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Production of Polyvinylpyrrolidone Nanowebs Containing Zinc Cyclohexane Mono Carboxylate via Electrospinning and Investigation of Antibacterial Efficiency

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Abstract

*In this study, after the synthesis of zinc cyclohexane mono carboxylate, its chemical structure was analysed with FTIR and TGA. Then electrospun polyvinylpyrrolidone nanofibres containing zinc cyclohexane mono carboxylate were produced and antibacterial properties of the nanowebs obtained were investigated for their use in the textile field. When the FTIR results of the nanofibres containing different concentrations of zinc cyclohexane monocarboxylate are examined, an –OH peak similar to that of PVP fibres is noticed. These results clearly indicate that zinc cyclohexane monocarboxylate is included in PVP. When the TGA spectra of CHMCZn doped nanofibres at different ratios are examined, it is seen that they give more similar results than polyvinylpyrrolidone nanofibres alone. According to the SEM-EDX analyses, it was observed that the fibre diameters obtained were in the range of 145-947 nm. On the other hand, antimicrobial activity against *B. subtilis*, *S. aureus* and *E. coli* strains was detected, regardless of the CHMCZn concentration.*

Key words: electrospinning, nanoweb, zinc cyclohexane mono carboxylate, antibacterial.

Introduction

Nanofibres have been attracting increasing attention in recent years. Nanofibres generally refer to fibres with diameters less than several hundred nanometers [1]. Electrospinning is a simple and versatile technique that relies on the electrostatic repulsion between surface charges to continuously draw nanofibres from a viscoelastic fluid [2]. It is a very effective, simple and inexpensive method of producing nanofibres from polymer solution as compared to others, such as self-assembly and phase separation, and has the potential for large-scale manufacturing [3]. Although the term “electrospinning”, derived from “electrostatic spinning”, has been used only relatively recently (in around 1994), its fundamental idea dates back more than 70 years [4]. Nanofibres that are produced by using electrospinning have a diameter and morphology that depend on many factors, including the dissolution, dispersion, viscosity, and surface tension of polymers, the volatility and electrical conductivity of the solvents, as well as the electrospinning voltage, collection distance, ambient temperature and humidity [5]. Recently, a significant number of studies have concentrated on this technique due to its potential applications in diverse fields [6]. Both natural and synthetic polymers can be used as raw materials to prepare polymer solution which is used to produce a non-woven fibre structure by electrostatic force [7]. The diameter

of nanofibres produced via the electrospinning technique usually ranges from several nanometers to several microns. The porosity, ductility, high specific surface area, controllability and design of the fibre formation of electrospun nanofibres can be considered as advantages of using this technique [8]. Polymer nanofibres have a broad range of applications, particularly in tissue engineering, sensors, protective clothing, filters, etc [9].

Several papers have reported that various types of nanoparticles or additives can be entrapped in the nanofibre membranes or encapsulated within the nanofibres. The incorporation of antimicrobial nanoparticles into nanofibres prepared using the electrospinning process has attracted a great deal of attention because of the almost unlimited variability of nanofibre membranes properties [10]. When the literature is investigated, it is seen that there are many studies on the production of silver nanoparticle doped electrospun nanofibres. Zinc nanoparticles were also used for this aim. Quirós et al. were studied the use of electrospun polyvinylpyrrolidone (PVP) nanofibres containing silver, copper, and zinc nanoparticles to prepare antimicrobial mats using silver and copper nitrates and zinc acetate as precursors. The efficiency of metal-loaded mats against *Escherichia coli* and *Staphylococcus aureus* bacteria was tested. The results indicated a strong inhibition against silver-loaded fibres; on the other hand, copper and zinc-load-

ed mats also decreased the metabolic activity and cell viability, although to a lesser extent [11]. Pan et al. produced PVA/zinc citrate nanofibre mats and reported that the incorporation of zinc citrate results in a fibre diameter of 70-100 nm for PVA/zinc citrate nanofibre mats. In addition, the mats also have optimal antibacterial efficacy and cell attachment when they are made with 3 wt % zinc citrate [5].

