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Synthesis, Characterization and Antimicrobial Evaluation of Polyacrylic acid Drug Conjugates Using Benzidine as a Spacer

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ABSTRACT

Background & Objective: Polyacrylic acid can serve as an effective drug-delivery platform when conjugated with pharmaceutical compounds via a reliable spacer. This study aimed to synthesize and characterize benzidine-linked polyacrylic acid drug conjugates and evaluate their antimicrobial activity.

Materials & Methods: Conjugates were prepared by reacting polyacrylic acid with benzidine, followed by attachment of carboxylic-acid-containing drugs. The resulting polymers were characterized for structural and physicochemical properties. Antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* was assessed.

Results: FTIR and ^{1}HNMR confirmed successful conjugation. Polymers showed pH-dependent drug-release behavior and varying molecular weights. AB1 and AB6 exhibited strong inhibition against *S. aureus* (20 and 18 mm) and moderate activity against *E. coli*, while AB2 demonstrated the highest effect against *E. coli* (28 mm). Most conjugates displayed enhanced antibacterial activity compared to the free drugs.

Conclusion: Spacer-mediated polyacrylic acid drug conjugates effectively modulate drug release and enhance antibacterial performance, highlighting their potential as controlled drug-delivery systems.

Keywords: Polyacrylic Acid, Carboxylic Acid Pharmaceuticals, Biological Activity, Antibacterial, Drug Release

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1. Introduction

The fact that the novel products created have several applications in different spheres of daily life has led to increased interest in polymer science and engineering. The main goal of the research is to increase durability and performance. Polyacrylic acid (PAA), one of the many polymers, is of interest to researchers due to its numerous uses in a variety of industries, such as electrochemical (1), electronic (2), biomedical (3, 4), and others.

Polyacrylic acid, is a valuable substance for use in pharmaceutical and medical applications. High swelling degree, biocompatibility, non-toxicity, pH sensitivity, and mucoadhesive qualities are characteristics of this polyelectrolite. It is an adaptable polymer that can be used as a protective system and for drug administration (5-7).

One limitation of polymer reactions is that direct bonding of a drug to the polymer backbone may result in reduced reactivity with the polymer chains. The nearby side groups' steric barrier could be the source of this. The restricted loading and excessively sluggish hydrolysis of the drug from the polymer backbone actually contribute

to the low efficacy of polymeric prodrugs. By using spacer arms to separate the reactive groups from the main chain, this issue has been resolved. "To facilitate the labile bonds' hydrolysis or enzymatic breakdown, the bridging groups or spacer arms must be added. Altering the kind and extent of substitution within the structure of a chosen polymer can also regulate activity. The molecular weight and molecular weight distribution of the polymer have already been determined, which is an advantage of such polymer processes (8).

At room temperature, benzidine, a biphenyl amine, is a crystalline grayish-yellow, white, or reddish-gray substance. It dissolves easily in less polar solvents like ethanol and diethyl ether, is somewhat soluble in cold water, and is more soluble in hot water. When exposed to light and air, it becomes darker. An intermediary in the synthesis of azo dyes, sulfur dyes, rapid color salts, naphthol, and other dye compounds, benzidine is a di-amine that was produced as a synthetic aromatic hydrocarbon with two benzene rings covalently bound to one another. Benzidine was selected as a spacer due to its rigid biphenyl structure and two reactive amine groups, which enable effective covalent attachment between the polyacrylic acid backbone and drug molecules. The incorporation of benzidine as a spacer increases the distance between the polymer chain and the drug, reducing steric hindrance and enhancing drug release and biological activity. In the synthesized systems, benzidine remains covalently bound within the polymer structure (9-12).

On the other hand, medications that include carboxylic acid, such as statins, β-lactam antibiotics, and nonsteroidal anti-inflammatory medicines (NSAIDs), have transformed the twentieth-century medical treatment of illnesses and pain (13).

Several methods can be used to release therapeutic compounds bound to polymers from the polymeric matrix at a regulated rate. A drug's transport to tissues over a certain length of time takes advantage of several polymer characteristics. One well-known example is the release of the medication via stimuli-sensitive polymers only in response to a pH or temperature change (12-14).

This study aims to develop polyacrylic acid–based polymer–drug conjugates linked through benzidine spacers and to investigate their chemical structure, physicochemical characteristics, and antibacterial activity.

