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Research Article

A new polymer synthesis: structural characterization, antimicrobial and antiproliferative activity

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ABSTRACT

In this study, homo-polymerization of N-(4-nitrophenyl) acrylamide (NPA) monomer was performed and firstly poly(NPA) homopolymer was synthesized in the literature. The structure of synthesized homopolymer was chemically characterized by FT-IR, 1H-NMR and 13C-NMR spectroscopic techniques and elemental analysis. Thermal behavior was investigated with DSC, and TGA/DTA/DTG simultaneous system. Biologically, antimicrobial activity (E. coli, S. aureus, B. megaterium, E. aeroginosa, C. tropicalis) test research was performed. Followed the cytotoxic effect of polymer was investigated using XTT assay on HeLa cells.

Keywords: Homopolymer, thermal stability, antimicrobial activity, antiproliferative activity.

1. INTRODUCTION

Because of their many benefits, including affordability, chemical resistance, and ease of manufacture, polymeric materials play a significant role in our daily lives. However, because polymer raw ingredients are derived from petroleum, there are issues with cost and sustainability in the production of these materials due to the finite supply of these oil resources.[1] For new polymeric product development effort, scientific studies on polymers are also increasing. Polymeric materials have many advantages such as high/weak electrical conductivity or high/weak thermal conductivity, good mechanical properties, light, easy portability, and costs not as high as ceramic and metal materials.[2,3]

In order to increase these functions of polymers, first, it is necessary to change the functional structure of the monomeric product.[3,4] When the monomeric unit changes, its homopolymer also changes, and then the copolymer structures that can be obtained become possible. In this way, many types of polymers with different functional structures can be obtained.[5,6] One of the most widely used types to improve the functionality of polymers is amide/methamide containing compounds.[7,9] Our team has been working on meth/acrylate and meth/amide structures for many years.[10,11] In this study, poly(NPA) homopolymer was synthesized and characterized from an amide derivative, N-(4-nitrophenyl)acrylamide (NPA) monomer, and its results were discussed. Then, the thermal stability study of this new polymer was carried out. Finally, some of its biological properties have been investigated *in vitro*.[12] In our previous study, we found the toxic ratio of NPA monomer in HeLa cells to be 1mM.[11] In this study, after the HeLa result we found, a homopolymer of the same monomer was prepared and cytotoxic investigation was carried out with HeLa cancer cells. The first malignant cell line, HeLa, exhibits many characteristics of normal cells. Because they have features like other cells. HeLa cells have unquestionably provided practical models study.[13,14]

2. EXPERIMENTAL

2.1. Materials for synthesis

In the synthesis stage, p-Amino nitrobenzene (Across), Acryloyl chloride (Merck), $(Et)_3N$, azobisisobutyronitrile chemicals and solvents were used.

2.2. Synthesis of NPA homopolymer

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The monomer NPA (1 mmol) with the radical initiator azobisisobutyronitrile in N,N-Dimethylformamide solution were added into flask (Figure 1). The reaction was carried out in an inert environment. Ethyl alcohol was used to crystallize the resultant homopolymer. Synthesis of NPA homopolymer are shown in Figure 1. The chemical structure of the homopolymer was characterized, its thermal properties were investigated, and its antimicrobial properties were examined.

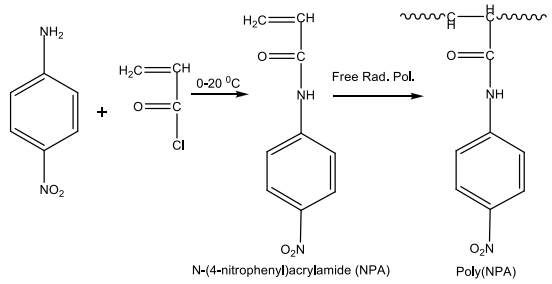

Figure 1. The synthesis of NPA monomer and its homopolymer.