In addition to the anti-microbial and antibacterial properties of zinc, the cell renewal effect is well-known in the medical world. In order to benefit from these properties, zinc oxide-containing wound dressings are also used all over the world so that wounds dry quickly, are healed quickly and are protected from bacteria. Although there are many studies in the literature on the use of zinc [12, 13], there is not any study on nanofibre production, in which zinc is used in a complex compound form. In this study, a zinc cyclohexane monocarboxylate compound (CHMCZn) was synthesised to prevent the possibility of zinc ions passing into human skin, owing to their complex form. Furthermore, the complex compound is also intended to exhibit better antibacterial activity due to the cyclohexane monocarboxylate's own antibacterial property. As is known, beyond the effects on the human and the environment, the cost is also of great importance for a chemical to be used in textile applications. Although it is possi-

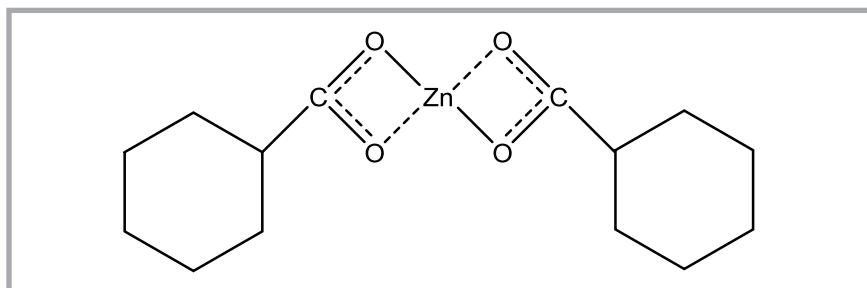


Figure 1. Chemical structure of CHMCZn compound.

ble to obtain very good results with silver compounds, it is thought that it would be advantageous in terms of economics if good results could also be achieved with zinc compounds.

In our previous study, we examined the usage possibility of various metal (copper, zinc, cobalt, nickel, potassium and sodium) mono carboxylates (metal naphthenates) as antibacterial agents in textile applications [14]. In the current study, after the synthesis of CHMCZn, its chemical structure was analysed with FTIR and TGA. Then electrospun PVP nanofibres containing CHMCZn were produced and antibacterial properties of the nanowebs obtained were investigated for their use in the textile field.

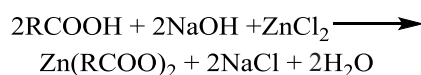
Material and method

Synthesis and characterisation of CHMCZn compound

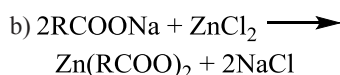
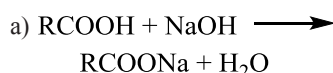
In this study, firstly the synthesis of CHMCZn was made.

For this aim a thermometer was placed on the first neck of the three-neck glass flask, a condenser on the second neck, and a dropping funnel on the third. A solution of 0.01 mol cyclohexane mono carboxylic acid (Merck, 98%) of 10% diethyl ether (Tekkim, 99.5%) was put into the flask, and 10% solution of NaOH (Merck, 99%) was put into the dropping funnel. The magnetic stirrer (Hot-Plate 300 °C 15 cm Circular M15 type) was switched on, the heat raised up to 40-45 °C, the dropping funnel opened, and stirring took place by dropping NaOH for 60 min. The pH value of the setting was adjusted between 7 and 8. 10% aqueous solution of 0.01 mol $ZnCl_2 \cdot 7H_2O$ (Tekkim, 98%) was added over the resulting sodium carboxylate salt under reflux by stirring for 1 h at room temperature. The stirring was then continued for 1 h, and the mixture was allowed to stand for one day. Then the

solution was put into an extraction flask, and the organic and liquid phases were separated. After the the organic phase was taken away, CHMCZn, which is insoluble in water and soluble in organic solvents, was obtained. CHMCZn is obtained by the following reaction [14]:



This reaction is carried out in two stages:



The chemical structure of the CHMCZn compound is shown in **Figure 1**.

FTIR and TGA analyses were performed to clarify the structure of the CHMCZn obtained.

