Polyethylene Succinate-Based Polymeric Nanoparticles Bearing Azobenzene Moieties as Potential Agents for Hypoxia-Targeted Cancer Treatment

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ABSTRACT

Numerous studies have been conducted to develop effective methods to combat cancer and reduce its severity. One of the most promising approaches involves using potent chemotherapeutic agents that minimize damage to healthy cells. Scientists have also explored materials capable of distinguishing between healthy and cancerous cells and responding accordingly. By utilizing these differences, researchers aim to design materials that can release antitumor drugs specifically at tumor sites. One such distinguishing factor is the difference in oxygen levels between normal and cancerous tissues, leading to hypoxia.

In this research, a methoxy-poly(ethylene glycol)-azobenzene-poly(ethylene succinate) (mPEG–Azo–PES) copolymer was synthesized using a one-pot method. To investigate the structure of the polymer nanoparticles, Fourier transform infrared (FT-IR) as well as proton nuclear magnetic resonance (^{1}H-NMR) measurements were employed to confirm the chemical structure of the materials. The results of the investigation indicated that these polymer nanoparticles have potent applicability as drug release agent in hypoxic tumor environments. This strategy offers a targeted approach that could reduce side effects on healthy tissues and contribute to more selective cancer therapies.

Keywords: Polyethylene glycol, Polyethylene succinate, Azobenzene, One-pot synthesis, Drug delivery, Targeted cancer therapy, Hypoxia-responsive nanoparticles

1. INTRODUCTION

Recent advancements in nanotechnology have significantly influenced various scientific and industrial fields, particularly in pharmaceutical sciences [1]. Researchers are continuously exploring innovative methods to improve cancer treatment and reduce its associated toxicity [2, 3]. Traditional therapeutic strategies such as chemotherapy and radiotherapy remain the most common clinical approaches for cancer management; however, these treatments are often limited by poor selectivity, systemic side effects, and rapid clearance from the body [4, 5]. Drug delivery systems based on nanotechnology have drawn a lot of attention lately because of their potential to get around the drawbacks of traditional chemotherapy [6-8].

Nanocarrier-based systems enable precise drug delivery, controlled release, enhanced bioavailability, and improved therapeutic efficiency by facilitating selective accumulation of drugs within tumor tissues [9-12]. Various nanomaterials, including organic, inorganic, and hybrid nanoparticles, have been developed for this purpose. Among them, polymeric nanoparticles, liposomes, micelles, dendrimers, and polymersomes are the most widely investigated due to their tunable physicochemical properties and biocompatibility [1, 13]. These nanocarriers improve drug stability, reduce degradation, and minimize systemic side effects while enhancing accumulation at the tumor site [1, 3].

Polymers are versatile materials widely used in biomedical applications because of their biocompatibility, biodegradability, and tunable structural properties [14, 15]. Polymersomes, which are vesicular nanoparticles

created by the self-assembly of amphiphilic block copolymers, have become one of the most promising polymeric nanocarriers for drug delivery due to their superior mechanical stability and capacity to encapsulate both hydrophilic and hydrophobic agents [11, 16, 17]. Polymeric and amphiphilic block copolymer systems, such as PEG-b-PCL, which combines poly(ethylene glycol) and poly(caprolactone) blocks; PLA, a homopolymer of lactic acid; as well as PLGA, a copolymer composed of lactic and glycolic acid units, have shown remarkable potential for targeted and sustained drug delivery [18, 19]. The PEG component provides steric stabilization, prolongs circulation time, and prevents opsonization, thereby creating long-circulating "stealth" nanoparticles capable of achieving enhanced permeability and retention (EPR) effects in tumors [16, 18, 20]. Additionally, polymersomes can be modified with stimuli-responsive segments that enable site-specific and controlled drug release under physiological or pathological conditions [17, 21].

However, hypoxia is a common symptom observed in more than 60% of solid tumors within the tumor microenvironments [13, 22]. In healthy tissues, the partial pressure of oxygen is normally between 40 and 60 mm Hg, whereas it is often <10 mm Hg in tumor tissues [23, 24]. This arises from increased oxygen consumption by rapidly proliferating cancer cells [25, 26] and the presence of abnormal, inefficient vasculature that restricts oxygen diffusion [27]. Hypoxia-inducible factors (HIFs), especially HIF-1α, stabilize under hypoxic conditions and cause the production of genes related to glycolysis, angiogenesis, and metastasis, thereby enabling cancer cells to survive and proliferate in oxygen-deficient environments [28, 29].