2.3. Instrumental measurements

FT-IR studies were performed using Perkin-Elmer Two (UATR) spectroscope (4000-400 cm^{-1}) ^{1}H-NMR measurements was conducted on AVENCE III Bruker and Bruker Top Spin Ultra Shilt 400 MHz in DMSO-d_6 as solvent with TMS as the internal reference. With a Hitachi 7000 TGA/DTA/DTG simultaneous system, a thermal analysis of the homopolymer was performed at a heating rate of 10°C /min in a N_2 gaze, from room temperature to 600°C temperatures. DSC measurements was performed with a Shimadzu DSC-50 analyzer under a nitrogen flow, at a heating rate of 20°C/min to 200°C. A Leco CHNS-O model 932 elemental micro analyzer was used to perform elemental studies.

2.4. Antimicrobial activity study

Escherichia coli ATCC 66032, *Staphylococcus aureus* COWAN 1, *Bacillus megaterium* DSM 32, *Enterobacter aeregenes* CCM 2531, and *Candida tropicalis* ATCC 13803 were obtained from the Microbiology Laboratory culture collection. By using the disc diffusion method, antimicrobial tests were performed using 100 L of solution containing 10^6 cells/mL of bacteria and 10^4 cells/mL of yeast in accordance with the McFarland standard. The discs were placed on the infected Mueller Hinton Agar (Difco), and Malt Extract Agar (Difco), and then impregnated with 100 µg of the chemical. For two hours, sterile petri plates were kept at 4°C. The infected plates were incubated for 24 hours at 37 °C for bacterial strains and for 72 hours at 25 °C for yeasts. By evaluating the zone of inhibition against the test bacterium, antimicrobial activity was assessed. Streptomycin sulphate and Nystatin were used as standard antibiotic.[12]

2.5. Antiproliferative effect of homopolymer on HeLa cell line

In the study you are reading, XTT assay was used to measure cytotoxicity (Biological Industries, USA). 5.10^4 Hela cells per well were seeded onto 96-well plates, and they were then treated to various concentrations of polymer for 48 hours. The XTT solution was added after aspirating the culture solution from each well. The results of the absorbance measurement at 450 nm were directly proportionate to the number of viable cells in each treatment when utilizing a micro culture plate reader (BioTEK). From the results, the 50% inhibitory concentration (IC50) was estimated.[11]

3. RESULTS AND DISCUSSION

3.1. Spectroscopic characterization of NPA homopolymer

The synthesis of NPA homopolymer was carried out by free radical polymerization of NPA in the preference of initiator. The reaction scheme of polymer is given on Figure 1.

3.1.1. FT-IR spectrum

The first spectroscopic technique for locating functional groups inside a molecule is the FT-IR technique. The spectrum of homopolymer shows the characteristic bands at 1683 cm^{-1} (C=O amide stretch), 1613 cm^{-1} (C=C stretch), 1592 cm^{-1} (N–H bending vibrational), 1502 and 1332 cm^{-1} (antisymmetric and symmetric stretch NO_2 bonds) were observed (Figure 2) .[10,11,15,16]

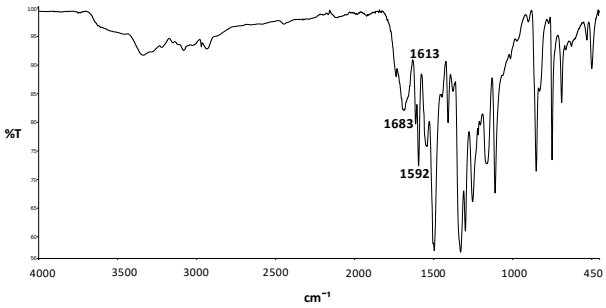

Figure 2. The FT-IR spectra of NPA homopolymer.