Production of CHMCZn doped polyvinylprolidone nanofibres via electrospinning and their characterisation

For the production of CHMCZn doped polyvinylprolidone nanofibres, 10% polyvinylpyrrolidone in ethanol (C_6H_9NO)X, MW = 1.300.000) was stirred until it is completely dissolved. As an antibacterial agent, 5%, 7.5% and 10% CHMCZn solutions in diethyl ether were prepared in a stirrer. 1 ml of this solution was added to 10 ml of PVP solutions and stirred for 5 hours. Then nanofibres were obtained from the polymer solutions containing CHMCZn in varying proportions on an INNOVENSA electrospinning device (Model: Basic system) (Voltage range 0-40 kV, Spinning types: Bottom-up, top-down, side-by-side spinning), on which the spinning distance, flow rate and voltage applied are all easily controlled in the horizontal or vertical spin-

ning mode. In our study working conditions were as follows: 14.5 kV energy at 26 °C, 6 ml/hr supply and 15 cm distance in the horizontal position. These operating conditions for the nanofibre production had been determined by pre-trials. FTIR, TGA and SEM analyses were performed to clarify the structure of the nanofibres obtained. Samples were measured using a ThermoFisher brand fourier transformed infrared spectrophotometer and Perkin Elmer TGA 4000 thermogravimetric analyser. SEM photographs of the samples were taken using an FEI brand Quanta FEG 250 model scanning electron microscope.

Determination of the antibacterial activity of CHMCZn doped polyvinylprolidone nanofibres

Antibacterial properties of the nanofibres obtained were investigated for their use in the textile field. For this purpose; antibacterial properties against various Gram (+) (*Bacillus subtilis* NRRL NRS-744, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 43300) and Gram negative (-) (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 ve *Proteus mirabilis* ATCC 12453) bacteria were investigated.

Nutrient agar cultures were incubated at 37 ± 2 °C for 16-24 hours for activation of bacteria. At the end of the incubation, the nutrient uptake was adjusted to be 0.5 MacFarland. For the disk diffusion method, the nutrient agar was planted with each bacterium of 10^4 CFU/ml.

In the study, nanofibre material (5%, 7.5%, 10%) was impregnated onto sterile filter paper (5 μ l) of 5 mm dimensions. PVP was used as a negative control, and a gentamicin (GM120) disc was utilised as a positive control. Filter papers impregnated with planted petrels were placed and incubated at 37 ± 2 °C for 16-24 hours. Zone diameters (mm) formed at the end of the incubation were evaluated.

Results and discussion

Results related to synthesis and characterisation of CHMCZn compound

FTIR analysis results of the CHMCZn compound are given in **Figure 2**.

The FTIR spectra in **Figure 2** confirm the successful modification of zinc with

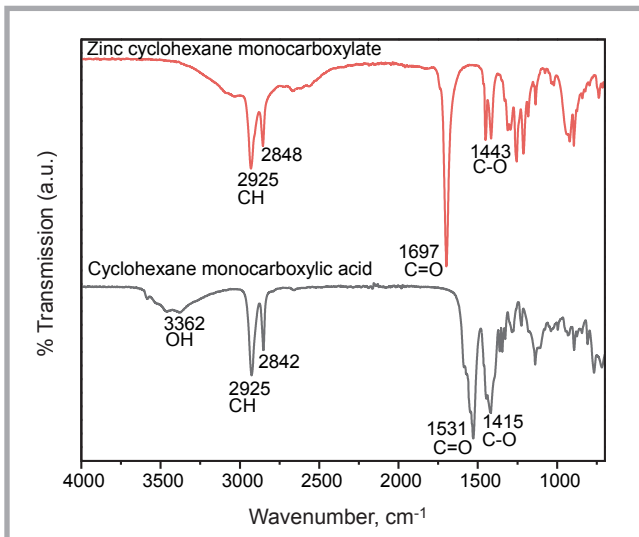


Figure 2. FTIR analysis results of CHMCZn compound.

