Black Sea Journal of Engineering and Science
doi: 10.34248/bsengineering.1501951

Open Access Journal
e-ISSN: 2619 – 8991

Research Article
Volume 7 - Issue 5: xxx-xxx / September 2024

SYNTHESIS AND EVALUATION OF PH AND TEMPERATURE STIMULI-RESPONSIVE MAGNETIC NANOHYDROGELS FOR GENE DELIVERY

Ferzane VALİOĞLU[1,3]*, Fereshteh VALIPOUR[2], Sibel SÜMER[3], Mustafa TÜRK[4], Soodabeh DAVARAN[2]

[1]*Sakarya University, Technology Development Zones Management CO, 54050 Sakarya, Türkiye*
[2]*Tabriz University of Medical Sciences, Faculty of Pharmacy, Department of Medicinal Chemistry, Tabriz, Iran*
[3]*Hacettepe University, Faculty of Sciences, Department of Molecular Biology, 06800, Ankara, Türkiye*
[4]*Kırıkkale University, Faculty of Bioengineering, Department of Bioengineering, 71451, Kırıkkale, Türkiye*

Abstract: The offer of gene delivery technologies as a promising approach to treating a variety of diseases has revolutionized human medicine over the last two decades. So, the application of suitable vectors, particularly polymers with substrates with unique physicochemical properties for the transfer of targeted genes to logical sites for effective treatment, plays an indispensable role for more personalized medicine and improves the safety profile in response to continuing to use new medical technologies. For this purpose, we synthesized nanocarriers with a two-block cationic hydrogel, magnetic and non-magnetic, based on N-isopropyl acrylamide (NIPAM) and quaternary alkyl ammonium halide salts of DMAEMA (DMAEMAQ) with pH and temperature responsiveness via the free radical polymerization technique. The bulk properties of these co-polymers were characterized by using Fourier transform infrared spectroscopy, ^{1}H NMR spectroscopy, zeta potential, lower critical solution temperature (LCST), and gel electrophoresis to show the loading of nanoparticles with the gene. In the results, magnetic P[NIPAM-DMAEMAQ] hydrogel showed controllable responsive properties determined by the nature of the cationic charge +24.7 mV incorporated, nanosize around 86.95 and 91.22 nm, and efficiency loaded with the gene more than 95%. As well, the synthesized nanohydrogel exhibited a sharp volume-phase transition in water at a LCST of ~40 °C. So, the combination of both monomers yielded an interesting system with high transfection efficiency and compliant biocompatibility characteristics, which could effectively achieve gene loading. Also, the magnetic potential of nanohydrogel was determined as a vector to deliver genes to localized sites. Notably, the synthesized combination P[NIPAM-DMAEMAQ] nanohydrogel has been considered a transfection of the biodegradable and biocompatible magnetic nanoparticle sensitive to tunable pH and temperature responsiveness, demonstrating that it will hold a promising approach as a potential carrier to improve gene delivery therapeutic efficacy in cancer and different disease treatments.

Keywords: Gene delivery, Biodegradation, Magnetic P(NIPAM-DMAEMAQ), pH and Thermoresponsive hydrogels

***Corresponding author:** Sakarya University, Technology Development Zones Management CO, 54050 Sakarya, Türkiye
E mail: valioglu.ferzane@gmail.com (F. VALİOĞLU)
Ferzane VALİOĞLU https://orcid.org/0000-0002-9388-3214
Fereshteh VALIPOUR https://orcid.org/0000-0002-4126-3945
Sibel SÜMER https://orcid.org/0009-0005-2048-8597
Mustafa TÜRK https://orcid.org/0000-0001-8202-090X
Soodabeh DAVARAN https://orcid.org/0000-0002-7072-2362

Received: June 16, 2024
Accepted: July 16, 2024
Published: September 15, 2024

Cite as: Valioğlu F, Valipour F, Sümer S, Türk M, Davaran S. 2024. Synthesis and evaluation of pH and temperature stimuli-responsive magnetic nanohydrogels for gene delivery. BSJ Eng Sci, 7(5): xxx-xxx.

1. Introduction

The delivery issue of genes (nucleic acids) and gene therapy in the biomedical field is a hopeful strategy with futuristic applications in the treatment of illnesses ranging from tumors to infectious diseases to genetic syndromes and a crucial role in the treatment of numerous ailments, including cancer, malignant tumors, hereditary diseases, and neurodegenerative diseases (Sung, 2019). Nevertheless, there are several disadvantages, such as no targeted delivery (Sayed et al., 2022), fast degradation (Shahryari et al., 2021), inadequate effectiveness, and many adverse effects following nucleic acid entry into the bloodstream. In the last few years, significant advancements in knowledge of the importance of effective specific gene transfer at its origin site through different vectors have drawn attention, and numerous carriers have been introduced. In stimuli-responsive gene delivery systems, targeted delivery and application of biodegrading nanoparticle-based carriers are one of the most important treatment strategies in delivery applications is to ferry genes into the target site with minimizing the side effects and maximizing their therapeutic effects while protecting the gene from nucleases during transit, providing an option for a substantial increase in treatment efficiency compared to conventional methods (Hamimed et al., 2009; Zhang et al., 2023). Because improving transcript splicing, inhibiting or regulating gene expression, and restoring the expression of a damaged protein are all effective methods that might have a significant impact on

biological research and therapy (Shillitoe, 2009; Yang et al., 2020).