3.1.2. ^{1}H and ^{13}C NMR spectra

^{1}H-NMR spectrum of the polymer peaks appear at 10.3 ppm for N-H, 7.6 and 7.9 ppm for aromatic ring protons, polymer chain protons appear at 1.8 ppm, and 3.4 and 2.5 ppm for DMSO-d_6 protons. ^{13}C-NMR spectrum of the polymer peaks appear at 174 ppm for O=C, ring carbons at 162, 142, 125 and 119 ppm, polymer chain carbons at 36 and 31 ppm, and 40 ppm for DMSO-d_6 carbons (Figure 3, Figure4).[10,11,16]

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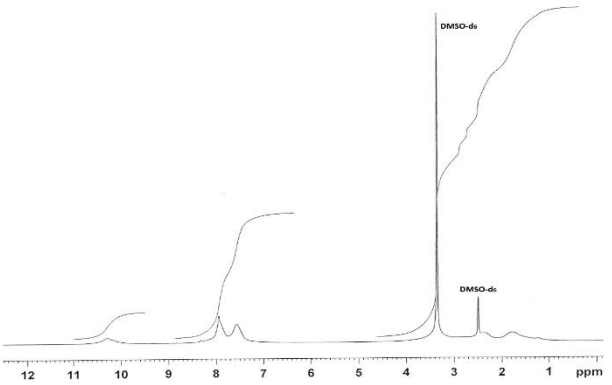

Figure 3. The ^{1}H-NMR spectrum of NPA homopolymer.

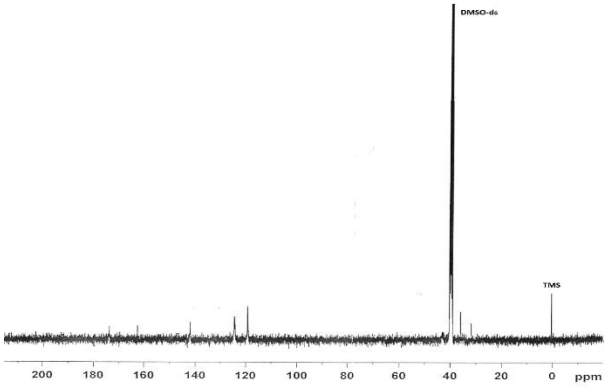

Figure 4. The ^{13}C NMR spectrum of NPA homopolymer.

The new polymer's elemental analysis values were within % of the theoretical data computed using the suggested formulas. The results of the polymer's elemental analysis are as follows: Experimental (%): C: 58.52, H: 5.9, O: 23.1, N: 14.0. The findings indicated that experimental and theoretical values were in good accord.[17]

3.2. Thermal characterization of NPA homopolymer

Utilizing thermal analysis techniques, one can learn more about the thermal behaviour of polymers and their thermal stabilities. The glass transition temperature, initial decomposition temperature and temperatures at some weight loss are important values for thermal stability. The glass transition temperature (T_g) of the homopolymer determined from the DSC thermogram, and the other thermal properties are determined by the TGA/DTA/DTG. T_g temperature of the homopolymer was determined to be about 153 °C, and the DSC curve is given in Figure 5.[18,19]

Important thermal results for polymer; decomposition temperature at 50%, 25% and 20% are 457 °C, 334 °C, and 326 °C. Weight loss at 500 °C, 450 °C, and 400 °C are 54%, 49%, and 45%. Residue at 600 °C, 550 °C and 500 °C are 40%, 42% and 46%. It was seen from the DTA curve that T_k (Crystallization temperature) was 344 °C, and from the DTG curve, the maximum decomposition temperature was 340 °C. Figure 6 shows the homopolymer thermal curves.[3,7,8,10,17-20]

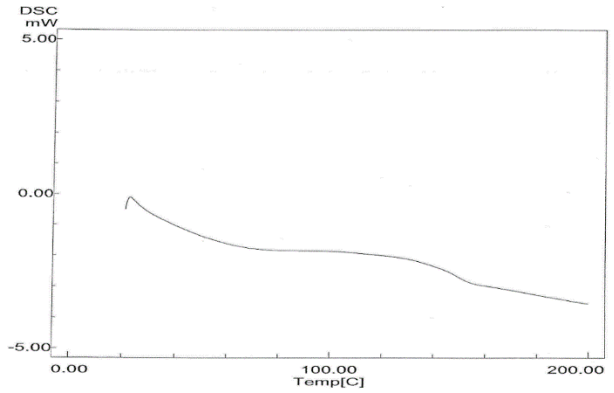

Figure 5. DSC curve of the NPA homopolymer.

