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Production and characterization of zinc oxide nanoparticles in microsystems via green synthesis

Yeşil sentez yoluyla mikrosistemlerde çinko oksit nanopartiküllerin üretimi ve karakterizasyonu

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Abstract
Nanoparticles (NP), which can be produced for many different fields and purposes; are at size vary from nanometer to a micrometer. These particles can be prepared by different approaches such as chemical, physical and biological methods. However, the biological approach is the most promising approach among other methods due to its simplicity and environmentally friendly conditions. The production of the nanoparticle by means of a biological approach does not require any high temperature, acidic/basic conditions and hazardous chemicals. Therefore, this method is named "green synthesis". Development in microtechnology has had profound effects in many areas such as drug discovery, biology, diagnosis, and tissue engineering. Microfluidics which focuses on the processing of liquids in microscale systems has attracted wide attention in the production process of nanoparticles due to precise control and fast mixing. The main purpose of this study is to produce and characterize metal nanoparticles by combining microsystems and green synthesis approaches. For this purpose, in the first step, the design of microsystems that suitable for the flow of different phases was performed. In the second step, ZnO nanoparticle synthesis was carried out using the red cabbage (*Brassica oleracea* var.*capitata f. Rubra*) extracts. The synthesis of nanoparticles achieved by optimizing different parameters such as; extract: zinc ratio, zinc solution concentration, flow rates, and flow rates ratio in the microsystem and temperature. The results showed that the herbal red cabbage components can be used for the green synthesis of ZnO nanoparticles.

Keywords: Green synthesis, Microfluidics, Nanoparticle, Red cabbage, Zinc

Öz
Birçok farklı alan ve amaç için üretilebilen nanopartiküller (NP); nanometreden milimetrenin binde biri kadar değişen boyutlara sahiptirler. Bu partiküller farklı kimyasal, fiziksel ve biyolojik yaklaşımlarla hazırlanabilir. Ancak yöntemler arasında biyolojik yaklaşım, basit olması ve çevre dostu koşulları nedeniyle en umut verici yaklaşımdır. Biyolojik yaklaşım ile nanoparçacık üretimi, yüksek sıcaklık, asidik / bazik koşullar ve tehlikeli kimyasallar gerektirmez. Bu nedenle, bu yöntemler "yeşil sentez" olarak adlandırılmaktadır. Mikroteknolojideki gelişmeler, ilaç keşfi, biyoloji, teşhis ve doku mühendisliği gibi birçok alanda derin etkiler yaratmıştır. Mikro ölçekli sistemlerde sıvıların işlenmesine odaklanan mikroakışkanlar, hassas kontrol ve hızlı karıştırma sayesinde nanopartiküllerin üretim sürecinde büyük ilgi görmüştür. Bu çalışmada temel amaç mikrosistemler ve yeşil sentez yöntemlerini birleştirilerek metal nanopartiküllerin üretimi ve karakterizasyonunu gerçekleştirmektir. Bu amaçla ilk adımda farklı fazlarını akışına uygun mikrosistemlerin tasarımı gerçekleştirilmiştir. İkinci adımda kırmızı lahana (Brassica oleracea var.capitata f. rubra) ekstreleri kullanılarak ZnO nanopartikülleri sentezi gerçekleştirilmiştir. Bitki ekstraktı: çinko oranı, çinko çözeltisi derişimi, mikrosistemde akış hızları ve akış hızları oranı ve sıcaklık gibi farklı parametreler optimize edilerek en uygun üretim koşulları belirlenmiştir. Sonuçlar, kırmızı lahana bileşenlerin ZnO nanopartiküllerin yeşil sentez yoluyla üretilebileceğini göstermiştir.

***Anahtar kelimeler**: Yeşil sentez, Mikroakışkan, Nanopartikül, Kırmızı lahana, Çinko*

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1. Introduction

Nanotechnology is a highly interdisciplinary field of application to control the structure of matter at the nanoscale. It involves a variety of scientific and engineering knowledge including chemistry, physics, biology, electrical engineering, mechanical engineering (Aslani, 2012). Nanoparticles are used in many fields from basic materials research to biomedical applications. Sensing applications such as protein, metal, DNA, and virus analysis, are frequently using nanoparticles for the advantage of their optical properties. Nanoparticles have also been used in applications such as bacteria detection in food and seafood and are suitable carriers for various biological agents such as drugs, viruses, cells, etc. Nanoparticles are synthesized from a diversity of common chemical assemblies such as metal, silicates, non-oxide ceramics, polymers, carbon, and biomolecules (Xu et al., 2006).