Nanocarrier systems could be developed to generate coating NPs using a variety of materials, including polymeric nanoparticles (Rai et al., 2019), inorganic nanoparticles (Lin et al., 2021), metals (He et al., 2023), semiconductors, and other substances, so that these features can be used for specific purposes and are possible to attach to a targeted therapeutic gene to correct a genetic defect (Jacob et al., 2024). A type of nanocarrier system such as hydrogels, by crosslinking water-soluble polymers into a network, can create hydrogels' porous and hydratable structure in order to deliver genes to specific sites in response to external stimuli such as temperature, magnetic field, pH, ionic strength, or electric field (Thang et al., 2023). Also recently in nanomedicine, nanoparticulate systems have seen considerable improvement in the development of an assortment of nanoparticles known as magnetic nanoparticles (MNPs) that are produced with a blend of metal ions and polymer coatings. Magnetic cores could be functionalized or made of porous polymers loaded with magnetic nanoparticles (Bi et al., 2020; Materon et al., 2021). One of the most significant components of magnetic nanoparticles as superparamagnetic iron oxide nanoparticles (SPIONs) is oxidized iron, which has a primarily crystalline core made of Fe_3O_4 or Fe_2O_3 with a dimension of 10–100 nm. These particles can be guided by an outside magnetic field, and nanoparticle-based gene delivery can deliver them to the target origins, which creates promising therapeutic effects for a wide range of diseases (Roy, 2022). Therefore, magnetic nanoparticles have been created to improve the essential features and attributes of capability applications for carrying active biomolecules and gene delivery systems and play a critical role in advance stability, controlling their release, and giving higher curative effects in biomedical applications. Moreover, the nanoscale dimension includes a high surface area-to-volume ratio, which is an important characteristic of nanoparticles (NPs), and in order to qualify as nanoparticles, they have to possess at least one dimension in the nanometer range, up to around 100 nm, that improves their therapeutic and diagnostic forms (Satalkar et al., 2016). Hence, the significance and nano-biotechnology's demand for magnetic nanoparticles due to their potential to improve treatment techniques and bring up new avenues for biomedicine have enabled us to produce and create the magnetic pH and thermosensitive nanohydrogel in this study. So, we initially created a copolymer of 2-(dimethylamino)ethyl methacrylate (DMAEMA) with poly (N-isopropylacrylamide) and its quaternary ammonium quart salt (DMAEMAQ). Next, we synthesized a magnetic P(NIPAM-co-DMAEMAQ) nanohydrogel that could react to temperature and pH stimuli simultaneously. The effect of PNIPAM-co-DMAEMA content was assessed in terms of surface charge features, particle size, construction, and loading of copolymers with the gene. NIPAM, as a temperature-responsive hydrogel, is a nonionic polymer, but loading a gene into nanomateriyal needs a positive charge because acidic nuclease groups have negative charges (Fussell et al., 2019). So, cationic copolymers of P[DMAEMAQ] with strong positively charged groups that could condense genes through electrostatic adsorption have been used as transfectants in which protonated amines of their capability to bind the desired gene into submicron complexes, which safeguard and preserve the gene from nucleases during transfer (Manouras et al., 2021). In addition, in order to use PNIPAM in gene delivery processes, it is necessary to improve its swelling value by copolymerizing it with hydrophilic and/or ionic comonomers such as poly (PDMAEMAQ). Also, when NIPAM is copolymerized with an ionic monomer, the resultant copolymer reacts to pH and temperature (Fussell et al., 2019; Kim et al., 2019). Thus, cationic polymer-poly (PDMAEMAQ) is utilized as a regulator of the binding or release of genes due to its satisfactory loading capacity. These unique characteristics contribute to targeted and controlled gene release in tumor cells (Yang et al., 2015). Overall, the aim herein was to synthesize and describe the biomedical potential of cationic stimuli-responsive thermo-responsive hydrogels with potential positive charge and magnetic properties for gene delivery to provide for the development of nanoplatforms that will be clinically applicable in medicine. Besides, synergistic therapy could be achieved when combined with other therapeutic regimens. The uniqueness of this study offers the possibility of creating intrinsic magnetic hydrogels through the copolymerization of a quart salt of dimethylaminoethyl methacrylate (DMAEMAQ) with a well-known thermoresponsive monomer, N-isopropylacrylamide (NIPAM), to create hydrogels via precipitation polymerization (radical polymerization) for gene delivery. As a result of this study, we determined that magnetic proportion with dual thermo-pH-sensitive potential copolymer hydrogels could load gene activity and have good physical properties besides maintaining thermos-responsiveness. Also, nano-vectors for gene therapy can be extensively researched and used owing to their facile application, targeting ability, high bioavailability, and good biocompatibility in biomedicine. Finally, by mentioning this case, the in-vitro investigation of these studies in breast cancer cells has been done and will be published soon.

2. Materials and Methods

2.1. Materials

N-isopropyl acrylamide (NIPAM) was obtained at Acros Organics and purified by recrystallization from n-hexane-toluene (90:10 v/v) under vacuum at 25 °C. 2-(Dimethylamino)ethyl methacrylate (DMAEMA) monomers (Sigma–Aldrich Co., Steinem, Germany) were distilled under reduced pressure in nitrogen atmosphere. Tin (II) 2-ethyl hexanoate (stannous octoate, $Sn(Oct)_2$),

benzoyl peroxide (BPO), ferrous chloride tetrahydrate ($FeCl_2.4H_2O$), ferric chloride hexahydrate ($FeCl_3.6H_2O$), ammonium hydroxide (32 wt %), N,N'-methylenebisacrylamide (MBA), tetrahydrofuran (THF), dimethyl sulfoxide (DMSO), sodium monohydrogen phosphate, dihydrogen phosphate, methyl iodide (CH_3I), and ethidium bromide (EtBr) were from Sigma-Aldrich, n-hexane, 1, 4 dioxan, and diethyl ether were obtained at Merck Chemical Co. Distilled deionized (DDI) water was prepared with a Millipore system and used for dilution purposes throughout. Other chemicals were reagent-grade commercial materials and used without further purification.

2.2. Synthesis of Magnetic P(NIPAM-CO-DMAEMAQ) Hydrogel Copolymers

2.2.1. Synthesis of superparamagnetic magnetite nanoparticles

Superparamagnetic magnetite nanoparticles were prepared via the improved chemical co-precipitation method. According to this method, 3.1736 g of $FeCl_2{\cdot}4H_2O$ (0.016 mol) and 7.5684 g of $FeCl_3{\cdot}6H_2O$ (0.028 mol) were dissolved in 320 mL of deionized water. The mixed solution was stirred under N_2 at 80 °C for 1 h. Then, 40 ml of $NH_3{\cdot}H_2O$ was injected into the mixture, rapidly stirred under N_2, then cooled to room temperature as shown in Figure1. The precipitated particles were washed five times with hot water and separated by magnetic decantation. Finally, magnetic nanoparticles were dried under vacuum at 70 °C (Wulandari et al., 2022).

$2\ FeCl_3 + FeCl_2 + 4\ H_2O + 8\ NH_3 \rightarrow Fe_3O_4 + 8\ NH_4Cl$

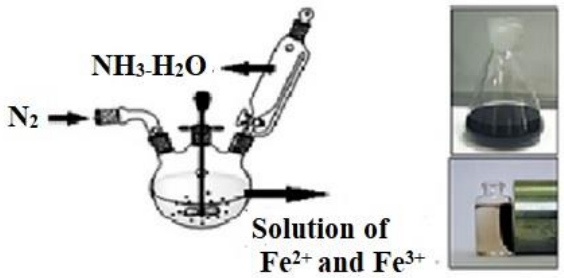

Figure 1. Schematic synthesis of superparamagnetic magnetite nanoparticles.

2.2.2. Synthesis of cationic quaternary ammonum alkyl halide monomers (Q)

Quaternary ammonium alkyl halide monomer (DMAEMAQ) was prepared by dissolving 19.1 mmol of DMAEMA in 5 mL of dry THF under stirring. The next step is the quaternization of the tertiary amine of DMAEMA by addition of 22 mmol of CH_3I, dissolved in 5 mL of THF, via the N-alkylation reaction using methyl iodide, as shown in Figure 2. The reaction was carried out at room temperature with magnetic stirring (Moselhy et al., 2009). The crude products were filtered, washed twice with 10 mL aliquots of cold hexane, and dried in vacuum to yield the DMAEMAQ products (white crystalline solid, yield: 90%).

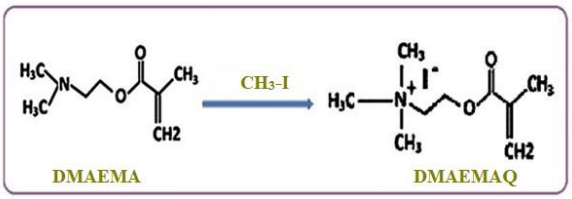

Figure 2. Synthesis scheme of quaternary ammonium alkyl halide monomers (DMAEMAQ).

2.2.3. Synthesis of magnetic P(NIPAM-DMAEMAQ) nanohydrogel

Magnetic thermoresponsive cationic nanogel networks P(NIPAM-DMAEMAQ) based on N-isopropylacrylamide (NIPAM) and quaternary alkyl ammo-nium halide salts of 2-dimethylaminoethyl methacrylate (DMAEMAQ) by molar ratio of 80:20, respectively, Fe_3O_4 nanoparticles, N,N′-methylene-bis-acrylamide (MBA) as a crosslinking agent, and benzoyl peroxide (BPO) as a copolymerization initiator (0.5% w/w) were synthesized by free radical copolymerization of monomers in 1,4-dioxane (50 ml) under nitrogen atmosphere. The specified quantity of reagents and Fe_3O_4 nanoparticles (1% of the total weight monomers) were sonicated with 1,4-dioxane for about 30 min to prepare magnetic nanocomposites. The mixture added to the flask was magnetically stirred and degassed under a nitrogen atmosphere for 30 minutes before polymerization. The reaction was carried out at 70 °C for 18 h under a nitrogen atmosphere. After polymerization, the synthesized polymer was cooled, and the resultant polymers were purified by dissolution/precipitation with a tetrahydrofuran (THF)/diethyl ether solution and dried in a vacuum. Then the purified solution was frozen in liquid nitrogen and then lyophilized to obtain dry powder with a yield of 87%.