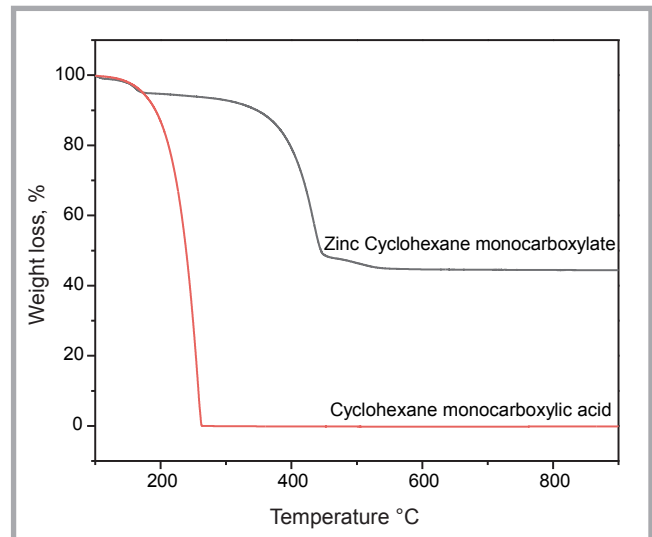


Figure 3. TGA analysis results of CHMCZn compound.

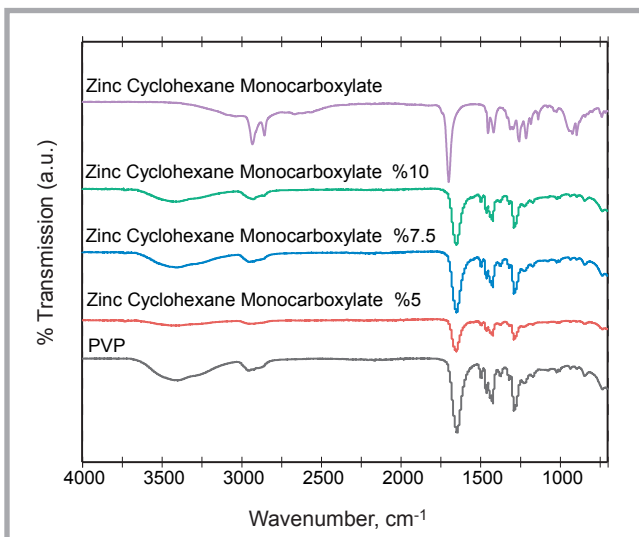


Figure 4. FTIR analysis results of PVP nanofibres containing various amounts (5, 7.5 and 10 wt %) of the CHMCZn compound.

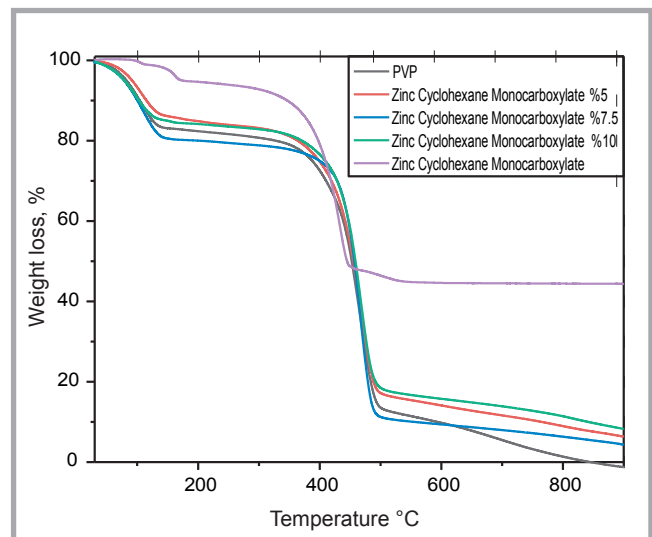


Figure 5. TGA analysis results of PVP nanofibres containing various amounts (5, 7.5 and 10 wt %) of the CHMCZn compound.

