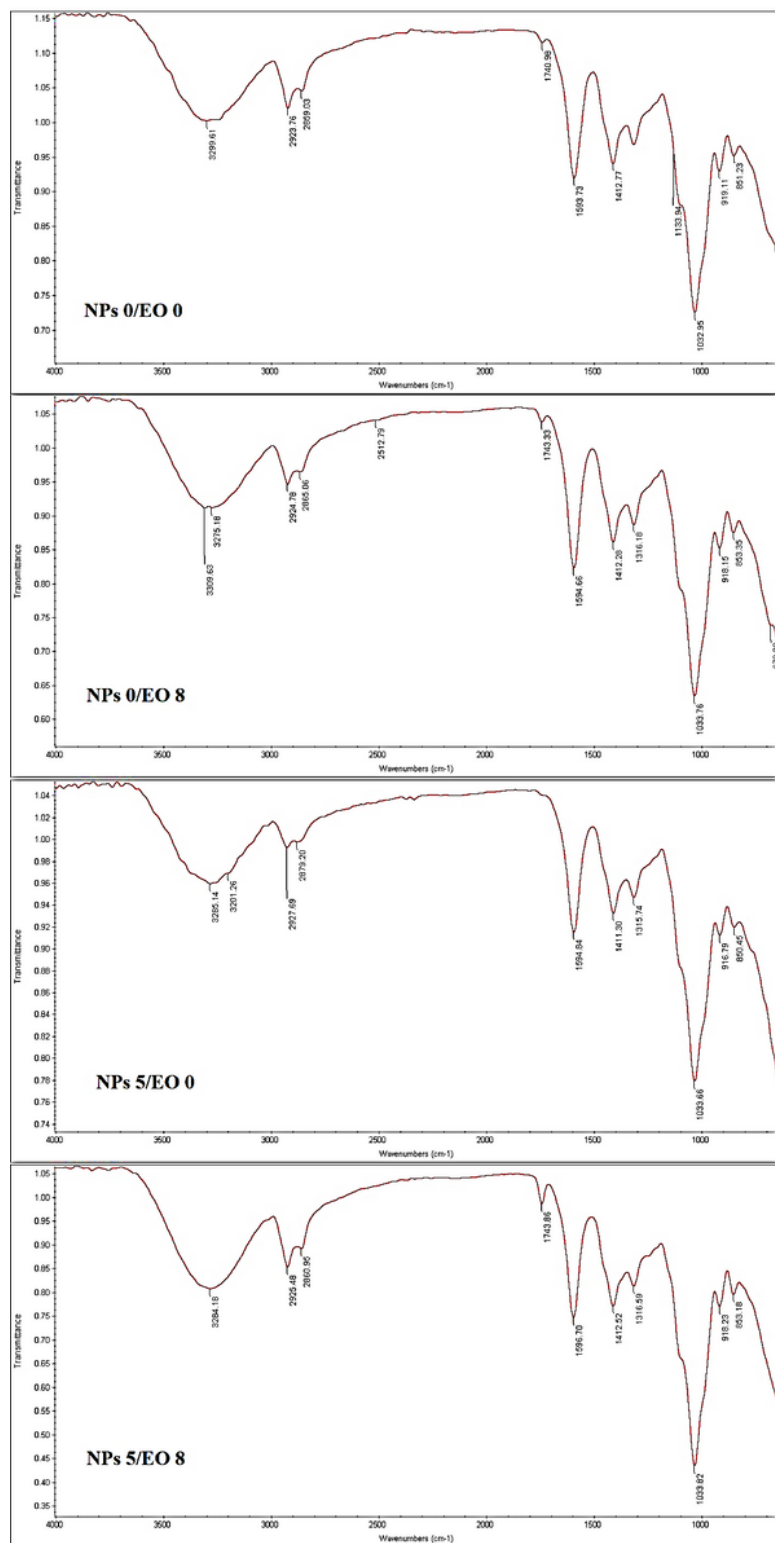


Figure 2

FTIR spectrums of films: CMC/PMSG, CMC/PMSG + FSEO 8, CMC/PMSG + CeO₂ NPs 5%, CMC/PMSG + CeO₂ NPs 5% + FSEO 8%