2.2.4. Characterization of P(NIPAM- DMAEMAQ) nanohydrogel

Fourier transforms infrared (FT-IR) spectroscopy

The chemical structures of the synthesized polymers were investigated by FT-IR spectroscopy. Upon having the synthesized product, all samples were mixed with the dry potassium bromide (KBr) powders and pressed to the disk. Infrared (IR) spectra of the samples were scanned in the range of 400 to 4000 cm^{-1} at a resolution of 4 cm^{-1}, with a minimum of 256 scans per spectrum at room temperature (Nicolet-Fisher Scientific, Inc., USA). The spectra of water, CO_2, and KBr were subtracted from the sample spectrum, and the procedure was done under nitrogen gas to prevent humidity interference.

Hydrogen nuclear magnetic resonance (^{1}HNMR) spectroscopy

The chemical composition of the synthesized polymers was determined by ^{1}H NMR in DMSO at 300 MHz by using Gemini 300 NMR Spectrometer (Varian, Palo Alto, CA, USA).

Particle size and zeta potential measurements

The analysis of the Zeta potential for size-distribution

and surface charge of PNIPAM-DMAEMAQ nanoparticules were performed using a Zetasizer Nano series ZS Dynamic Light Scattering (DLS) (Malvern. Instruments Ltd., UK). The sample of nanoparticules were dissolved in deionized water and ultrasonicated for 5 minutes. It was measured using a Zetasizer Nano Z at 37 °C and at a neutral pH. (Malvern Instruments, UK). The measurement was repeated in triplicate per sample, and measurements were reported as mean ± S.D.

Scanning electron microscopy studies

The size, surface morphology and appearance of the cationic NIPAM-DMAEMAQ hydrogels were assessed by using a scanning electron microscope (JEOL, JSM 5600). Before investigation, the hydrogels were dried at -45 °C and 38.10^{-3} mmHg using a vacuum freeze-dryer (Armfield SB4), and then powder sample was spread on a SEM stub and sputter-coated with gold. Particle size was obtained by measuring the diameters of at least 300 particles shown in SEM using image analysis software (Image-Pro plus 4.5; Media Cybernetics, Silver Spring, USA).

Measurement of LCST of P[NIPAM-DMAEMAQ] nanohydrogel

The phase transition temperature of aqueous P[NIPAM-DMAEMAQ] nanohydrogel was determined using a UV-visible spectrophotometer (UV-160 Shimadzu) at the wavelength of 500 nm over the temperature range (18–50 °C) by using cloud point measurement (turbidimetry) method. The heating rate was 1 °C/min. At each step, the samples were stabilized for 10 min before the next measurements (Valipour et al., 2011). Values for the LCST of polymeric solution were determined as the temperature at the inflection point in the normalized absorbance versus the temperature curve.

Evaluation of the binding P(NIPAM-DMAEMAQ) nanoparticles with the gene

The binding and interaction abilities of the gene with the magnetic P(NIPAM-DMAEMAQ) nanohydrogels of the complex formed by using the negative charge of the gene and the positive charge of the nanoparticle were determined using agarose gel electrophoresis. For this, firstly, nanoparticles (magnetic and non-magnetic) in non-toxicity concentration proportions were prepared. The prepared NP and MNP nanoparticles were loaded with 1 µg of the desired gene (Bcl-2 siRNA) mix in the RPMI 1640 medium without FBS by stirring for 30 min at 37 °C. It was left at room temperature for half an hour for the gene to interact with the nanoparticle. Then, 15 µL of the NP-gene and MNP-gene samples were mixed with 5 µL of 6× loading dye, and the final volume was made up to 20 µL, respectively. The naked gene was used as the control. The complexes were loaded onto a 4% agarose gel of EtBr and run using Tris-Agarose EDTA (TAE) buffer at 100 V for 45 min. The experiments were performed in triplicate. The gene bands were visualized by irradiation with UV light using the ChemDoc™ XRS Gel Documentation System (BioRad, California, USA).

3. Results and Discussion

3.1. Magnetic P(NIPAM-DMAEMAQ) Nanohydrogel

The synthesized bases of magnetic P (NIPAM-DMAEMAQ) and non-magnetic P (NIPAM-DMAEMA) are demonstrated schematically in Figure 3. Co-polymer magnetic P(NIPAM-co-DMAEM) nanohydrogels were synthesized using a radical polymerization process in order to create magnetic and thermoresponsive PNIPAM-based nanocomposites with quaternary alkyl ammonium halide salts of DMAEMA (Kim et al., 2019). Superparamagnetic magnetite nanoparticles were created in the first stage, and then, via the amend chemical co-precipitation technique, cationic quaternary ammonium alkyl halide monomers were synthesized by reacting methyl iodide (CH_3I) and the amine moiety of DMAEMA. Next, in the presence of NIPAM monomers, graft-free radical polymerization was initiated by using the vinyl bond of the DMAEMAQ moiety of a cationic monomer. Subsequently, copolymer P(NIPAM-DMAEMAQ) was mixed with superparamagnetic magnetite nanoparticle polymers. Poly (N,N dimethylaminoethyl methacrylate) (PDMAEMA) carries secondary amine groups that are both thermos-sensitive and pH-sensitive. Then in this way, the cationic magnetic nanoparticulated hydrogels that are pH- and thermos-sensitive were made ready for gene delivery. As known, magnetic nanoparticles, also known as iron oxide nanoparticles, have been shown to be more effective as gene delivery vehicles due to their stronger proton relaxation properties, reduced toxicity, lower detection limit, and active biomolecules tailored to their particular uses (Patra et al., 2018). As shown in Figure 3, the successful synthesis of P(NIPAM-DMAEMAQ) was confirmed by FT-IR spectroscopy. PNIPAM chain was grafted onto the nanogels to serve as pH sensitive and thermos-sensitive nanovalves (Liu et al., 2009). DMAEMAQ as a cationic polymer can condense negatively charged gene into nano sized particles through electrostatic interactions. In the result of nanocomplex formation, the polymers can protect gene from nuclease degradation and facilitate cellular uptake to induce high gene transfection.

3.2. Characterization

3.2.1. Fourier-transform infrared spectroscopy (FT-IR) analysis

FT-IR was first performed to confirm that the copolymerization of NIPAM and DMAEMAQ was successfully achieved. The FT-IR spectrum of the P(NIPAM-DMAEMAQ) polymer network are presented in Figure 4 the range of 4000 to 400 cm^{-1}. The spectrum exhibited two characteristic absorption signals of amide functional groups of poly(NIPAM) at 1650 cm^{-1} (NH-CO stretching) and 1550 cm^{-1} (N-H bending). Additionally, the broad absorption band at 3284 cm^{-1}referred to (–NH secondary amid stretching), and an absorption band at 2960 cm^{-1} (–CH_3 asymmetric stretching) of NIPAM was observed.