Different chemical and physical approaches have been applied for the synthesis of nanoparticles. Many of them are consist of toxic chemicals and harsh conditions since the biocompatibility of synthesized particles are suspicious (Katmıs et al., 2018). Because this hazardous materials and techniques lead to significant toxic problems, alternative approaches are required for synthesis of nanoparticles with the important properties such as size, physicochemical properties, low cytotoxicity, and biocompatibility maintained. One of the most promising method is green synthesis which using plants, bacteria, fungi, yeast, algae, viruses, etc. (Shivaji et al., 2018).

Green synthesis, known as cheap, efficient, and environmentally friendly "green" production, has become an alternative to other methods in recent years (Makarov et al., 2014; Sefaoğlu, 2020). Green synthesis is not only an economical and environmentally friendly method but also can be applied to large- scale production. Eliminating the use of high pressure, high temperature and toxic chemicals in the production process is another advantage of green synthesis methods (Chintamani et al., 2018; Garibo et al., 2020).
Among numerous natural sources used for the synthesis of nanoparticles, different parts of plants such as leaves, roots, and stems are the most proper candidates, and nanoparticles obtained from these sources are found to be more stable, have various sizes and shapes, and the production is faster than other biological agents. Moreover, nanoparticles produced by green synthesis are reported as more effective than chemically synthesized nanoparticles regarding bioactivity. Therefore, many plants and their extracts have been reported to be used in the production of various metal nanoparticles, especially copper, silver, and zinc (Mittal et al., 2013; Igwe and Ejiako, 2018). Although the exact mechanism that takes place in metal nanoparticle synthesis from plant extracts is not known, it is reported that nanoparticle formation occurs in the form of reduction, aggregation, and nanoparticle development (Jain and Mehata, 2017). A wide variety of metabolites and phytochemicals such as flavones, ketones, organic acids, aldehydes, and amides found in plant extracts are thought to be responsible for the reduction of metal ions due to their antioxidant and reducing effects (Bhuyan et al., 2015).

In the last decade, inorganic metal oxides have been widely used as antioxidants, antibacterial, antifungal, and anticancer agents. Zinc oxide (ZnO) nanoparticles are one of the leading nanostructures due to their diverse properties and widespread utilization in a variety of applications field such as electronics (sensors), diagnostics (UV- and photodetection), energy (solar cells), and biomedical (antimicrobial or antitumor agents) (Deng et al., 2008; Anbuvannan et al., 2015; Singh et al., 2018).

Compared to traditional methods, microsystems stand out as they having the advantage of allowing high heat and mass transfer. The rapid mass and heat transfer in a microenvironment enable the chemical synthesis with enhanced efficacy and reproducibility. In addition, the reduction of sample volumes and the ability to strictly control the reaction parameters (e.g temperature), are the other superiorities of microsystems (Luan et al., 2008; Akay et al., 2017). The most employed method for fabricating microdevices is Soft photolithography. However, this technique comprises time-consuming steps such as mask design, substrate preparation, photoresist development, UV exposure, etc, and most of these steps have to be completed in a clean room.

Many studies reporting red colored plants, which are an indispensable part of our diets, are rich in phytochemicals such as vitamins, beta-carotene, and lycopene (Çöteli and Karataş, 2016). Phenolic compounds constitute the largest group of phytochemicals (Güzel and Akpınar, 2019). Red cabbage (*Brassica oleracea* var.*capitata f. Rubra*) is a plant rich in polyphenols and anthocyanins. So far, about 36 anthocyanins have been identified in red cabbage and it has been found that the bioactivity of red cabbage is directly related to the total amount of anthocyanin. Recent studies show that red cabbage is effective in preventing some types of cancer, and is certain heart diseases (Dyrby et al., 2001; Wu et al., 2006; Charron et al., 2007).

Different approaches for the production of red cabbage extract directed silver and gold nanoparticles have been reported (Demirbas, et al., 2016; Ocsoy et al., 2017; Unal, et al., 2020). In this study, it was aimed to synthesize Zinc Oxide nanoparticles in microsystems by green chemistry techniques using aqueous extracts of red cabbage. Furthermore, the effects of parameters such as zinc concentration, flow rate, and temperature on nanoparticle synthesis were investigated.

2. Materials and methods

Red cabbage (*Brassica oleracea* var.*capitata f. Rubra*) used in this study was obtained from local markets. All the chemicals such as Zinc Nitrate ($Zn(NO_3)_2$, Sodium Hydroxide (NaOH) Acetic Acid, Ethanol were of analytical grade and obtained from Sigma Aldrich (Germany) and Merck (Germany).