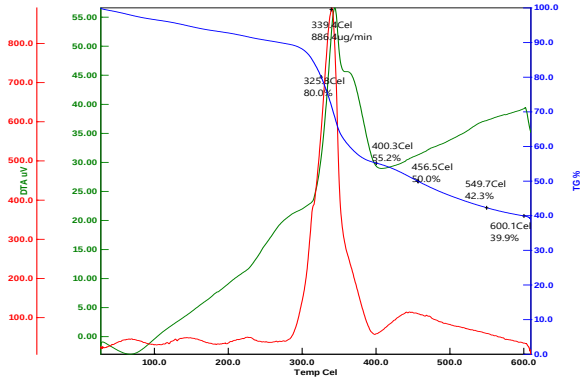

Figure 6. The thermal curves of the NPA homopolymer.

3.3. Antimicrobial properties of NPA homopolymer

On the plates, the resulting inhibition zones were measured (mm). The results were standardized against streptomycin sulfate (antibacterial, 10 µg/disc) and Nystatin (antifungal, 30 µg/disc) under the same conditions.[12,21,22]

The antibacterial effectiveness of homopolymer against microorganisms was examined. However, the polymer (0.1 mg/disc) did not show selective antimicrobial activity against *E. coli*, *S. aureus*, *B. megaterium*, *E. aeregenes*, and *C. tropicalis*. Homopolymer did not affect the growth of microorganisms. In a previous study by our team, N-(4-nitrophenyl)acrylamide (NPA) monomer used in the production of NPA homopolymer also did not have any activity against the same microorganisms.[12] It was observed that the material being in monomeric or polymeric structure did not change the result.

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3.4. Antiproliferative activity of NPA homopolymer on HeLa cell line

The cytotoxic effect on the homopolymer was tested by XTT assay up to concentration of 2000 µg/mL, and the results are given in Figure 7. Our findings demonstrated that polymer had no impact in preventing HeLa cell proliferation at 48 hours. In our team's previous study, the toxicity value of NPA monomer was determined as 1mM.[11] When switching from monomeric structure to polymeric structure, the number of repeating units of polymers increases and their molecular weights also increase. Although derived from the same substance, homopolymers are very different from their monomers. It can be said that the reason why the synthesized homopolymer does not have cytotoxic activity against HeLa cells is due to the fact that it has switched to a polymeric structure. In the studies carried out, we see that the synthesized materials have non-toxic properties.[14]

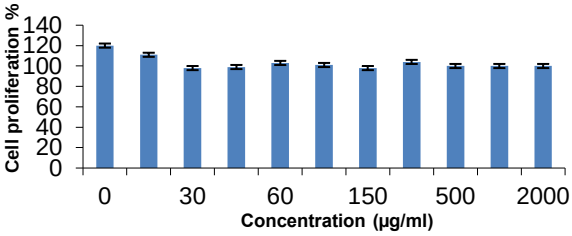

Figure 7. Cytotoxicity analyses of the NPA homopolymer.

4. CONCLUSION

In this study, poly(N-(4-nitrophenyl)acrylamide) homopolymer has been synthesized via free-radical solution polymerization. The resulting polymer was characterized by FT-IR and NMR spectroscopy techniques and elemental analysis. When the spectroscopy results were examined, it was seen that the results were in accordance with the literature. The findings of the elemental analysis demonstrated good concordance between experimental and theoretical data. Thermal properties of the polymer were investigated by the DSC and TGA/DTA/DTG system. It can be said that the thermal stability of the polymer is good. Some biological research of the homopolymer has also been done. It was tested for its antimicrobial activity against microorganism. However, the polymer did not show selective antimicrobial activity. In addition, the cytotoxic effect of homopolymer was investigated using XTT assay on HeLa cells. According to the findings, polymer exhibited a non-cytotoxic response; it may be used safely in a variety of research fields, including agriculture, medicine, and cosmetics. We believe that the obtained findings will guide some biological properties of the substances to be synthesized in the future. It is thought that the newly synthesized poly(NPA) homopolymer will attract the attention of those working in the materials and biomaterials sectors.

Conflict of interests

I declare that there is no a conflict of interest with any person, institute, company, etc.

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