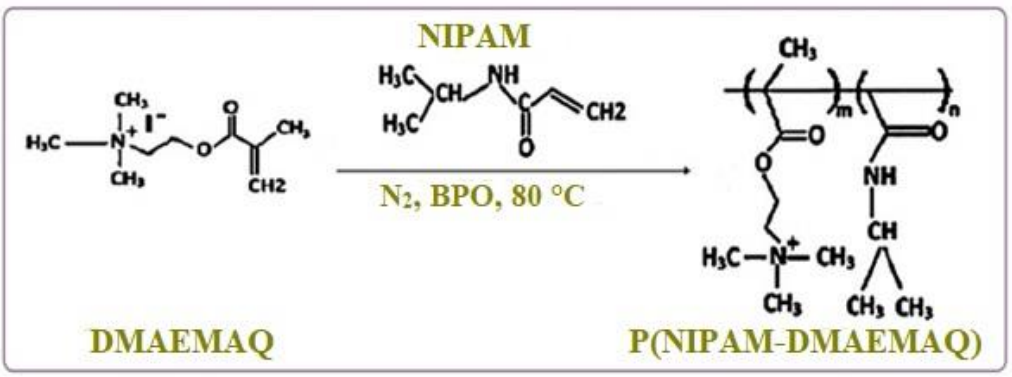

Figure 3. Synthesis scheme of P(NIPAM-DMAEMAQ) copolymer.

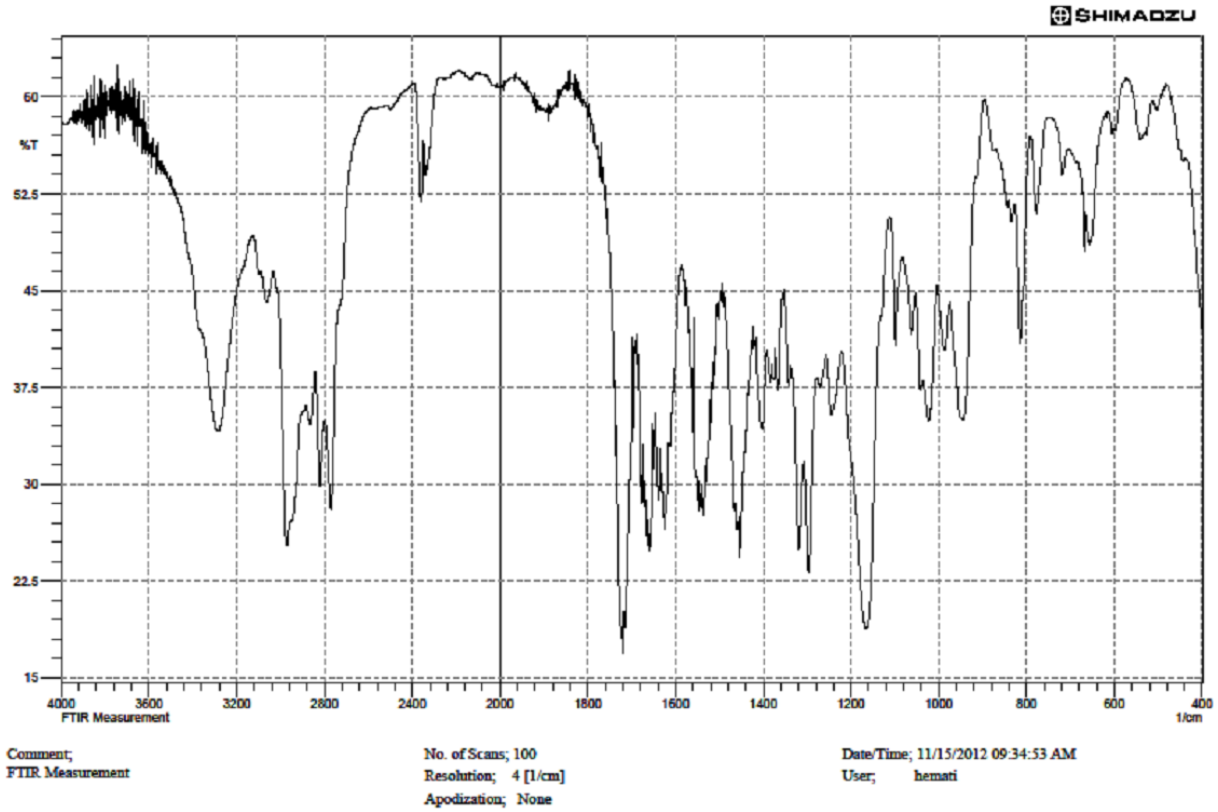

Figure 4. FT-IR spectrum of P(NIPAM-DMAEMAQ) nanohydrogel.

Finally, a characteristic stretching vibration peak for the ester carbonyl (C=O) at 1735 cm^{-1}, which confirms the successful copolymerization of PNIPAM with DMAEMA.

3.2.2. Hydrogen nuclear magnetic resonance (^{1}HNMR) spectrum

The ^{1}HNMR spectrum (in DMSO) of the P(NIPAM-DMAEMAQ) copolymer was used to determine the structure and composition of the copolymer (Figure 5). The chemical shifts at 1.16 ppm (6H, -CH-($\underline{CH_3}$)$_2$), 3.89 ppm (1H, -$\underline{CH}$-(CH_3)$_2$), and 7.42 ppm (1H, $\underline{NH}$-C=O) correspond to protons in the PNIPAAm segment. The signals appearing in 2.58 ppm (9H, -N^+-(CH_3)$_3$), 3.89 ppm (2H, -O-CH_2-CH_2-N(CH_3)$_2$), 2.82 ppm (2H, CH_2-N(CH_3)$_2$) related to protons of DMAEMA moiety of polymer, respectively (De Jesus-Tellez et al., 2020). The removal of the vinyl protons peaks at 5.58 ppm and 6.11 ppm demonstrates that end double bonds were converted into carbon-carbon signal bonds to form the main chain during polymerization (Valipour et al., 2021). All the evidence proved a graft copolymer was successfully synthesized.

3.2.3. Zeta potential (ζ) measurements

After magnetic copolymer P(NIPAM-DMAEMAQ) nanohydrogels were created, measurements of the surface charge distribution of the nanoparticles were taken with the Zeta-Sizer device (3000 HSA model MALVERN). Figures 6 and 7 show the surface charge graph of nanoparticles measured with the Zeta-Sizer. Initially, we analyzed the produced microgels and ascertained how the NIPAM affected their surface charge density (zeta potential). The PNIPAM hydrogel zeta potential has been shown to be near the isoelectric point (ISP = 0 mV). Then we determined the effect of DMAEMAQ content on the surface charge density of the

prepared microgels and analyzed how the queerization of the tertiary amine of the DMAEMA with different alkyl agents affects the hydrogel's surface charge. When DMAEMAQ was incorporated into the system, as observed in Figure 6, surface charge became positive and increased in value as the content of DMAEMAQ increased, and quaternized hydrogels with methyl showed even higher values of zeta potential: 12 mV. So, it was seen that the zeta potential of non-magnetic P(NIPAM-DMAEMAQ) nanoparticles had a positive surface charge in the surface charge analysis results. This could be caused by the increased carboxyl groups of DMAEMAQ in P (NIPAM-DMAEMAQ). Also, it was seen that the nanoparticles combined with magnetite had a more positive surface charge of +24.7 mV as shown in Figure 7. This difference could be caused by the Fe^{+3}-containing groups interacting between the polymer layers, which have the most positive charge in magnetic P(NIPAM-DMAEMAQ) hydrogel. It was remarkable that the high zeta potential value was indicative of the colloidal stability of NPs. Thus, magnetic and non-magnetic nanoparticles, which are determined to have a positive surface charge by zeta-potential analysis, will be able to bind and interact with negatively charged genes. Thus, magnetic and non-magnetic nanoparticles, which are determined to have a positive surface charge by zeta-potential analysis, will be able to bind and interact with negatively charged genes.