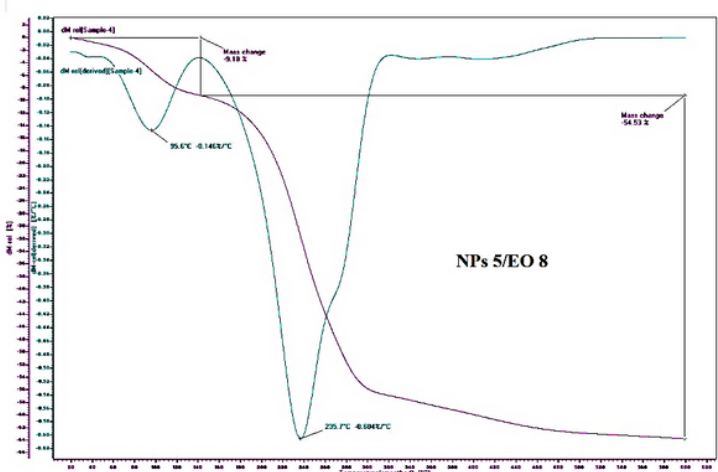
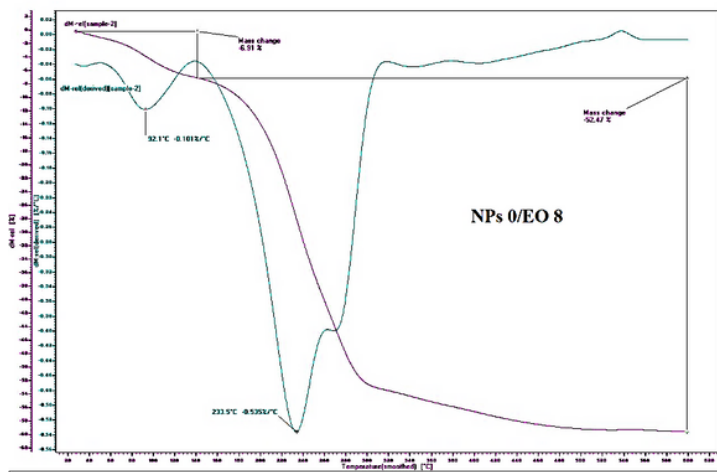
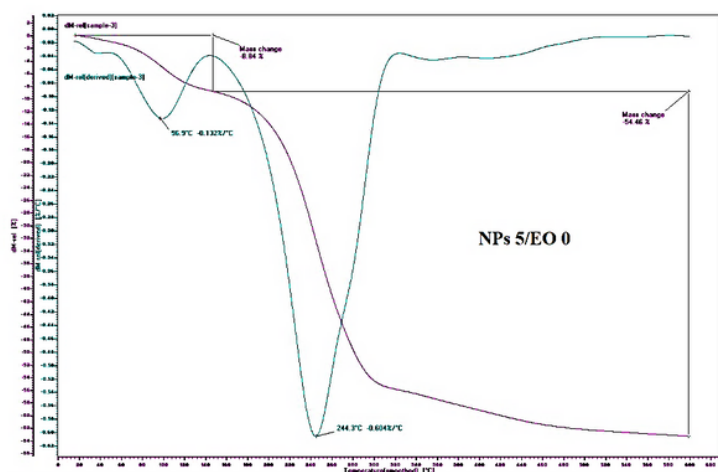
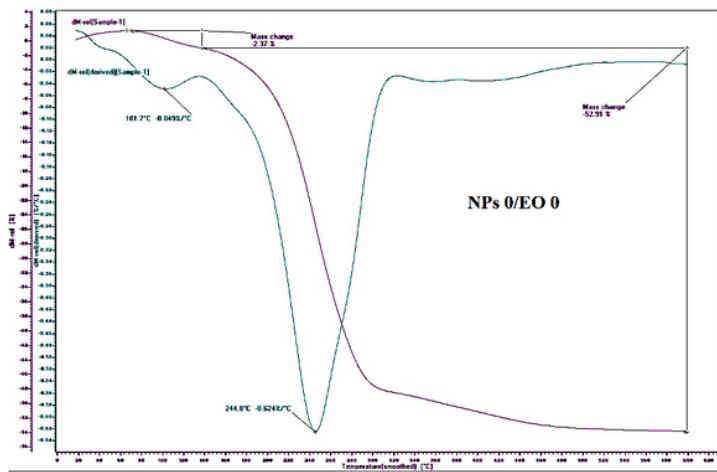