2.1. Preparation and extraction of herbal materials

Red cabbage obtained from local markets was cut into small pieces and left to dry at 40 ^{0}C for 2-7 days. The dried samples were grounded into powder using a laboratory blender (Waring 8011 EB, USA). 1 g of powder was taken and extracted with 50 ml of distilled water for 20 minutes at room temperature using ultrasonic bath (Wisd WUCD10H, Korea). Subsequently, the samples were filtered through filter paper, and the plant pulp was discarded. The obtained extract was then subjected to centrifugation (4500 rpm for 5 minutes) to remove possible residues (Beckman Coulter Allegra X-30R, USA). The supernatant obtained was used in the production of nanoparticles.

2.2. Green synthesis of nanoparticles

In order to eliminate problems of conventional microfabrication techniques, two alternative methods that can be applied to all laboratories were developed. A microsystem based on the flow focusing principle has been designed for nanoparticle production. Simply, a 1 mm channel was opened in a plexiglass layer with the help of a drill and an insulin syringe needle with an inner diameter of 120 µm (31G) was inserted horizontally into the drilled channel. In addition, microfluidic platforms consist of 250 µm channels and different inputs and outputs were also designed and fabricated using 3D printer technology and tested (Figure1).

For the synthesis of Zinc Oxide nanoparticles, it was aimed to produce nanoparticles by mixing the herbal extract obtained from red cabbage with the different concentrations of the Zinc Nitrate in microchips. The aqueous extract (dispersed phase) was fed from one of the microchip's inlet, while the Zinc Nitrate solution (continuous phase) connected to the other inlet, and it was expected the two solutions mixed along the channels of the microchip to form the nanoparticles.

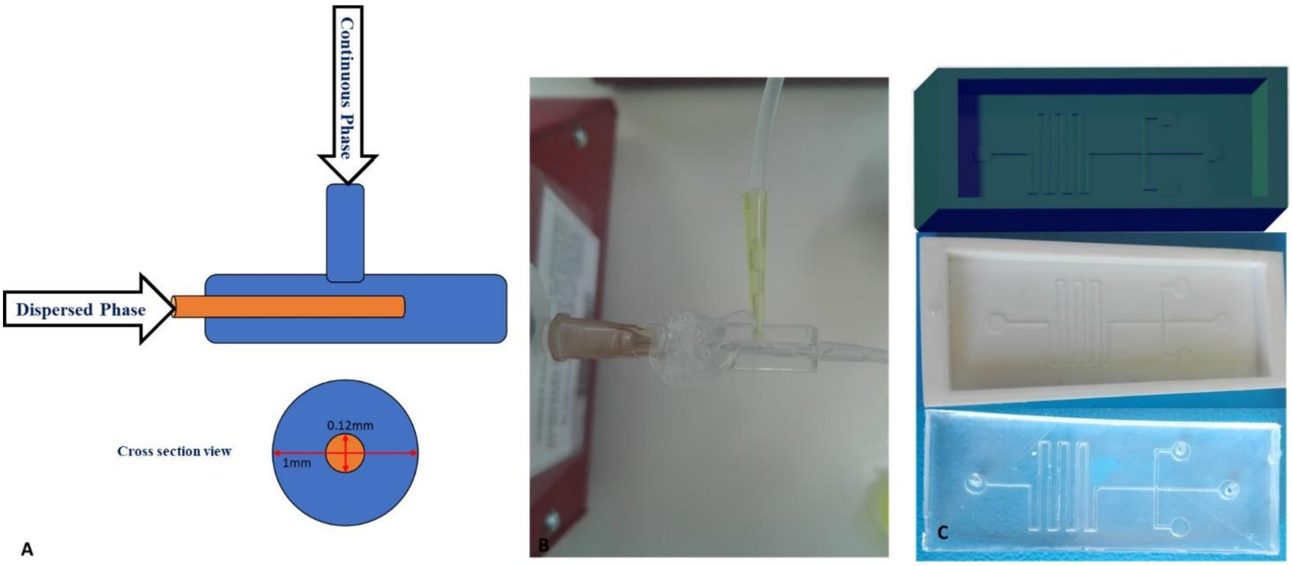

Figure 1. Microsystem used for nanoparticle sythesis; A-B; Syringe based flow-focusing microdevice, C; 3D printed microchannel

The samples collected from the microchips were centrifuged (14000 rpm for 10 min.) to separate the nanoparticles. This step was repeated 2 times by adding distilled water to remove the extracts completely. The samples were suspended in distilled water for further characterization. During the synthesis of nanoparticles; the effect of different parameters such as flow rate, extract: Zinc Nitrate ratio, Zinc Nitrate concentration, and temperature in the channels have been investigated. The experimental conditions are summarized in Table 1.