2. Materials and Methods

Analytical-grade solvents and reagents for analysis were supplied by Fluka, Sigma-Aldrich, CDH, and Riedel-deHaen. The SDI-Samarra Company supplied aspirin, ciprofloxine, ampicillin, folic acid, cephalexin, and amoxicillin. Fourier Transform Infrared (FTIR) spectra were recorded using a Bruker Tensor II Fourier Transform Infrared Spectrometer. In the 400–4000 cm-1 range, the ATR FTIR spectra were recorded using a Bruker Tensor II Fourier Transform Infrared Spectrometer Promoter ATR-FTIR. UV absorption was measured using the PG CECIL CE7200 double-beam spectrophotometer. A Varian INOVA 500 MHz NMR spectrometer was used to produce 1H NMR spectra in dimethyl sulfoxide (DMSO) with TMS as the reference standard. Parts per million (ppm) were used to describe chemical changes.

2.1 Modification of polyacrylic acid with benzidine (AB)

(0.5g, 0.00025 mole) Polyacrylic acid was dissolved in 10ml of DMF (1.29g, 0.007 mole) of benzidine and (6 drop, 0.082 mol) was added from $SOCL_2$, the mixture was stirred vigorously at room temperature for 1 hr. A precipitate was obtained, the solvent was evaporated, washed with ether and dried at room temperature (13, 14).

2.2 Synthesis of AB1-AB6 Derivatives

The drugs were prepared by modifying them with thionyl chloride to react with AB using the following method: Thionyl chloride (6 drop, 4.2 mmol) was added to (2.1 mmol of [cephalexin (0.76 gm), folic acid (0.92 gm), ampicillin (0.78 gm), ciprofloxine (0.69 gm), amoxicillin (0.77 gm), and aspirin (0.5 gm)] respectively, and left at room temperature for (30 mins.) with stirring. After that 0.5g, 0.075 mmole of prepared compound (AB) was dissolved in 15ml of DMF, and (2.1 mmole) of prepared carboxylic acid drug was added, the mixture was refluxed with stirring for 2hrs. The solvent was evaporated under a vacuum; the product was washed with water three times, dried under a vacuum oven, to produce compounds AB1 to AB6 (Figure 1) (15).