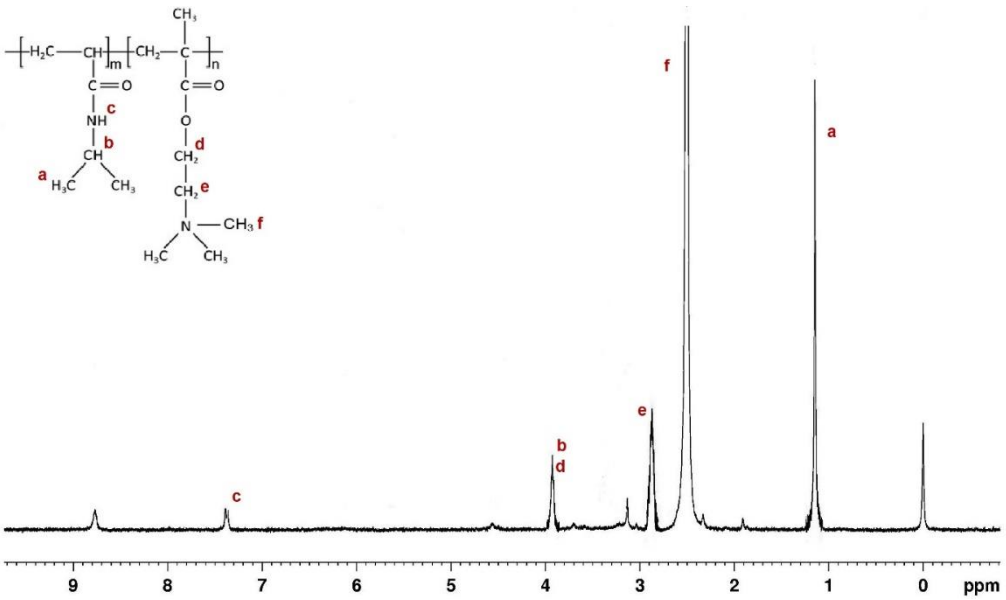

Figure 5. ^{1}HNMR spectrum of P(NIPAM-DMAEMAQ) nanohydrogel.

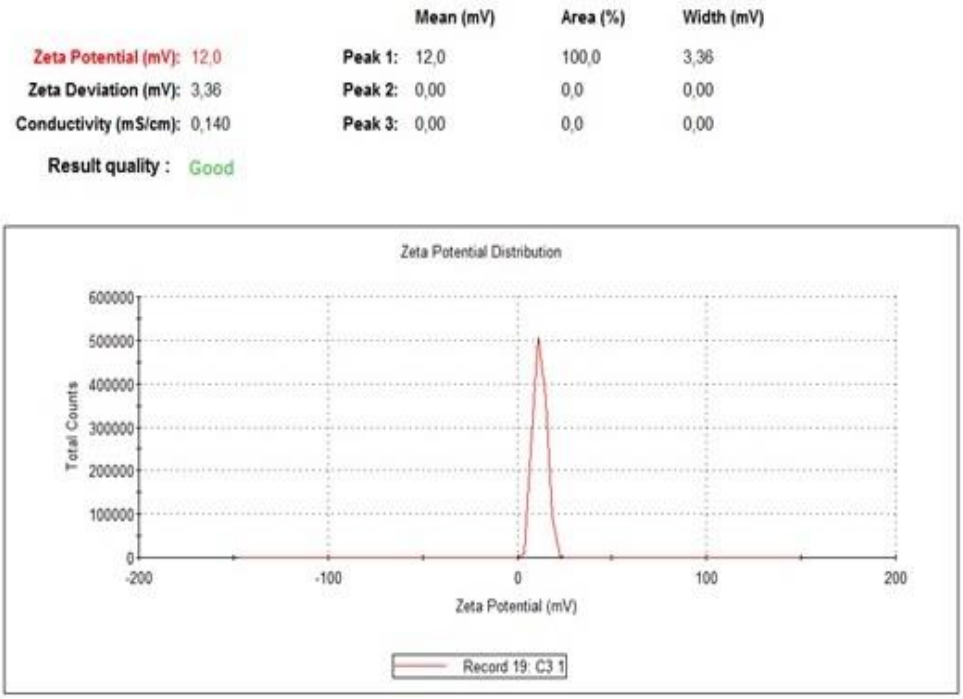

Figure 6. Surface charge distribution of P(NIPAM-DMAEMA) nanoparticles by zeta potential analysis.

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): 24,7	Peak 1:	25,5	96,5	5,37
Zeta Deviation (mV): 6,98	Peak 2:	-0,989	3,5	3,30
Conductivity (mS/cm): 1,22	Peak 3:	0,00	0,0	0,00
Result quality : Good				

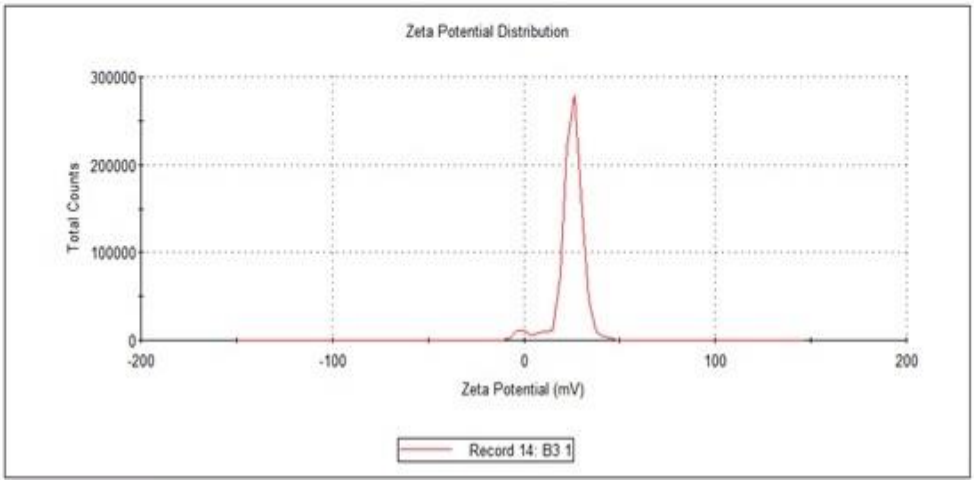

Figure 7. Surface charge distribution of magnetic P(NIPAM-DMAEMAQ) nanoparticles by zeta potential analysis.

3.2.4. Morphology with SEM

The structural morphology of non-magnetic P(NIPAM-co-DMAEMAQ) and magnetic P(NIPAM-co-DMAEMAQ) hydrogels was investigated by scanning electron microscopy (SEM) after the hydrogels in the swelling state were freeze-dried. Figure 8 shows that the interior morphology of the hydrogels clearly exhibits a macro-nano structure. And the particle size and distribution of both copolymer nanohydrogels were porous, with a relatively uniform pore size distribution and a similar trend. Additionally, although there is no apparent difference between the SEM micrographs of both of the magnetic and non-magnetic copolymers, in Figure 9, the SEM results of the nanoparticles' dimensions in non-magnetic nanoparticles were approximately around 77.06 and 77.16 nm, and magnetic nanoparticles were around 86.95 and 91.22 nm. So, the size of magnetic-loaded P(NIPAM-DMAEMAQ) increased slightly, indicating that the magnetic particles were loaded in P(NIPAM-DMAEMAQ) nanocomposites. As a result, it was confirmed that the synthesized P(NIPAM-DMAEMAQ) particles are nanosized.