Consequently, hypoxic tumors exhibit reduced therapeutic efficacy and poor responses to chemotherapy and radiotherapy [30, 31]. To address this, researchers have developed hypoxia-activated drugs that are enzymatically triggered in low-oxygen regions for targeted treatment of hypoxic tumors [32, 33].
Despite these advantages, many small-molecule agents display short circulation half-lives due to renal filtration, yielding insufficient intratumoral concentrations [34].

To overcome the therapeutic limitations caused by tumor hypoxia, researchers have focused on developing hypoxia-responsive (HR) drug delivery systems capable of releasing therapeutic agents selectively under oxygen-deficient conditions [35, 36]. Polymeric nanocarriers have been engineered to respond to hypoxic stimuli by incorporating reducible linkages such as azobenzene groups, which undergo cleavage in low-oxygen environments [37, 38]. The azobenzene (-N=N-) bond can be reduced to amine derivatives by azoreductase enzymes, leading to structural disassembly and controlled drug release from the carrier [39]. Moreover, azo-functionalized amphiphilic copolymers, particularly PEG-based systems, exhibit high stability and tunable release profiles, along with fluorescence activation upon azo-bond reduction [38]. Nitroaromatics and azo derivatives are frequently attached to amphiphilic copolymers to form self-assembled hypoxia-responsive nanocarriers [26, 40]. These groups are sensitive to oxygen deficiency, and the resulting chemical transformations promote rapid and selective drug release [25, 41]. Such self-assembled HR polymeric nanoparticles demonstrate excellent stability and preferential accumulation in tumor tissues, facilitating efficient and localized drug delivery [42].

Based on these principles, numerous investigations have documented the evolution of hypoxia-responsive nanocarriers and azo-functionalized polymers exhibiting selective drug release and improved therapeutic outcomes under oxygen-deficient conditions. A PEG–Azo-based AIEgen molecular probe was created by Li et al. [43] for hypoxia-mediated tumor imaging and photodynamic treatment. The azo linker between PEG and the photosensitizer was cleaved under hypoxic conditions, activating fluorescence and singlet oxygen generation. The system showed excellent biocompatibility, tumor specificity, and hypoxia-triggered phototoxicity, providing a model for designing multifunctional PEG–Azo nanocarriers. Similarly, Mahmudi et al. [44] reported the development of hypoxia-responsive nanoparticles (HRNPs) based on mPEG–Azo–Chitosan for selective delivery of Fingolimod to triple-negative breast cancer. The azobenzene linker enabled PEG detachment and charge reversal under hypoxic conditions, leading to enhanced tumor penetration and controlled drug release. Spectroscopic analyses confirmed azo bond cleavage and amine formation under reductive environments, verifying the system's hypoxia sensitivity and self-activation potential. In another study, Yuan et al. [45] synthesized an amphiphilic block copolymer PCL_{3k}–TPE–Azo–PEG_{5k}, where the azobenzene linkage connected hydrophilic (PEG) and hydrophobic (PCL) segments via a tetraphenylethylene (TPE) bridge. The polymer self-assembled into rod-like micelles capable of encapsulating doxorubicin (DOX). Under hypoxic or reductive conditions, azoreductase cleaved the azo bonds, resulting in micelle disassembly, controlled drug release, and activation of fluorescence. This "turn-on" fluorescence behavior confirmed the polymer's potential for simultaneous imaging and hypoxia-triggered drug delivery. Additionally, Thambi et al. [26] developed hypoxia-responsive carboxymethyl dextran nanoparticles (CMD NPs) by conjugating nitroimidazole derivatives to the polymer backbone. These nanoparticles exhibited selective drug release under

hypoxic conditions, demonstrating enhanced cytotoxicity and tumor accumulation while maintaining excellent biocompatibility.

However, there is still a need to develop novel hypoxia-sensitive copolymers with improved responsiveness and structural stability for targeted cancer therapy. Therefore, this study focuses on the synthesis and structural characterization of an mPEG–Azo–PES copolymer, proposed as a potential hypoxia-sensitive platform for targeted cancer drug delivery.