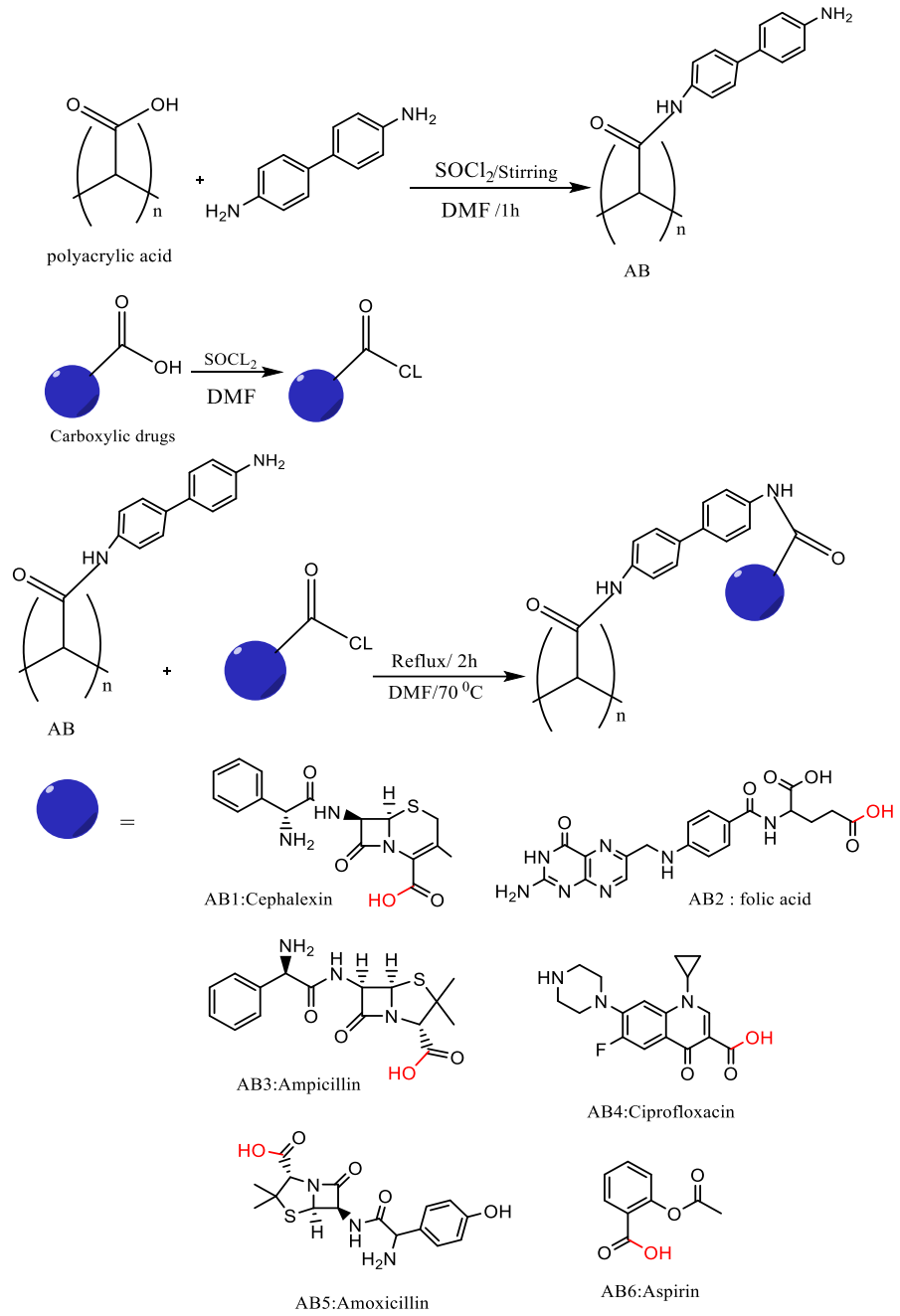


Figure 1. Synthesis of compounds (AB1_AB6) **(15)**.

3. Result

Polyacrylic acid–based polymer–drug conjugates (AB1–AB6) were successfully synthesized via reaction with benzidine as a spacer, followed by conjugation with cephalexin, folic acid, ampicillin, ciprofloxacin, amoxicillin, and aspirin. The reactions proceeded in good yields, and the obtained polymers were characterized by FTIR and ^{1}HNMR spectroscopy, solubility studies, viscosity measurements, controlled drug-release experiments, and antibacterial evaluation against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria **(13-15)**.

Table 1 describes the physical properties of the synthesized polymers after conjugation with drugs, in terms of color and melting points of the resulting compounds.

3.1 Spectroscopic Characterization

FTIR spectra of all polymers confirmed successful conjugation through the appearance of new amide carbonyl bands in the range 1610–1690 cm^{1} and the disappearance of the characteristic –OH stretching bands of the carboxylic acid groups of both polyacrylic acid and the parent drugs. Additional absorption bands corresponding to aromatic C=C stretching (≈1500–1585 cm^{1}), aliphatic and aromatic C–H stretching (≈2850–3160 cm^{1}), and characteristic functional groups of each drug

(e.g., C–F, S–C, ester C=O) further supported the proposed structures.

The ^{1}HNMR spectra of AB1–AB6 showed characteristic signals for the polymer backbone (CH–CH at ~1.2–1.6 ppm), amide-linked methylene groups (O=C–CH at ~2.1–2.3 ppm), aromatic protons (6.5–8.0 ppm), and amide protons (6.9–8.9 ppm). Drug-specific resonances such as phenolic –OH, ester methyl, and sulfur-containing groups were also observed, confirming the successful incorporation of the drug moieties into the polymer chains. This can be explained in detail as follows **(12-16)**:

FTIR spectrum of Polymer AB1 shows: NH_2- 3396.64 cm^{-1}; NH- 3332.99 cm-1; new C=O amide group at 1610-1678 $cm^{-1}$and disappearing of –OH carboxyl group of drug and polyacrylic acid ; CH=CH (Ar.) 1500-1585 cm^{-1}; -CH (Alph.) 2926.01cm^{-1} ;-CH_2 (Alph.) 3219.19 cm^{-1} ; -CH (Ar.) 3039.81 cm^{-1}; S-C 600-800 cm^{-1} (Figure 2). For Polymer AB1 the ^{1}HNMR spectrum shows: CH-CH_2 1.6 ppm; O=C-CH 2.1 ppm; (Ar.) CH=CH 6.8-7.9 ppm; HC-NH_2 5 ppm; O=C-NH 8- 8.9 ppm.