Figure 3

TGA thermograms of films: CMC/PMSG, CMC/PMSG + CeO₂ NPs 5%, CMC/PMSG + FSEO 8%, CMC/PMSG + CeO₂ NPs 5% + FSEO 8%

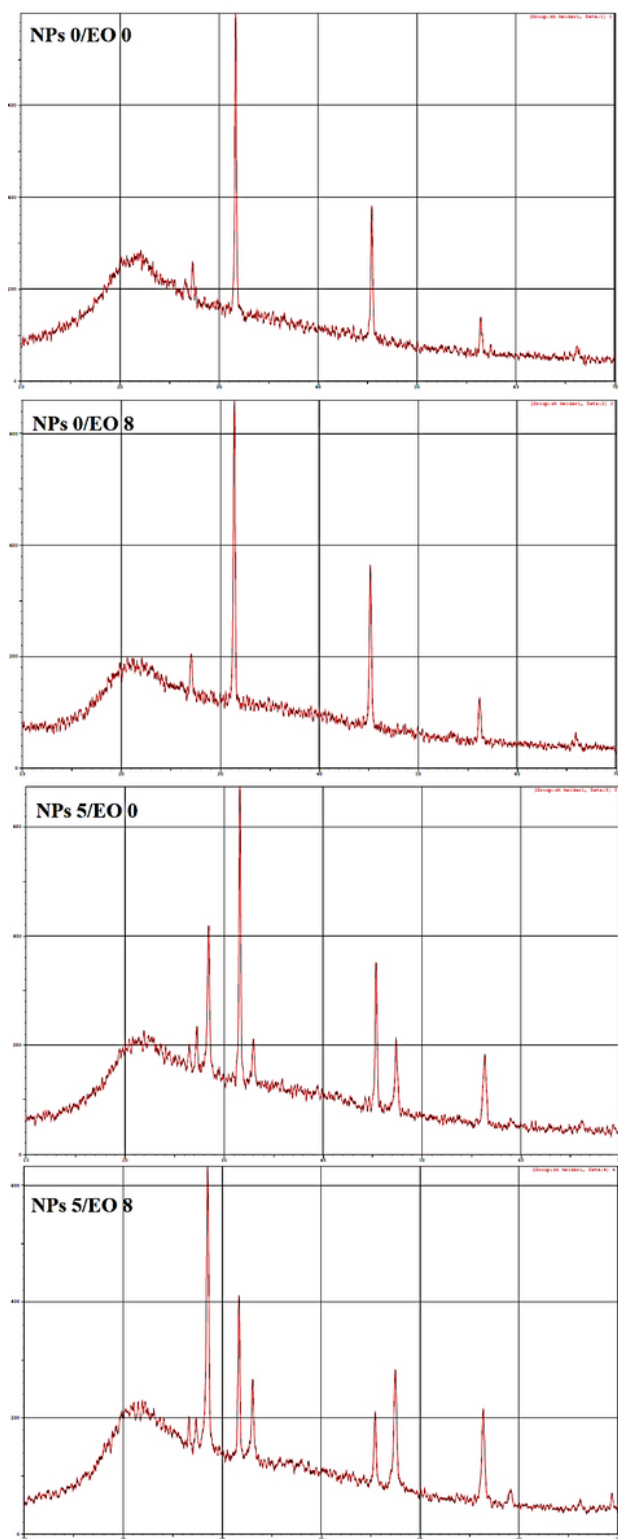


Figure 4

XRD diffractograms of films: CMC/PMSG, CMC/PMSG + CeO_2 NPs 5%, CMC/PMSG + FSEO 8%, CMC/PMSG + CeO_2 NPs 5% + FSEO 8%