Tablo 1. The experimental conditions for nanoparticle synthesis

Sample	Microsystem	Dispersed Phase	Continuous Phase	Dispersed Phase flow rate (μl/min)	Continuous Phase Phase flow rate (μl/min)	Flow rate ratio	Temperature
M7	3D printed	red Cabbage extract	$Zn(NO_3)_2$ 5mM	20	40	1:2	Room
M8	3D printed	red cabbage extract	$Zn(NO_3)_2$ 5mM	20	100	1:5	Room
M9	3D printed	red cabbage extract	$Zn(NO_3)_2$ 5mM	100	100	1:1	Room
M15	3D printed	red cabbage extract	$Zn(NO_3)_2$ 2.5mM	5	10	1:2	40
M16	3D printed	red cabbage extract	$Zn(NO_3)_2$ 2.5mM	5	20	1:4	40
M17	3D printed	red cabbage extract	$Zn(NO_3)_2$ 5mM	10	20	1:2	40
IS1	Syringe flow focusing	red cabbage extract	$Zn(NO_3)_2$ 10 mM	25	50	1:2	50
IS2	Syringe flow focusing	red cabbage extract	$Zn(NO_3)_2$ 10 mM	50	100	1:2	50
IS3	Syringe flow focusing	red cabbage extract	$Zn(NO_3)_2$ 10 mM	2	10	1:5	50
IS4	Syringe flow focusing	red cabbage extract	$Zn(NO_3)_2$ 10 mM	75	75	1:1	50
IS27	Syringe flow focusing	red cabbage extract	$Zn(NO_3)_2$ 10 mM	2	4	1:2	50
IS28	Syringe flow focusing	10 mM Zn(NO3)2	red cabbage extract	25	50	1:2	50

2.3. Characterization of ZnO nanoparticles

UV-visible Spectroscopy Analysis: the synthesized ZnO nanoparticles were suspended in distilled water to measure their absorbance which could be the sign of ZnO nanoparticle formation. The UV–Vis measurements were carried out within the wave-length range of 200–800 nm using a UV–Vis spectrophotometer (Thermo, USA).

Dynamic Light Scattering (DLS) Analysis: was performed for measurement of size and size distribution of produced zinc nanoparticle using Particle Size Analyzer (Malvern Zetasizer Nano ZSP, USA). Distilled water was used as a dispersant.

Scanning Electron Microscopy Analysis; ZnO nanoparticles were further characterized for surface morphological properties using Scanning Electron Microscope (SEM) coupled with an EDX (Energy dispersive X-Ray spectrometer) probe (Zeiss Sigma 300). Composition and particle size were also investigated.

2.4. Antimicrobial activity of ZnO nanoparticles

The prepared nanoparticles were tested to evaluate the antimicrobial activity against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria. For this purpose, the agar well assay was performed. 6 mm diameter wells were opened on the agar layer in the Petri dishes and 80 μL of samples to be tested were added to the wells (0.2-1 mg/ml,). After 24 hours of incubation at 37 °C, the plates were observed. Ampicillin (50 mg / mL), red cabbage extract, Zinc Nitrate (5 mM), and the supernatant obtained from the particles were used as control.

3. Results and discussion

3.1. Synthesis and optimization of nanoparticles

The simplest method to understand the formation of nanoparticles is the maximum absorption wavelengths. Therefore, samples have been examined first by UV spectrophotometry. The maximum UV peaks for ZnO nanoparticles were recorded at around 330 nm (Figure 2). Since, all oxide materials, particularly at the nanoscales, have shorter wavelengths (Naseer et al., 2020) the observed results fit the typical ZnO absorption pattern.