FTIR spectrum of Polymer AB2 shows: NH2- 3255.84 cm-1; NH- 3327.21 cm-1; O=C carboxyl group at 1718.58 cm-1 ;OH 2601.97-3327.21 cm-1 ; new C=O amide group at 1633.71-1689.64 cm-1 and disappearing of –OH carboxyl group of drug and polyacrylic acid ; CH=CH (Ar.) 1496.76 cm-1; -CH (Alph.) 2852.72cm-1 ;-CH2 (Alph.) 3037.89 cm-1 ; -CH (Ar.) 3070.93 cm-1; N-C 350-1000 cm-1 ; N=C 1640-1690 cm-1 (Figure 2). For Polymer AB2 the 1HNMR spectrum shows: CH-CH2 1.2 ppm; O=C-CH 2.1 ppm; (Ar.) CH=CH 6.5-8 ppm; HC-NH2 5.3 ppm; O=C-NH 8.7 ppm; O=C-OH 10.3 ppm.

FTIR spectrum of Polymer AB3 shows: NH_2- 3437.15 cm^{-1}; NH- 3394.72 cm^{-1}; new C=O amide group at 1660.71 cm^{-1} and disappearing of –OH carboxyl group of drug and polyacrylic acid; CH=CH (Ar.) 1500.62 1531.48 cm^{-1}; -CH (Alph.) 3321.41 cm^{-1}; -CH_2 (Alph.) 3037.89 cm^{-1}; -CH (Ar.) 3035.96 cm^{-1}; S-C 600-800 cm^{-1} ; C-N 350-1000 cm^{-1} (Figure 2). For Polymer AB3 the ^{1}HNMR spectrum shows: CH-CH_2 1.2-1.4 ppm; O=C-CH 2.1 ppm; (Ar.) CH=CH 7.1-8 ppm; HC-NH_2 4.9-5 ppm; O=C-NH 8.6 ppm; S-CH 2.9 ppm; C-CH_3 0.8- 1.1 ppm.

FTIR spectrum For Polymer AB4 shows: NH- 3421.72 cm^{-1}; new C=O amide group at 1627.92 cm^{-1} and disappearing of –OH carboxyl group of drug and polyacrylic acid ; CH=CH (Ar.) 1500.62 cm^{-1};C=O ketone 1732.08 cm^{-1} ; -CH (Alph.) 3332.99 cm^{-1} ;-CH_2 (Alph.) 3022.45 cm^{-1} ; -CH (Ar.) 3159.40 cm^{-1} ;C-F 1000-1400 cm^{-1} ; C-N 350-1000 cm^{-1} (Figure 2). For Polymer AB4 the ^{1}HNMR spectrum shows: CH-CH_2 1.3 ppm; O=C-CH 2.1 ppm; (Ar.) CH=CH 7.2-8 ppm; O=C-NH 8.7 ppm; N-CH 2.7-2.9 ppm.

FTIR spectrum of AB5 shows: NH_2- 3437.15 cm^{-1}; NH- 3394.72 cm^{-1}; new C=O amide group at 1668.43 cm^{-1} and disappearance of –OH carboxyl group of drug and polyacrylic acid; CH=CH (Ar.) 1500.62 cm^{-1}; (Alch.) OH 3502.73 cm^{-1}; -CH (Alph.) 3319.44 cm^{-1} ;-CH_2 (Alph.) 3041.74cm^{-1} ; -CH (Ar.) 3163.26 cm^{-1} ;S-C 600-800 cm^{-1} ; C-N 350-1000 cm^{-1} ; CH_3 2929.87 cm^{-1} (Figure 2). Polymer AB5 the ^{1}HNMR spectrum shows: CH-CH_2 1.4 ppm; O=C-CH 2.3 ppm; (Ar.) CH=CH 7-7.7 ppm; HC-NH_2 5 ppm; O=C-NH 8-8.9 ppm; Ar -OH 6.7 ppm; C-CH_3 0.8; N-CH 2.7-2.9 ppm.