cyclohexanemonocarboxylic acid. When the FTIR spectrum of pure cyclohexane monocarboxylic acid is investigated, it can be said that the peaks at $\sim 1400\text{ cm}^{-1}$ are attributed to $-\text{C}-\text{O}$ stretching vibrations, those at $\sim 1500\text{ cm}^{-1}$ to $-\text{C}=\text{O}$ stretching vibrations, the peaks at $3000\text{--}2800\text{ cm}^{-1}$ correspond to $\text{C}-\text{H}$ vibrations and a very broad band centered at $\sim 3300\text{ cm}^{-1}$ related to the $\text{O}-\text{H}$ stretching (ν) vibrations. When the FTIR spectrum of the CHMCZn compound is examined, it is seen that the OH peak disappears and the intensity of the $\text{C}=\text{O}$ peak increases. In addition, the wavelengths of the $\text{C}=\text{O}$ and $\text{C}-\text{O}$ peaks shifted. These shifts are due to the transition of zinc instead of the hydrogen atom attached to the oxygen in the carboxyl group.

TGA analysis results of the CHMCZn compound are given in **Figure 3**.

Figure 3 shows that cyclohexane monocarboxylic acid undergoes rapid weight loss at about $100\text{ }^{\circ}\text{C}$ and disappears completely at about $260\text{ }^{\circ}\text{C}$. Because the organic cyclohexane monocarboxylic acid is decomposed by the effect of temperature, it is disintegrated by the water and carbonmonoxide/carbondioxide. After the removal of water and carbonmonoxide/carbondioxide from the media, there is no material left. When the CHMCZn compound is examined, it is observed that it starts to lose weight at about $100\text{ }^{\circ}\text{C}$, where water is removed from the structure, and then it rapidly loses weight between $310\text{--}450\text{ }^{\circ}\text{C}$, where carbonmon-

oxide/carbondioxide is removed. However, this compound did not completely disappear because it contained inorganic zinc, thus zinc oxide remains at the end.

Results related to the production of CHMCZn doped polyvinylprolidone nanofibres

FTIR analysis results of PVP nanofibres containing various amounts (5, 7.5 and 10 wt %) of the CHMCZn compound are shown in **Figure 4**.

The surface chemistry of three different amounts of zinc cyclohexanemonocarboxylate (5, 7.5 and 10%) containing nanofibres was studied using the FT-IR spectrum. When the FTIR analysis results

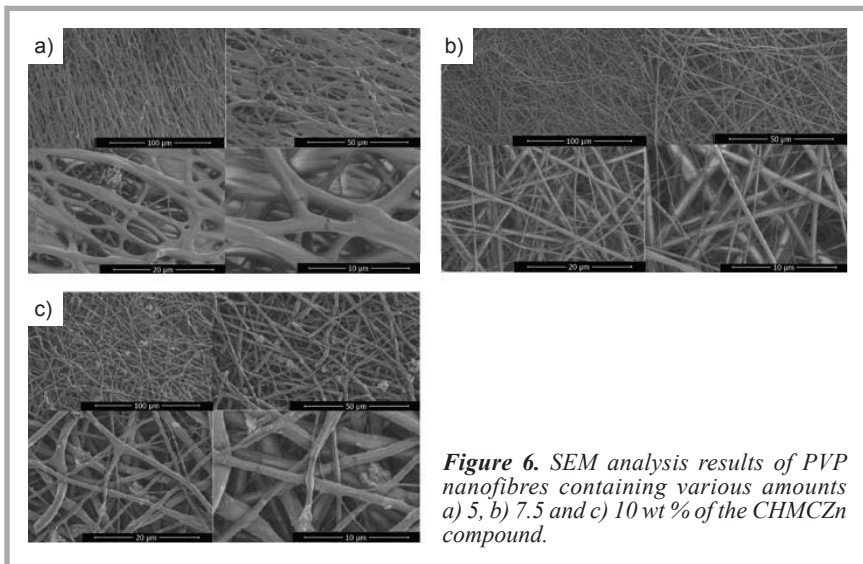


Figure 6. SEM analysis results of PVP nanofibres containing various amounts a) 5, b) 7.5 and c) 10 wt % of the CHMCZn compound.