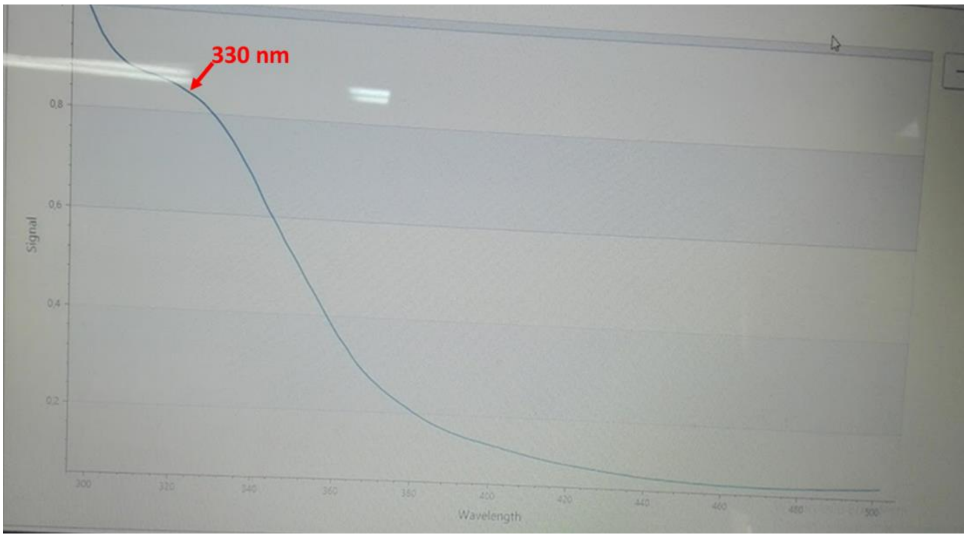

Figure 2. UV spectrum of ZnO nanoparticles

In the experiments performed on 3D printed microchips (Table 1), red cabbage extract was treated with the Zinc Nitrate at different concentrations and different flow rates. There was no particle formation observed at room temperature. However, although particle formation was observed in the experiments performed at 40^0C, it was detected that the synthesized particles had very different size ranges and did not have the proper homogeneity. In the experiment where the flow rate ratio was 1:2, the Zinc Nitrate concentration was increased to 5 mM (M17), size and monodispersity of particles have been improved but the expected nanoparticle range in terms of size still could not be reached (Data are not shown). The possible reason for these results might be related to 3D printing microchannel due to backpressure and leakage problems which lead to an unbalanced flow of phases. Because of these problems, these microchips could not be used for enough time for particle formation. On the other hand, results showed that increasing the ratio of flow rates, which means increasing the continuous phase flow rate, has a positive effect on the size and the distribution of particles. Similarly, it has been determined that keeping the Zinc Nitrate concentration high has a positive effect on both size and size distribution.

In the studies performed with the syringe-based flow-focusing microsystem, the zinc Nitrate concentration was selected as 10 mM and the temperature as 50^0C in light of the previous results mentioned above. In the experiment (Table 1.; IS1) where the flow rate of the red cabbage extract was 25 µl / min and the Zinc Nitrate flow rate was 50 µl/min (flow rate ratio 1:2), it was found that the produced particles were smaller (1044 nm) and more homogeneous compared to the particles produced in 3D printed microchips (Figure 3A).

In the case where the flow rate ratio was kept constant as 1:2 and the flow rates of both phases were increased by 2 times (Table 1; IS2), it was observed that the particle size increased slightly (1153 nm) compared to low flow rates, however, the particle size distribution was more homogeneous (Figure 3B). Flow rates ratio were then changed to 1:5, and both phases were fed at lower rates (Table 1; IS3). It was determined that both particle sizes (1354 nm) and homogeneity were negatively affected (Figure 3C). If the flow rate of the extract and the Zinc Nitrate are the same (75 µl / min) (flow rate ratio; 1:1) (Table 1; IS4), it was determined that the sizes of the obtained particles (980 nm) decreased significantly (Figure 3D). It has also been observed that for the very low flow rates (Table 1.; IS27. extract; 2 µl / min, zinc nitrate; 4µl / min), the size and homogeneity of the particles have been affected negatively (Figure 3E).

To see the dispersed and continuous phase effects, the feed of the Zinc Nitrate and the extract were replaced and the flow rates ratio were set to 1:2 (Table 1; IS28). Accordingly, the particles with relatively small size (718 nm) and homogeneity were produced under these conditions (Figure 3F and 4).