FTIR spectrum of AB6 shows: NH- 3327 cm^{-1}; new C=O amide group at 1666.50 cm^{-1} and disappearing of –OH carboxyl group of drug and polyacrylic acid ; CH=CH (Ar.) 1496-1527.62 cm^{-1};C=O (aster) 1720 cm^{-1} ; -CH (Alph.) 3282.84 cm^{-1} ;-CH_2 (Alph.) 3039.81 cm^{-1} ; -CH (Ar.) 3101.54 cm^{-1} ;CH_3 2873.94 cm^{-1} (Figure 2). Polymer AB6 the ^{1}HNMR spectrum shows: CH-CH_2 1.2 ppm; O=C-CH 2.2 ppm; (Ar.) CH=CH 7.2-8 ppm; O=C-NH 6.9 ppm; O-C=O-CH_3- 2.7 ppm.

The resulting compounds have polar properties, making them more compatible with polar solvents than nonpolar ones (Table 2) **(16, 17)**.

Table 1. Physical properties of polymers AB1-AB6.

POLYMER	Color	Melting point ºC
AB1	Orange	314-318 ºC
AB2	Brown Dark	300 ºC <
AB3	Orange	205-208 ºC
AB4	Light Brown	295-300 ºC
AB5	Maroon	245-248 ºC
AB6	Tan	290-293 ºC

Table 2. Solubility of the polymers AB1-AB6.

Polymer	THF	Acetone	benzene	Diethyl ether	ether	CH3Cl	DC-M	DMSO	DMF	MeOH	EtOH	H_2O
AB1	P.	P.	P.	+	+	P.	P.	-	P.	-	P.	P.
AB2	P.	P.	P.	+	+	P.	P.	-	P.	-	P.	P.
AB3	P.	P.	P.	+	+	P.	P.	-	P.	P.	P.	P.
AB4	P.	P.	+	+	+	P.	P.	-	P.	P.	P.	P.
AB5	P.	+	+	+	+	P.	P.	P.	P.	P.	P.	P.
AB6	P.	+	+	+	+	P.	P.	P.	P.	P.	P.	P.

Table 3. Antibacterial activity of (AB1-AB6).

Compounds	Inhibition zone for *E. coli* of Drug (mm)	Inhibition zone for staphylococcus of Drug (mm)	Drugs	Inhibition zone for *E. coli* of polymer (mm)	Inhibition zone for sample staphylococcus of polymer (mm)
AB1	20	10	Cephalexin	8	8
AB2	25	28	Folic acid	10	8
AB3	8	8	Ampicillin	15	15
AB4	8	8	Ciprofloxacin	20	25
AB5	15	16	Amoxicillin	18	18
AB6	18	8	Aspirin	15	15

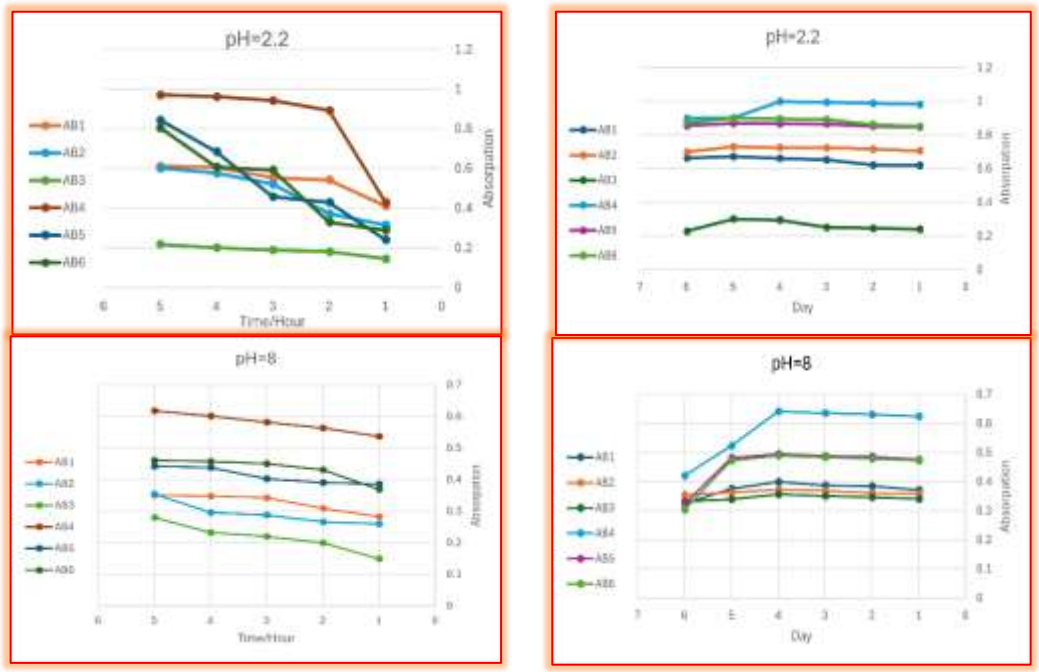


Figure 2. Survival a patients (**Prepared by Authors, 2026**).