of pure PVP nanofibres are examined, the peaks at 1300 and 1200 cm^{-1} are attributed to $-\text{C}-\text{O}$ stretching vibrations, that at 1500-1400 cm^{-1} to $-\text{C}=\text{O}$ stretching vibrations, and those at 3000-2800 cm^{-1} correspond to $\text{C}-\text{H}$ vibrations and a very broad band centered at 3600-3400 cm^{-1} related to the $\text{O}-\text{H}$ stretching (ν) vibrations. In the spectrum of the zinc cyclohexane monocarboxylate, the peaks at 3000-2800 cm^{-1} , 1500-1400 cm^{-1} and 1300-1200 cm^{-1} are attributed to the stretching vibrations of $-\text{CH}$ groups, $-\text{COO}$ groups and $\text{C}-\text{O}$ species, respectively. However, when the FTIR results of nanofibres containing different concentrations of CHMCZn are examined, an $-\text{OH}$ peak similar to that for PVP fibres is noticed. These results clearly indicate that zinc cyclohexane monocarboxylate is included in PVP.

TGA analysis results of PVP nanofibres containing various amounts (5, 7.5 and

10 wt %) of the CHMCZn compound are shown in **Figure 5**.

When the TGA spectra given in **Figure 5** are examined, it can be seen that the zinc cyclohexane monocarboxylate composition undergoes weight loss at about 100 $^{\circ}\text{C}$ and rapid weight loss at 340-450 $^{\circ}\text{C}$. When the TGA spectra of PVP nanofibres is investigated, it can be seen that PVP undergoes rapid weight loss at about 50 $^{\circ}\text{C}$ and disappears completely at about 850 $^{\circ}\text{C}$. Because the organic compound is decomposed by the effect of temperature, it is disintegrated in the water and carbonmonoxide/carbondioxide. After the removal of water and the carbonmonoxide/carbondioxide from the media, there is no material left. On the other hand, the TGA spectra of CHMCZn doped nanofibres at different ratios show that they give more similar results to those of polyvinylprolidone nanofibres alone. This means that CHMCZn mole-

Table 1. Zone diameters against bactericide (mm).

Bacteria	Amount of CHMCZn compound, %		
	5	7.5	10
<i>B. subtilis</i>	11	11	12
<i>E. faecalis</i>	0	0	0
<i>S. aureus</i>	10	10	10
<i>E. coli</i>	11	12	12
<i>P. aeruginosa</i>	0	0	0
<i>P. mirabilis</i>	0	0	0

cules were trapped in the PVP structure and that the resulting nanofibres exhibit similar properties to the external polymer structure – polyvinylprolidone. Another point which attracts attention is that in the case of CHMCZn doped PVP nanofibres, complete weight loss does not occur as zinc oxide remains at the end.

SEM analysis results of PVP nanofibres containing various amounts (5, 7.5 and 10 wt %) of the CHMCZn compound are shown in **Figure 6**.

From **Figure 6**, it is seen that the fibre diameters obtained are in the range of 145-947 nm.

SEM-EDX analysis results of PVP nanofibres containing various amounts (5, 7.5 and 10 wt %) of the CHMCZn compound are shown in **Figure 7**.

As can be seen from **Figure 7**, the Zn content of the nanofibres was increased with an increase in CHMCZn doping concentration.

Results related to determination of the antibacterial activity of CHMCZn doped polyvinylprolidone nanofibres