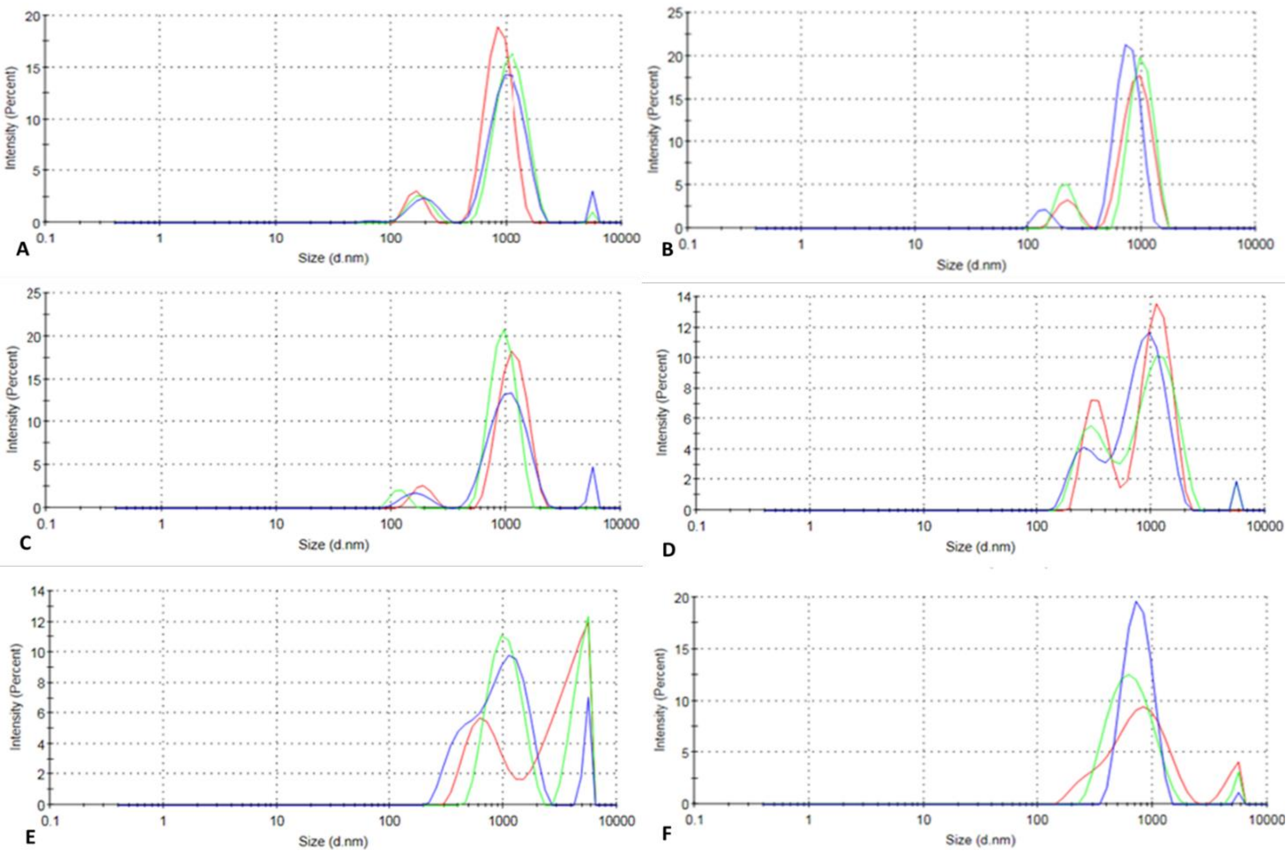

Figure 3. DLS analysis of nanoparticles produced under different conditions

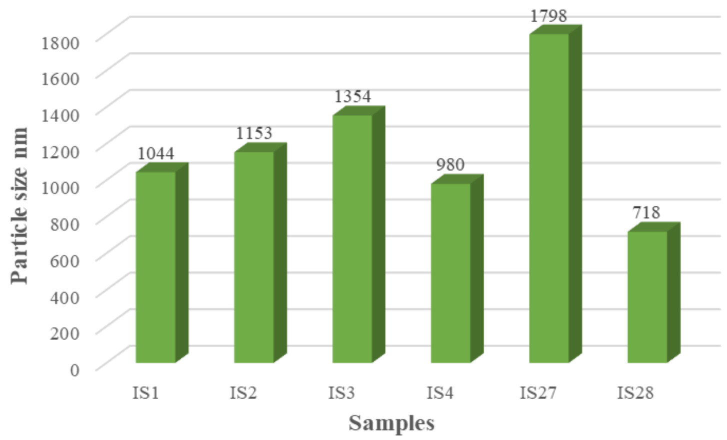

Figure 4. Mean particle size of samples produced under different conditions

The results showed that there is no strong relationship between the flow rate ratio and the particle size or distribution, but it can be proposed that feeding the phases at higher and near flow rates might be effective to obtain smaller size particles. There is no study on green synthesis methods of ZnO nanoparticles in microfluidic systems. On the contrary, in conventional chemical synthesis methods, the flow rate is mostly used to control the residence time and reaction time in the microchannel (Luan et al., 2008) and it has been reported that smaller nanoparticles are formed at lower flow rates (Ji et al., 2011). However, considering the flow-focusing microsystems, it has been reported that the synthesized nanoparticles are smaller when the flow rate (particularly the continuous phase flow rate) is high. It is predicted that mass transfer between phases occurs by convection rather than diffusion at high flow rates (Pessoa et al., 2017), and therefore the reducing compounds in red cabbage extracts interact with Zinc rapidly to form nanoparticles faster.