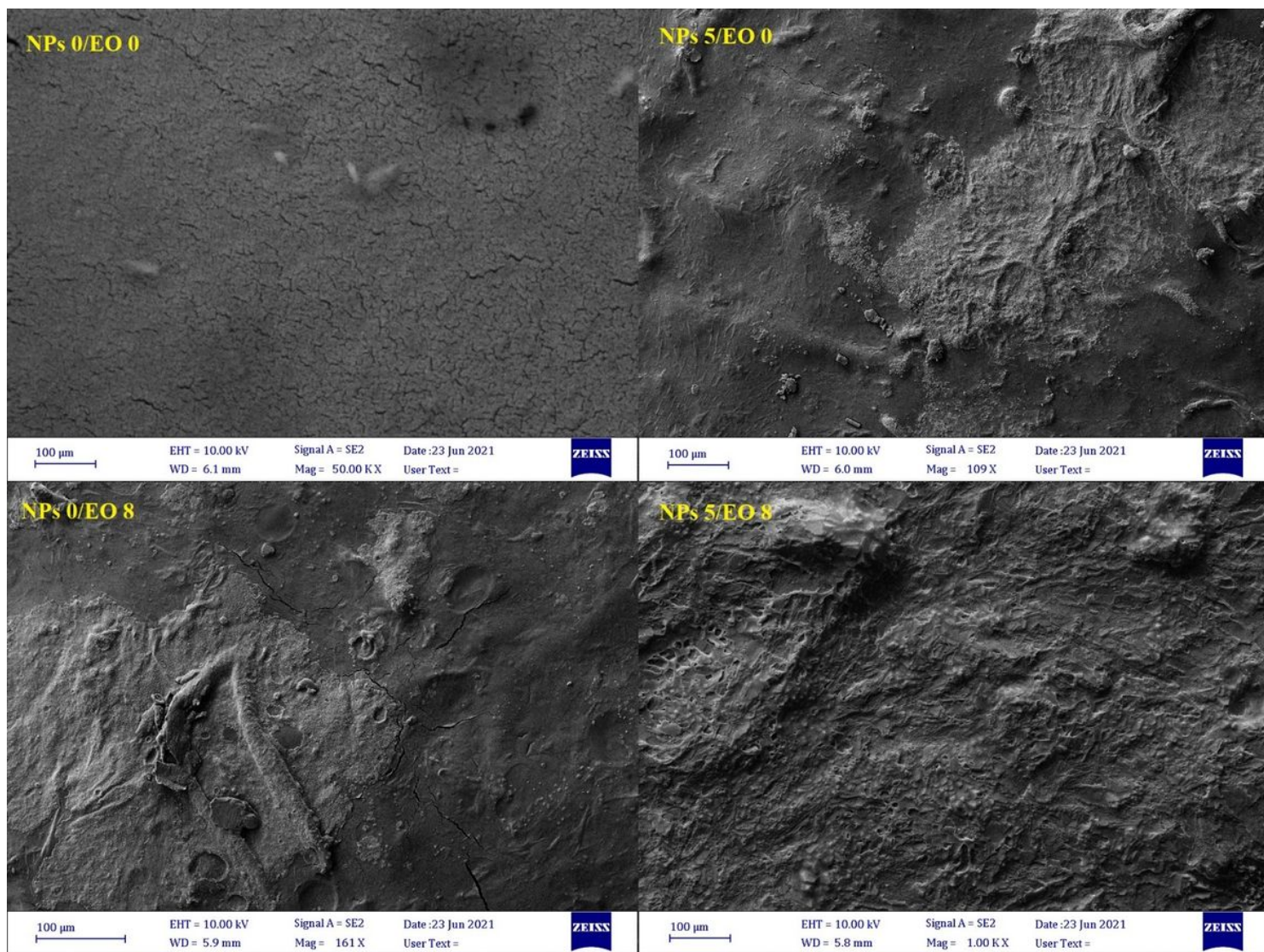


Figure 5

FESEM images of films: CMC/PMSG, CMC/PMSG + CeO₂ NPs 5%, CMC/PMSG + FSEO 8%, CMC/PMSG + CeO₂ NPs 5% + FSEO 8%