3.2.5. Phase transition behavior and LCST of the P(NIPAM-DMAEMAQ) nanohydrogel

Cloud point measurements were utilized for determination of LCST of P(NIPAM-DMAEMAQ) nanohydrogel by the turbidity method via monitoring the change in the optical absorbance as a function of temperature. Figure 10 exhibits the typical cloud point measurement of hydrogel with sharp phase transition for hydrogel with a small temperature change. The sharp reduction in UV transmittance indicates that the hydrogels become increasingly turbid with shrinking on increasing at higher temperatures, and the LCST is the point at which the network structure of polymer collapse and water expelled of the hydrogel. As depicted in Figure 10, P(NIPAM-DMAEMAQ) exhibited sharp volume phase transition at 40 °C, so the LCST of the hydrogel solution was about 40 °C, which was between the LCST of poly(NIPAM) (32 °C) (Lanzalaco, 2017) and poly(DMAEMA) (50 °C) (Gutarowska et al., 2015). In general, incorporation of hydrophilic monomers shift the hydrogel toward a more hydrophilic nature and the LCST should shift to a higher temperature. An admitted mechanism of the phase separation of PNIPAM as a thermo-sensitive moiety in the polymer backbone, is described as resulting from the breakdown of hydrogen bonds and the enhancement of hydrophobic interactions. Generally, a hydrophilic/hydrophobic balance exists in PNIPAM side chains. At room temperature, favorable hydrogen bonding interactions between hydrophilic amide groups in the polymers and water molecules result in the good solubility of PNIPAM. With further increasing temperature, the hydrophilic/hydrophobic balance shifts to a more hydrophobic nature, the hydrogen bonds are broken and the aggregation process takes place at around LCST. In addition, copolymerization of hydrophilic DMAEMAQ moiety with NIPAM may increase the LCST value, which caused by the changes of the hydrophilic/hydrophobic ratio and formed more hydrogen bonds between the polymer chains and water molecules (Huang et al., 2017).

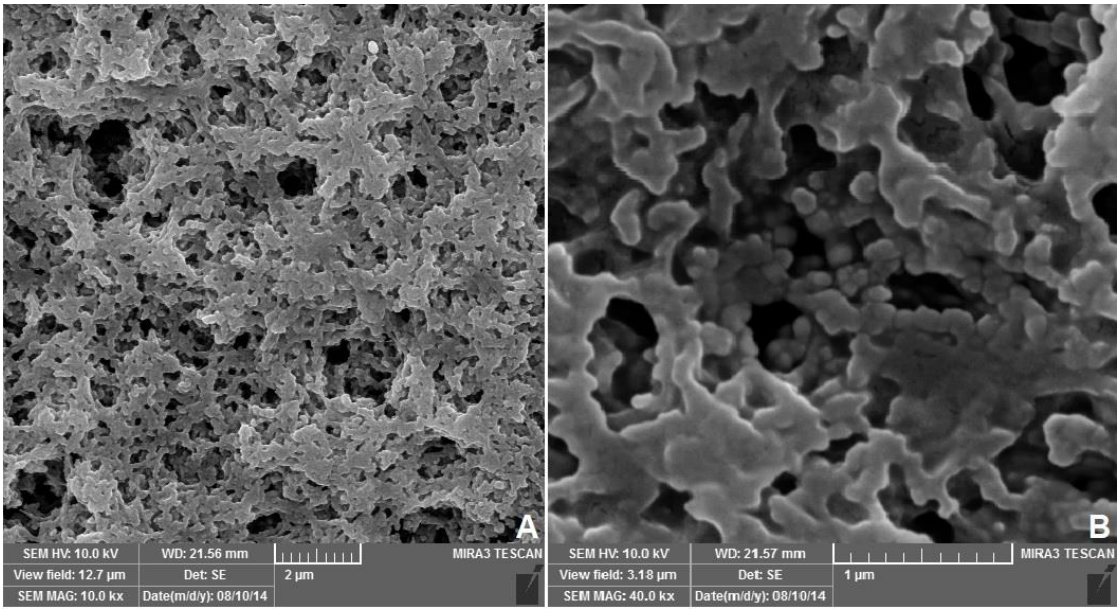

Figure 8. Surface image of the synthesized P(NIPAM-DMAEMAQ) nanohydrogel using SEM.

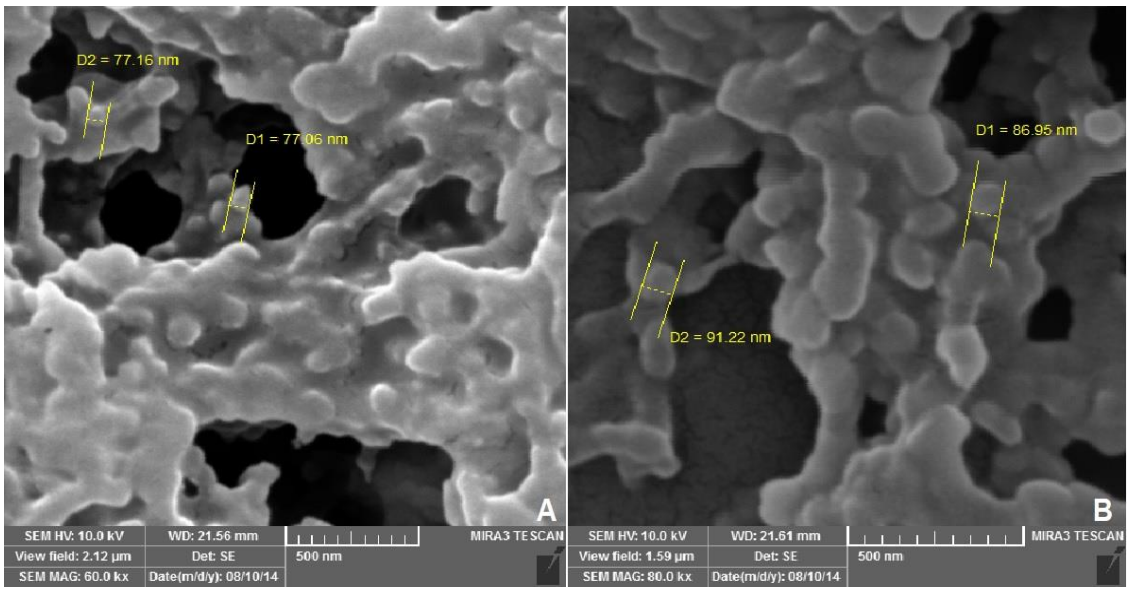

Figure 9. Demonstration of the dimensions of the synthesized P(NIPAM-DMAEMAQ) nanohydrogel using SEM. (A): Size of non-magnetic nanoparticle, (B): Size of magnetic nanoparticle.

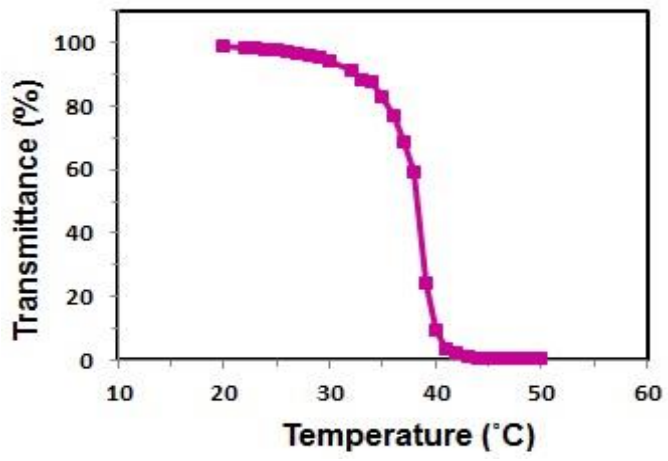

Figure 10. Transmittance-temperature curve of the P(NIPAM-DMAEMAQ) nanohydrogel determination by UV–Vis spectrum.