2. MATERIALS AND METHODS

Succinic anhydride, ethylene glycol, and the required solvents, including chloroform, n-hexane, tetrahydrofuran (THF), dimethylformamide (DMF), and thionyl chloride ($SOCl_2$), were obtained from Merck, Germany. Azobenzene-4,4'-dicarboxylic acid (linker) was purchased from Alfa Aesar, USA. Poly(ethylene glycol) monomethyl ether (mPEG, 550 g/mol) was obtained from Sigma Aldrich, USA. Several instruments were used in this study, including a universal centrifuge (Raymand T.N. Co., Iran), a vacuum oven (SHELDON LAB, USA), a rotary evaporator (Dragon Lab, China), and a magnetic stirrer (IKA, Germany). The FT-IR spectra of the synthesized materials were recorded using a Tensor 27 spectrophotometer (Bruker, Germany), and the ^{1}H-NMR spectra were also obtained on a 400 MHz Bruker Avance, NMR spectrometer.

2.1 Synthesis of PES

To synthesize the desired polyester, 2 g of succinic acid was mixed with 1.2 g of ethylene glycol. The mixture was heated under polycondensation conditions to form a low-molecular-weight prepolymer. The water generated during the reaction was separated using a Dean-Stark apparatus. Afterward, a small amount of THF was added to dissolve the prepolymer, followed by the addition of n-hexane to induce precipitation. The solvent was removed under reduced pressure using a vacuum oven, and the resulting white powder was dried to yield poly(ethylene succinate).

2.2 Chlorination of Azobenzene-4,4′-Dicarboxylic Acid Linker

To chlorinate the desired linker, a 50 mg portion of azobenzene-4,4'-dicarboxylic acid was mixed with appropriate amounts of DMF and $SOCl_2$ in a reaction flask with a total volume of 10 mL. Stirring was maintained at 500 rpm for 72 h under an argon atmosphere and in the dark using a magnetic stirrer. Due to the low thermal stability of azobenzene, the solvent was evaporated at low temperature (around 30 °C) with a rotary evaporator. The obtained product appeared as an orange solid.

2.3 Synthesis of mPEG–Azo–PES Nanoparticles

The polymeric nanoparticles were synthesized via a one-pot single-step method. In this process, the pre-synthesized polyester was mixed with mPEG and the chlorinated azobenzene linker in THF. The mixture was stirred at 500 rpm for 96 h using a magnetic stirrer, and the solvent was subsequently evaporated at approximately 30 °C using a rotary evaporator. The final product was obtained as an orange solid. The schematic mechanism of the polymerization reaction for mPEG–Azo–PES nanoparticles is illustrated in ***Fig. 1****.*

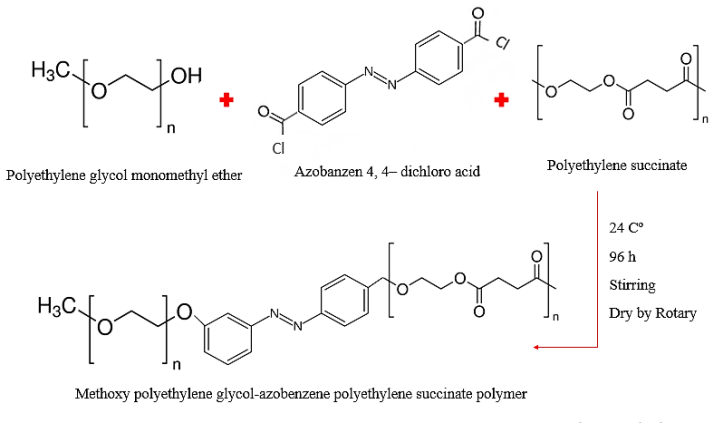

Fig.1. *Schematic overview showing the one-pot formation of mPEG–Azo–PES copolymer nanoparticles.*

3. RESULTS AND DISCUSSION

3.1 FT-IR Spectrum of PES

The infrared spectrum recorded for the produced PES sample is presented in ***Fig. 2****., where a band at approximately 3446 cm^{-1} is assigned to the stretching vibration of hydroxyl (O–H) groups. A band appearing at 2964 cm^{-1} can be attributed to the aliphatic C–H stretching vibrations in the methylene units. A strong and sharp band at 1733 cm^{-1} confirms the existence of ester carbonyl (C=O) bonds, indicating the successful formation of the polyester backbone. A weaker band near 1686 cm^{-1} can be assigned to the carbonyl vibration associated with carboxylic acid groups, with a slight shift likely caused by hydrogen bonding interactions. Also, the peak at 1389 cm^{-1} is associated with the C–H bending vibrations, whereas the bands between 1120–1165 cm^{-1} are attributed to C–O–C stretching modes within the ester linkages. These observations verify the successful synthesis of poly(ethylene succinate) PES.*