The antibacterial properties of zinc cyclohexane monocarboxylate doped nano-

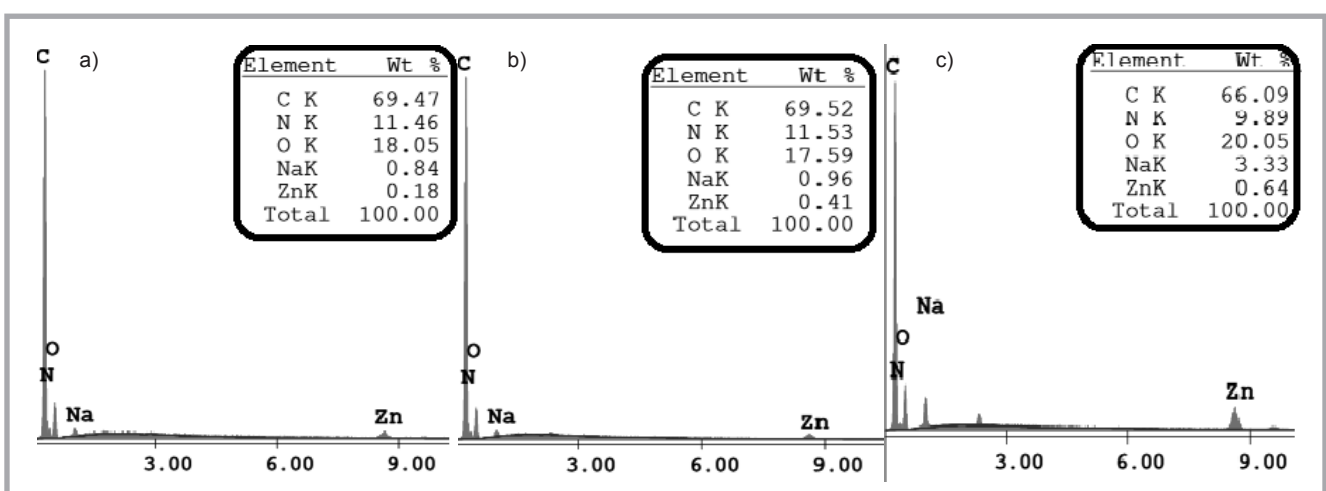


Figure 7. SEM-EDX analysis results of PVP nanofibres containing various amounts a) 5, b) 7.5 and c) 10 wt % of the CHMCZn compound.

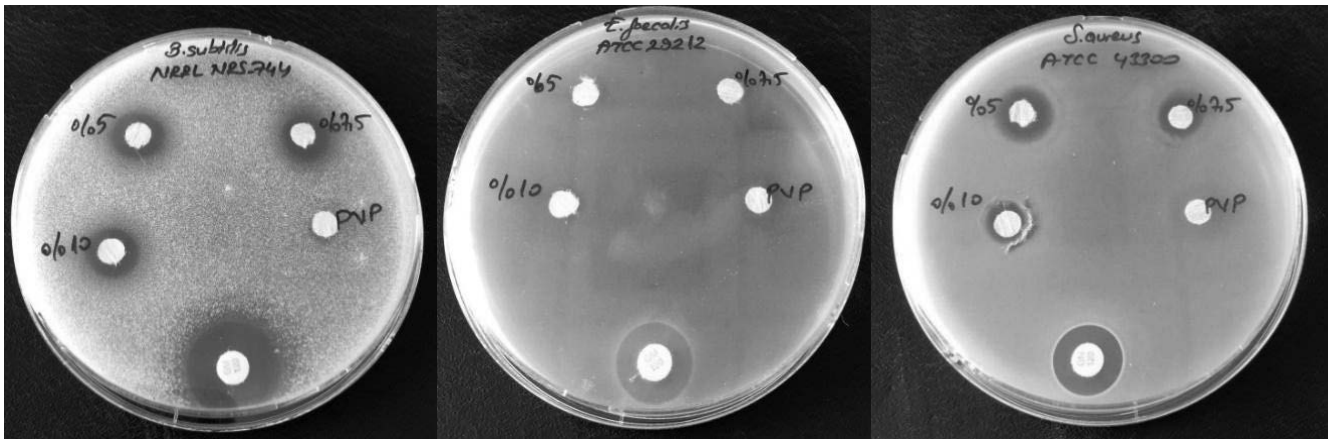


Figure 8. Test images of antibacterial activity against Gram (+) bacteria.