The sample produced in the optimized conditions (IS28) were subjected to further characterization.

3.2. SEM and EDX analysis of ZnO nanoparticles

The structural characterizations and elemental composition of synthesized ZnO nanoparticles were explored using SEM equipped with an EDX. The SEM investigation showed that nanoparticles with nearly spherical-like shapes and quite homogeneous distribution have been synthesized as is shown in Figure 5. Furthermore, it was observed that the size of nanoparticles was even smaller as it was determined by DLS analysis.

The existence of 3 different peaks was observed as expected in Figure 6 in EDX analysis. These peaks are C and O peaks most probably arising from organic compounds in herbal extracts used in green synthesis and Zn peaks representing the metal component.

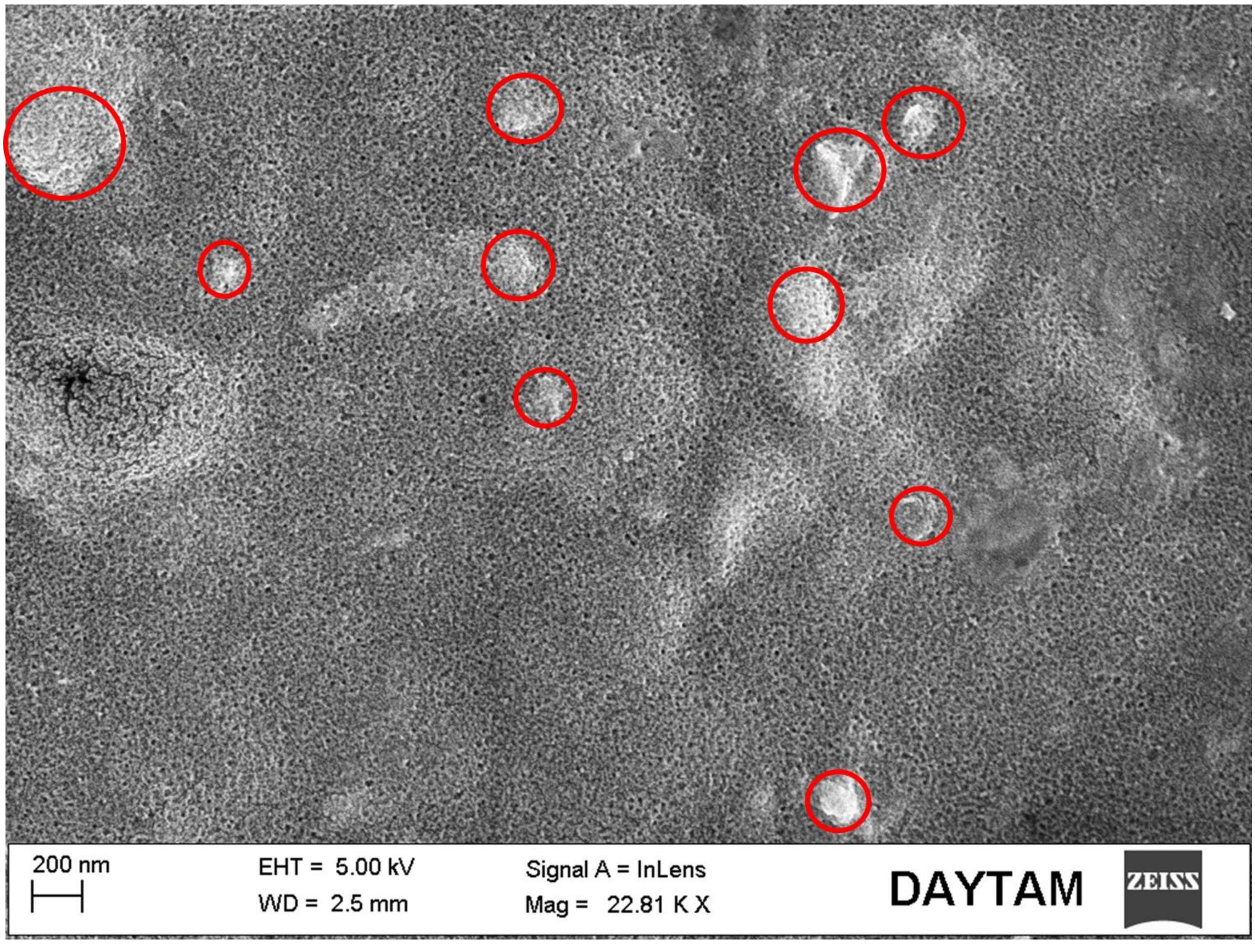

Figure 5. SEM images of selected sample (IS28)

Şekil 6. EDX analysis of ZnO nanoparticles

3.3. Antimicrobial activity of ZnO nanoparticles

It has been reported that extracts of red cabbage prepared by various methods show activity against both gram-negative and gram-positive bacteria (Abdel-Shafi et al., 2019). Similarly, ZnO nanoparticles at the size range of 5-140 nm obtained by biological methods have also been reported to have antibacterial activity (Bhuyan et al., 2015; Singh et al., 2018). Although the metal oxide nanoparticles smaller than 10 nm has been conveyed to have antimicrobial activities, there are also ZnO particles with size up to 2 microns reported to have bactericidal or bacteriostatic activities (Padmavathy