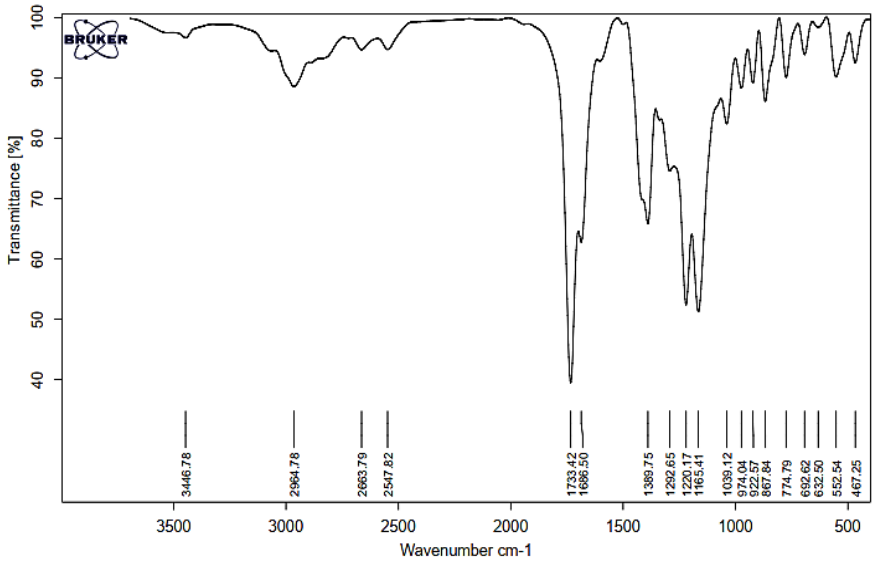

Fig. 2. *FT-IR spectrum of PES.*

3.2 FT-IR Analysis of mPEG–Azo–PES Polymer Nanoparticles

After purification, the FT-IR spectrum of the synthesized mPEG–Azo–PES polymer nanoparticles is shown in ***Fig. 3****. The absorption band at approximately 3441 cm^{-1} exhibits the stretching vibrations of O–H groups. The absorptions at 2928 and 2854 cm^{-1} can be attributed to the C–H stretching modes of aliphatic chains. The band near 1390 cm^{-1} is related to the C-H bending vibrations. The strong absorption at 1733 cm^{-1} represents the ester carbonyl group, confirming the formation of the polyester backbone, while the band at 1689 cm^{-1} is attributed to the C=O vibration corresponding to carboxylic functionalities. The peak at 1167 cm^{-1} is assigned to C–O–C (ether) stretching, indicating the presence of mPEG chains. Furthermore, the signals at 1509 cm^{-1} (N=N) and 1220 cm^{-1} (C–N) confirm the incorporation of the azobenzene linker into the copolymer nanoparticles. These spectral features collectively verify the successful one-pot synthesis of mPEG–Azo-PES polymer nanoparticles.*