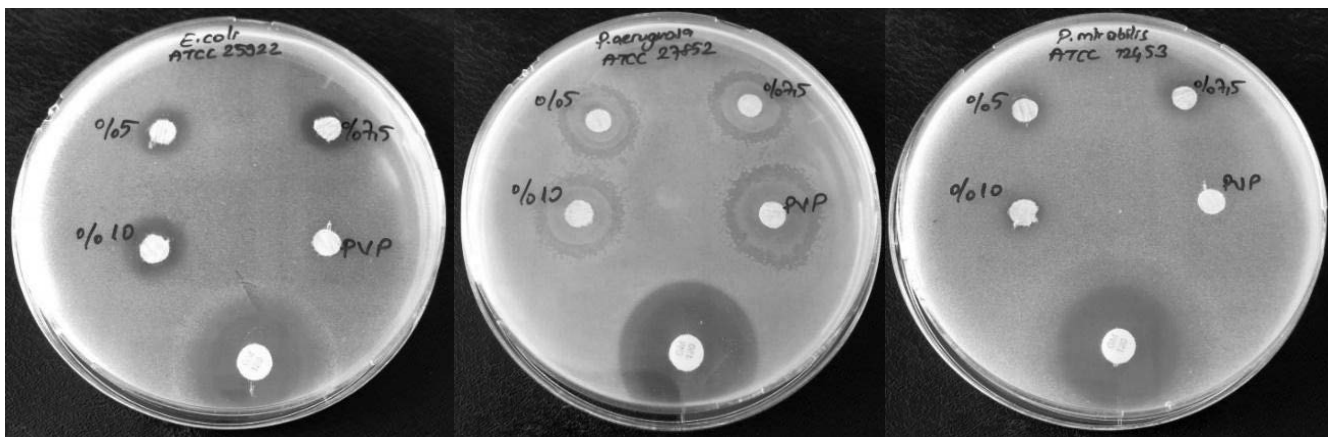


Figure 9. Test images of antibacterial activity against Gram (-) bacteria.

fibres at different ratios (5, 7.5 and 10% wt %) were tested against *E. faecalis*, *P. aeruginosa*, *P. mirabilis*, *B. subtilis*, *S. aureus* and *E. coli* bacteria, the results of which are shown in **Table 1**.

Table 1 shows that the nanofibres obtained did not show antimicrobial activity against *E. faecalis*, *P. aeruginosa* and *P. mirabilis* strains at the zinc cyclohexane monocarboxylate addition ratio. However, antimicrobial activity against *B. subtilis*, *S. aureus* and *E. coli* strains was detected regardless of CHMCZn concentration. Results can also be clearly seen from the images given in **Figures 8** and **9**.

Conclusions

In this study, the zinc cyclohexane monocarboxylate compound was synthesised and the structure of the material explained by FTIR and TGA. When the FTIR spectrum of the zinc cyclohexane monocarboxylate compound is examined, it is seen that the -OH peak disappears and the intensity of the C=O peak increases.

On the other hand, when the TGA spectrum of the zinc cyclohexane monocarboxylate compound is examined, it is observed that it starts to lose weight at 100 °C and rapidly so at 350 °C. However, this compound did not completely disappear because it contained inorganic zinc. By using the electrospinning method, nanofibres were produced from PVP containing different ratios (5, 7.5, and 10% wt %) of the CHMCZn compound. The structure of the nanofibres obtained was described using FTIR, TGA, SEM and EDX analyses. When the FTIR results of nanofibres containing different concentrations of zinc cyclohexane monocarboxylate are examined, an -OH peak similar to that for PVP fibres is noticed. These results clearly indicate that zinc cyclohexane monocarboxylate is included in PVP. When the TGA spectra of CHMCZn doped nanofibres at different ratios are examined, it is seen that they give more similar results to those of polyvinylprolidone nanofibres alone. This shows that zinc cyclohexane monocarboxylates are trapped in the PVP structure and that the resulting nanofibres

exhibit similar properties to the external polymer structure – polyvinylprolidone. According to the SEM-EDX analyses, it is seen that the fibre diameters obtained are in the range of 145-947 nm.

Antibacterial properties of the nanofibres obtained against various Gram (+) and Gram (-) bacteria were also investigated. Antimicrobial activity against *B. subtilis*, *S. aureus* and *E. coli* strains was detected regardless of the CHMCZn concentration.

In future studies it is advised to work on lower concentrations (less than 5%) of CHMCZn doping and determine histograms of the fibre diameters of nanofibres spun at different CHMCZn concentrations. □

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