4. Discussion

Following a good yield reaction with benzidine, cephalexin, folic acid, ampicillin, ciprofloxine, amoxicillin, and aspirin were utilized to synthesize a new medicinal polymer based on polyacrylic acid. These compounds were examined using several analytical methods, such as FTIR and ^{1}H NMR.

Benzidine was selected as a bifunctional aromatic spacer due to its rigid, planar structure, which can facilitate effective conjugation between polyacrylic acid and the drug molecules. Importantly, during the synthesis, benzidine undergoes chemical transformation and becomes covalently immobilized within the polymer backbone. As a result, no free benzidine molecules are present in the final polymer–drug conjugates. This immobilization minimizes potential toxicity, as the carcinogenic aromatic amine groups are chemically bound and not bioavailable, providing a safer platform for studying spacer-mediated polymer–drug delivery systems **(9-11)**.

The viscosity of the result compounds was measured to determine their molecular weight, and the solubility was measured in several solvents. To test the polymers' antibacterial properties, two gram-positive (*Staphylococcus*) and gram-negative (*E. coli*) bacteria were used **(17-19)**.

The synthesized polymers exhibited predominantly polar characteristics and showed good solubility in polar aprotic solvents such as DMSO and DMF, while displaying partial or poor solubility in less polar or nonpolar solvents. This behavior is attributed to the presence of polar functional groups (e.g., –NH and –C=O), which promote hydrogen bonding with polar solvents (Table 2).

The variation in viscosity-average molecular weights among the synthesized polymers reflects differences in drug structure, degree of substitution, and chain interactions introduced by the benzidine spacer. Polymers with bulkier or multifunctional drug moieties exhibited higher molecular weights and viscosities, indicating increased chain entanglement and intermolecular interactions. These structural differences are expected to influence swelling behavior, drug release, and antibacterial performance rather than serving merely as numerical values.

A viscometer calculates the viscosity-average molecular weight and measures intrinsic viscosity using the Mark-Houwink equation. The formula that illustrates how a polymer's intrinsic viscosity varies with its relative molecular mass, or molecular weight, is: $[\eta]=KM\alpha$, where [η] is the intrinsic viscosity, K and α are constants the values of which depend on the nature of the polymer and solvent as well as on temperature. M is usually one of the relative molecular mass averages. Because of its high molecular weight, the AB5 sample has a higher viscosity than the other produced polymers. According to the Mark Houwink equation, viscosity and molecular weight are directly correlated. The calculated molecular weight of the prepared polymers are: AB1, Mwt=10310.613; AB2, Mwt=10270.684; AB3, Mwt=6940.935; AB4, Mwt=14870.198; AB5, Mwt=22581.761; AB6, Mwt=25892.372 **(16, 17, 20)**.

The drug-release profiles of the polymer–drug conjugates were strongly influenced by environmental pH and polymer structure. In all cases, release occurred faster in the basic medium than in the acidic medium, with completion observed within approximately six days. This behavior can be attributed to enhanced nucleophilic attack of hydroxide ions on amide and ester linkages, leading to bond cleavage and drug liberation. Polymers with higher swelling capacity and lower crosslinking density exhibited faster release rates, while more rigid or highly substituted systems showed comparatively slower release. These findings demonstrate that polymer architecture and linker chemistry play key roles in controlling release kinetics.

Variations in drug release rates can be attributed to a number of factors that affect the locations of bands in the UV spectrum. The conjugation effect, which causes a red shift because of chain elongation, including conjugated bonds, is the most important of these effects. Electronic transitions to empty molecular orbitals need less energy as energy levels approach one another. A higher maximum wavelength is the result of more conjugation. This implies that drug release occurs more quickly in basic media than in acidic ones and requires six days. More nucleophilic than the proton or the water molecule, the hydroxide ion targets the carbon atom of the carbonyl group. The type of bond (amide or ester), the degree of swelling, the properties of the medication, and the degree of crosslinking within polymer chains are some of the factors that affect the pace of drug release. Both the rate of drug release and the degree of polymer swelling are reduced by crosslinking. Figure 2 shows that two different media (basic and acidic) were used to study the drug release process.