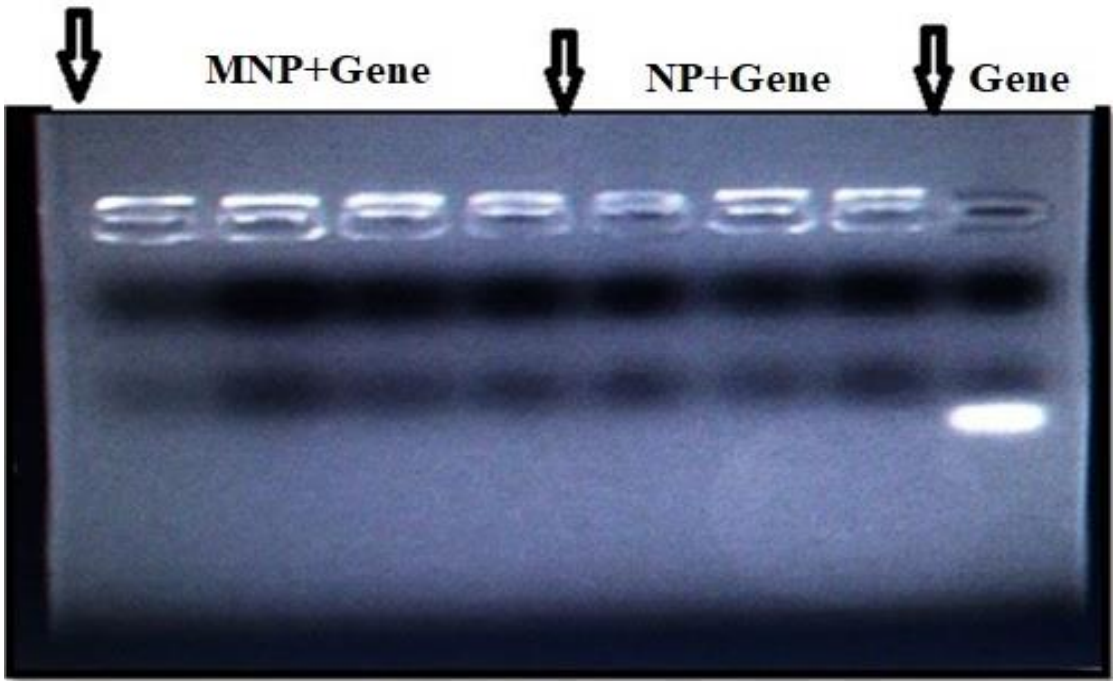

Figure 11. Demonstration of loading MNP and NP with the gene through an agarose gel electrophoresis assay.

3.3. Investigation of the P(NIPAM-DMAEMAQ) Nanohygrogel Loading with the Gene through Agarose Gel Electrophoresis Assay

In this stage of the research to confirm the application of NIPAM-DMAEMAQ-based nanocomposites with loading by gene, the interaction properties between magnetic and non-magnetic NIPAM-DMAEMAQ nanocomposites and the gene were examined by gel electrophoresis assay. As observed in Figure 11, wells 1–7 represent the presents of the gene and NIPAM-DMAEMAQ complex inside the well itself. The wells of 1-4 represent the presents of the gene with MNP, the wells of 5-7 represent the presents of the gene with NP, and well 8 presents the naked gene. The naked gene was used as the control group. As the results showed in well 8, the band didn't exist in the wall, indicating no banding of the naked gene in the well. So the naked gene has not shown any absorbance in the 8-well region. In the case of NP via gene for the wells (5, 6, and 7), a light band is observed. This clearly showed a weak interaction between nonmagnetic NP and the gene, but in the case of MNP with the gene for the wells (1, 2, 3, and 4), a potent band is observed. So, the complete binding of the gene to magnetic P[NIPAM-DMAEMAQ] nanoparticles clearly demonstrates a strong interaction between magnetic NP and the gene in wells 1–4, which has prevented the release of genes in the presence of the electrophoresis buffer. Also, due to the presence of Fe_3O_4, it causes a very positive charge and demonstrates a potent interaction between magnetic NP and gene. So that, the positive charges of magnetic P[NIPAM-DMAEMAQ] could neutralize the negative charges of the phosphate groups in the gene backbone, thus retarding gene mobility. This clearly suggests that the charge of nanoparticles plays a significant role in the loading and delivery of gene products (negative charge due to the presence of a phosphate group).Therefore, P[NIPAM-DMAEMAQ] NPs are more effective in the intracellular delivery of genes (nucleic acids) (Yang et al., 2015). On the other hand, non-magnetic versus magnetic samples were also tested to check the bonding pattern of nanoparticles with genes. Electrophoresis with gene-loaded NPs demonstrated a weak interaction between the NPs and genes. As a result, the high gene loading in nanoparticles can be attributed to the interaction between the positively charged group of the magnetic P(NIPAM-DMAEMAQ) and the negative charge part of the gene through electrostatic adsorption. Hence, according to the results, we chose the magnetic P(NIPAM-DMAEMAQ) due to its strong adsorption capabilities as a gene carrier, their magnetic properties to achieve control over the delivery of the gene, and their capacity for satisfactory loading to continue our research.

4. Conclusion

In gene delivery systems, the precise control of gene delivery and the right site-selective capability accompanying it are still a big challenge. Currently, the development of functional biosynthesis nanocarriers with safety, good biocompatibility, low cost of production, and effectiveness is of intense focus and importance for the delivery of the gene (nucleic acid) for gene therapy. According to the importance of the issue, our main goal herein was to build up a cationic carrier for targeted, specific gene delivery to maximize efficiency and minimize nano-related toxicity. For these purposes, we copolymerized NIPAM with a basic monomer quaternary ammonium quart salt of DMAEMA. Then, two kinds of dual temperature and pH-responsive polymer-coated magnetic nanohydrogels composed of magnetic P(NIPAM-DMAEMAQ) and non-magnetic P(NIPAM-

DMAEMAQ) polymers were successfully synthesized at an approximate size of nano. The nanoparticles' dimensions in non-magnetic nanoparticles were approximately around 77.06 and 77.16 nm, and magnetic nanoparticles were around 86.95 and 91.22 nm. As a result, MNPs with hyperthermia properties were loaded with specific genes as nanocarriers and used for targeted delivery. The resultant nanocarriers with positive charge were able to be efficiently loaded with the specific gene (more than 95%). Thanks to these unique properties, magnetic nanoparticles with metal-based configurations could be controlled by a varying external magnetic field current for various purposes, such as targeting nano-based genes or medications, promoting specific site targeting, and slowing tumor development. Therefore, combined with the specific gene release behavior and high loading capacity of genes, the present magnetic P (NIPAM-DMAEMAQ) could resolve the difficulties in loading and uncontrolled release of genes in cancer cells and different diseases. As a result, the targeted delivery of genes and drugs with smart nanocarriers could be used to treat different diseases and tumors and will be a promising alternative to cancer and other diseases.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	F.Valio.	F.Valip.	S.S.	M.T.	S.D.
C	50	15	20	10	5
D	50	35	5	5	5
S	25	5	35	25	10
DCP	50	35	5	5	5
DAI	50	35	5	5	5
L	50	35	5	5	5
W	50	30	10	5	5
CR	50	35	5	5	5
SR	50	35	5	5	5
PM	50	5	20	20	5
FA	40	5	25	20	10

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

Acknowledgment

The authors are thankful to Hacettepe University, Scientific Research Projects Coordination Unit. This article was written as a part of PhD thesis and was financially supported by Hacettepe University, Scientific Research Projects Coordination Unit. (Grant No. 610).