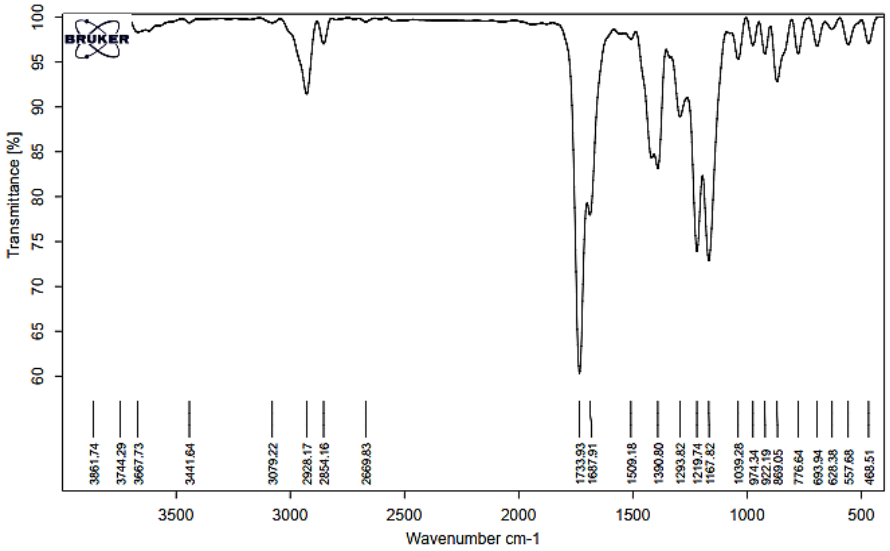

Fig.3. *FT-IR spectrum of mPEG–Azo–PES polymer nanoparticles.*

3.3 ^{1}H-NMR Analysis of mPEG–Azo–PES Polymer Nanoparticles

The ^{1}H-NMR spectrum of the synthesized mPEG–Azo–PES recorded in DMSO-d_6 is shown in ***Fig. 4****. A multiplet at 2.67 ppm (J ≈ 3 Hz) corresponds to methylene protons adjacent to the ester carbonyl groups (–CO–CH$_2$–CH$_2$–CO–) of the polyester backbone. Broad signals observed within the 3.6–3.8 ppm range correspond to protons of the methylene units (–CH$_2$–O–) of the mPEG segment. A resonance at 4.31 ppm is assigned to the methylene protons adjacent to the oxygen atom in the ester linkage (–COO–CH$_2$–CH$_2$–O–). Finally, aromatic signals at 7.1–7.3 ppm confirm the presence of azobenzene units within the copolymer structure.*

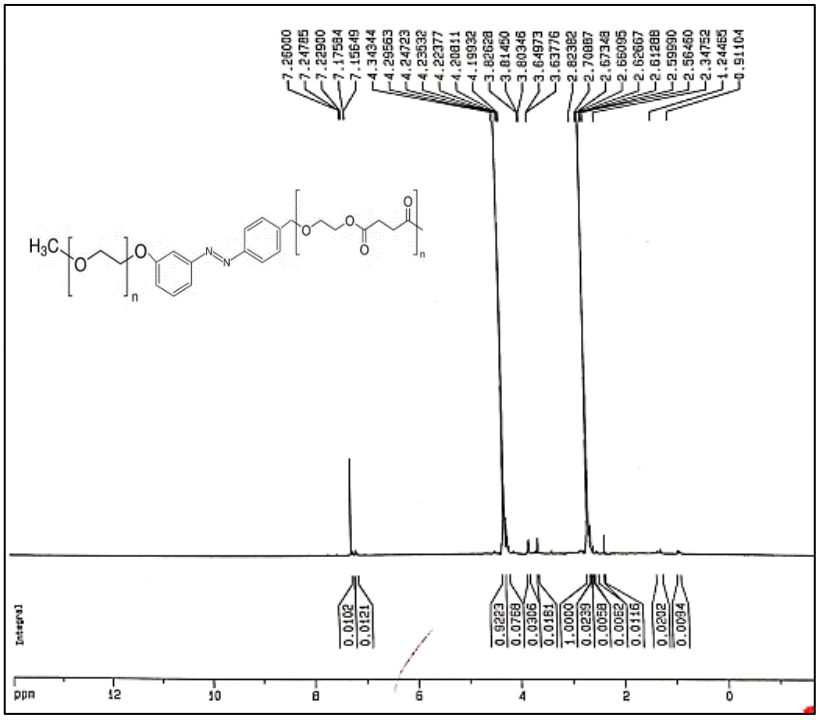

Fig.4. *^{1}H-NMR spectrum of mPEG–Azo–PES polymer nanoparticles in DMSO-d_6, confirming the expected proton resonances corresponding to the copolymer structure shown in the inset.*

4. CONCLUSION

In this study, methoxy polyethylene glycol-azobenzene-polyethylene succinate (mPEG–Azo–PES) copolymer nanoparticles were successfully synthesized via a one-pot single-step method. The structural characteristics of the obtained nanoparticles were verified using FT-IR ae well as ^{1}H-NMR analyses. The observed spectral features verified the presence of ester, azobenzene, and PEG linkages, confirming the successful formation of the designed amphiphilic copolymer. Based on their chemical composition and the presence of azobenzene linkers, these nanoparticles exhibit potential for use as hypoxia-responsive carriers in targeted drug delivery systems.

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