Where the medication is released from the sample because of hydrolysis. The results show that controlled drug release occurs more quickly in the basic media than in the acidic medium and that the drug release process ends after six days. This is due to the hydroxide ion's more potent nucleophilic attack on the carbonyl group than that of the proton or water molecule **(17-19)**.

The antibacterial evaluation revealed that polymer–drug conjugation significantly altered biological performance compared to the free drugs. AB1 and AB6 showed enhanced activity against *S. aureus*, while AB2 demonstrated pronounced efficacy against *E. coli*. The improved antibacterial behavior can be attributed to synergistic effects between the polymer backbone and the drug moieties, including increased local drug concentration at the bacterial surface and prolonged interaction time due to polymer adhesion. The higher activity observed against Gram-negative bacteria may be

related to improved polymer penetration or interaction with the outer membrane, rather than simple electrostatic attraction alone. These results suggest that polymer–drug conjugation can modulate antibacterial selectivity.

Overall, the present results are consistent with previously reported polymer–drug conjugate systems, where spacer-assisted attachment enhanced drug stability, controlled release, and antibacterial efficacy. Compared with similar poly (acrylic acid)-based carriers reported in the literature, the synthesized systems demonstrate comparable or improved antimicrobial activity and tunable release behavior, highlighting the effectiveness of benzidine as a spacer and the potential of these materials for controlled drug-delivery and antimicrobial applications.

The results, according to Table 3, show that AB1 and AB6 have a good inhibition zone (20 and 18 mm) against *S. aureus* and (10 and 8 mm) against *E. coli* in comparison to used drugs inhibitions. While good inhibition of growth for homopolymers AB2 against bacteria with a higher effect toward *E. coli*. The majority of the compounds made using DMSO (0.1 mg/ml) demonstrated greater antibacterial activity against *E. coli* and other Gram-negative bacteria than against Gram-positive bacteria. The reason might be that amide polymers are frequently positively charged, which makes them more successful at drawing in Gram-positive bacteria than Gram-negative ones, which are shielded by an outer shell **(17-19, 21, 22)**.

The present findings are in good agreement with previously reported polymer–drug conjugate systems based on poly (acrylic acid) and other hydrophilic polymer carriers. Earlier studies have shown that the incorporation of spacer molecules between the polymer backbone and drug moieties improves drug accessibility, modulates release kinetics, and enhances biological activity. Similar pH-dependent release behavior, with faster drug liberation in basic media, has been reported for amide- and ester-linked polymeric prodrugs. Moreover, enhanced or comparable antibacterial activity relative to free drugs has been observed in several polymer–drug conjugates, which was attributed to prolonged drug–bacteria interaction and controlled release. In comparison to these systems, the polymers synthesized in the present study demonstrate effective conjugation, tunable release profiles, and promising antibacterial activity, confirming the suitability of benzidine as a spacer and polyacrylic acid as a carrier matrix.

5. Conclusion

Novel polyacrylic acid–based polymer–drug conjugates were synthesized using benzidine as a covalently immobilized spacer. Structural analyses (FTIR and ^1H NMR) confirmed successful conjugation, and the polymers showed good compatibility with polar solvents. The conjugates exhibited pH-dependent drug-release behavior, with faster release under basic conditions. Antibacterial evaluation indicated that some conjugates enhanced activity relative to the free drugs, particularly against *Staphylococcus aureus*. These results highlight that spacer-assisted polymer–drug conjugation can provide a controlled drug-release platform with potential antimicrobial applications, while minimizing exposure to free toxic spacer molecules.

6. Declarations

6.1 Acknowledgments

The authors thank the University of Babylon for the support.

6.2 Ethical Considerations

The ethical rules of the journal are adhered to by this paper.

6.3 Authors' Contributions

All the authors have equal contributions and approved the final version of the manuscript.

6.4 Conflict of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

6.5 Fund or Financial Support

This research received no external funding.

6.6 Using Artificial Intelligence Tools (AI Tools)

The authors were not utilized AI Tools. (Please review and complete it.)

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