References

Bi Q, Song X, Hu A, Luo T, Jin R, Ai H, Nie Y. 2020. Magnetofection: Magic magnetic nanoparticles for efficient gene delivery. Chin J Chem, 31: 3041-3046.

De Jesus-Tullez MA, Sanchez-Cerrillo DM, Quintana-Owen P, Schubert US, Contreras-Lopez D, Guerrero-Sanchez C. 2020. Kinetic investigations of quaternization reactions of poly [2-(dimethylamino) ethyl methacrylate] with diverse alkyl halides. Macromol Chem Phys, 221: 1900543.

Fussell SL, Bayliss K, Coops C, Matthews L, Li W, Briscoe WH, Faers M, Royall C, Van Duijneveldt JS. 2019. Reversible temperature-controlled gelation in mixtures of pNIPAM microgels and non-ionic polymer surfactant. Soft Matter, 15: 8578-8588.

Gutarowska B, Stawski D, Sknra J, Herczynska L, Pielech-Przybylska K, Połowinski S, Krucinska I. 2015. PLA nonwovens modified with poly (dimethylaminoethyl methacrylate) as antimicrobial filter materials for workplaces. Text Res J, 85: 1083-1094.

Hamimed S, Jabberi M, Chatti A. 2022. Nanotechnology in drug and gene delivery. Naunyn Schmiedebergs Arch Pharmacol, 395: 769-787.

He Y, Li D, Wu L, Yin X, Zhang X, Patterson LH , Zhang J. 2023. Metal-Organic Frameworks for Gene Therapy and Detection. Adv Funct Mater, 33: 2212277.

Huang Y, Yong P, Chen Y, Gao Y, Xu W, Lv Y, Yang L, Reis RL, Pirraco RP, Chen J. 2017. Micellization and gelatinization in aqueous media of pH-and thermo-responsive amphiphilic ABC (PMMA 82-b-PDMAEMA 150-b-PNIPAM 65) triblock copolymer synthesized by consecutive RAFT polymerization. RSC Adv, 7: 28711-28722.

Jacob S, Kather FS, Morsy MA, Boddu SH, Attimarad M, Shah J, Shinu P, Nair AB. 2024. Advances in nanocarrier systems for overcoming formulation challenges of curcumin: Current İnsights. Nanomater, 14: 672.

Kim YK, Kim E-J, Lim JH, Cho HK, Hong WJ, Jeon HH, Chung BG. 2019. Dual stimuli-triggered nanogels in response to temperature and pH changes for controlled drug release. Nanoscale Res Lett, 14: 1-9.

Lanzalaco S, Armelin E. 2017. Poly (N-isopropylacrylamide) and copolymers: a review on recent progresses in biomedical applications. Gels, 3: 36.

Lin G, Revia RA, Zhang M. 2021. Inorganic nanomaterial-mediated gene therapy in combination with other antitumor treatment modalities. Adv Func Mater, 31: 2007096.

Liu R, Cellesi F, Tirelli N, Saunders B. 2009. A study of thermoassociative gelation of aqueous cationic poly (N-isopropyl acrylamide) graft copolymer solutions. Polymer, 50: 1456-1462.

Manouras T, Platania V, Georgopoulou A, Chatzinikolaidou M, Vamvakaki M. 2021. Responsive quaternized PDMAEMA copolymers with antimicrobial action. Polymers, 13: 3051.

Materon EM, Miyazaki CM, Carr O, Joshi N, Picciani PH, Dalmaschio CJ, Davis F, Shimizu FM. 2021. Magnetic nanoparticles in biomedical applications: A review. Appl Surf Sci Adv, 6: 100163.

Moselhy J, Vira T, Liu FF, Wu XY. 2009. Characterization of complexation of poly (N-isopropylacrylamide-co-2-(dimethylamino) ethyl methacrylate) thermoresponsive cationic nanogels with salmon sperm DNA. Int J Nanomed, 2009: 153-164.

Patra JK, Das G, Fraceto LF, Campos EVR, Rodriguez-Torres MDP, Acosta-Torres LS, Diaz-Torres LA, Grillo R, Swamy MK, Sharma S. 2018. Nano based drug delivery systems: recent developments and future prospects. J Nanobiotechnol, 16: 1-33.

Rai R, Alwani S, Badea I. 2019. Polymeric nanoparticles in gene therapy: New avenues of design and optimization for delivery applications. Polymers, 11: 745.

Roy I. 2022. Therapeutic applications of magnetic nanoparticles: recent advances. Mater Adv, 3: 7425-7444.

Satalkar P, Elger BS, Shaw DM. 2016. Defining nano, nanotechnology and nanomedicine: why should it matter? Sci Eng Ethics, 22: 1255-1276.

Sayed N, Allawadhi P, Khurana A, Singh V, Navik U, Pasumarthi SK, Khurana I, Banothu AK, Weiskirchen R , Bharani KK. 2022. Gene therapy: Comprehensive overview and therapeutic applications. Life Sci, 294: 120375.

Shahryari A, Burtscher I, Nazari Z, Lickert H. 2021. Engineering gene therapy: advances and barriers. Adv Therap, 4: 2100040.

Shillitoe EJ. 2009. Gene therapy: the end of the rainbow?. Head Neck Oncol, 1: 1-5.

Sung YK, Kim S. 2019. Recent advances in the development of gene delivery systems. Biomater Res, 23: 8.

Thang NH, Chien TB, Cuong DX. 2023. Polymer-based hydrogels applied in drug delivery: An overview. Gels, 9: 523.

Valipour F, Esmhosseini M, Nejati K, Kianfar H, Pasdaran A, Davaran S. 2011. Synthesis and antibacterial activity of silver nanoparticles embedded in smart poly (N-isopropylacrylamide)-based hydrogel networks. J Nanotechnol Eng Med, 2: 041001.

Valipour F, Valipour F, Rahbarghazi R, Navali AM, Rashidi MR, Davaran S. 2021. Novel hybrid polyester-polyacrylate hydrogels enriched with platelet-derived growth factor for chondrogenic differentiation of adipose-derived mesenchymal stem cells in vitro. J Biol Eng, 15: 1-14.

Wulandari AD, Sutriyo S, Rahmasari R. 2022. Synthesis conditions and characterization of superparamagnetic iron oxide nanoparticles with oleic acid stabilizer. J Adv Pharm Technol Res, 13: 89-94.

Yang HY, Van Ee RJ, Timmer K, Craenmehr EG, Huang JH, Oner FC, Dhert WJ, Kragten AH, Willems N, Grinwis GC. 2015. A novel injectable thermoresponsive and cytocompatible gel of poly (N-isopropylacrylamide) with layered double hydroxides facilitates siRNA delivery into chondrocytes in 3D culture. Acta Biomater, 23: 214-228.

Yang R, Chen F, Guo J, Zhou D, Luan S. 2020. Recent advances in polymeric biomaterials-based gene delivery for cartilage repair. Bioact Mater, 5: 990-1003.

Zhang Q, Kuang G, Li W, Wang J, Ren H, Zhao Y. 2023. Stimuli-responsive gene delivery nanocarriers for cancer therapy. Nanomicro Lett, 15: 44.