and Vijayaraghavan, 2008). In this study, it was observed that none of the nanoparticle samples show activity against both groups of bacteria (Figure 7). The possible explanation for not observing any bioactivity in this study could be that the sample amounts prepared are low and the concentration is not sufficient for the activity. Bioactivity studies at different concentrations could not be carried out due to the low amount of samples obtained in microsystems.

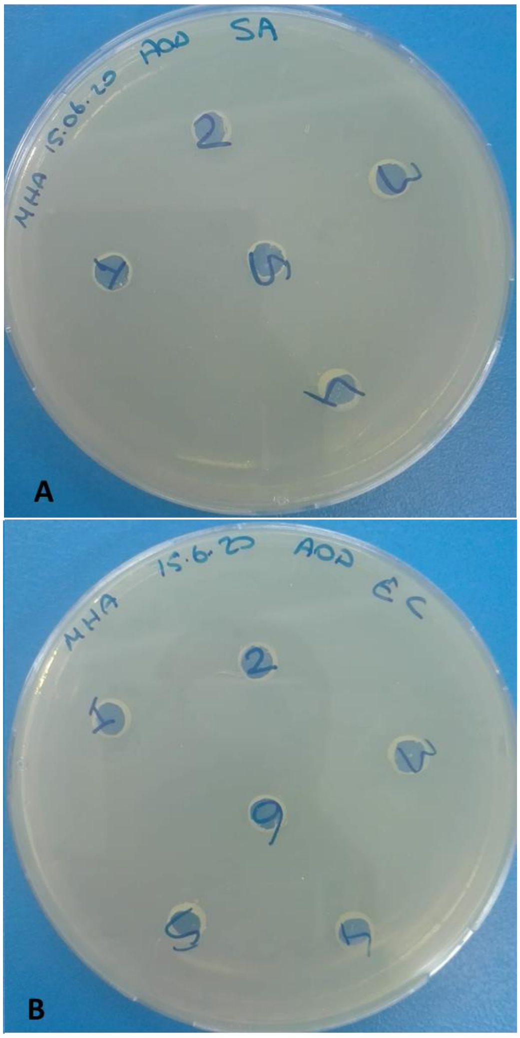

Figure 7. Antimicrobial activity against; A: *S. aureus*, B: *E.coli*.1;IS1, 2; IS28, 3;IS1 supernatant, 4; IS28 supernatant, 5; Zinc nitrate (5mM), 6; Red cabage raw extracts

4. Conclusion

In the present study, it was aimed to synthesis and characterization of the ZnO nanoparticles by green synthesis methods using aqueous extracts of red cabbage. In addition, due to the advantages of high heat and mass transfer provided by microsystems, the synthesis of nanoparticles was performed in flow-focusing based microsystems and the conditions have been optimized. From this aspect, in the present study, it was attempted to develop an alternative and eco-friendly method for the synthesis of ZnO nanoparticles by combining green synthesis and microfluidics. Because of achieved results, it can be concluded that reducing organic compounds in herbal extracts can be used to reduce metal ions and provide a green synthesis of nanoparticles. Thus, the method applied in the present work promise significant advantages for future studies.

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