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ABSTRACTS

of the dissertation for the degree of
Doctor of Science

SYNTHESIS AND INVESTIGATED MEMORY BASED NANOSTRUCTURED POLYMERS FOR IMMOBILIZATION AND LOCAL DELIVERY OF ENZYMES AND ANTIBIOTICS

Speciality:	2304.01 – Macromolecular chemistry
Field of science:	Chemistry
Applicant	**Shamo Zokhrab oglu Tapdiqov**

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The work was performed at «Nanostructured metal polymer catalysist» laboratory of the Institute Catalysis and Inorganic Chemistry named after acad. M.Naghiyev of Azerbaijan Republic Minstry of Science and Education.

Scientific consultans:
Full member of ANAS,
doctor of chemical sciences, professor
Dilgam Babir oglu Tagiyev
Doctor of chemical sciences
Nizami Allahverdi oğlu Zeynalov

Official opponents:
Full member of ANAS,
doctor of chemical sciences, professor
Adil Abdulkhalig oglu Garibov
Corresponding member of ANAS
doctor of chemical sciences, professor
Islam Israfil oglu Mustafayev
Corresponding member of ANAS
doctor of chemical sciences, professor
Bakhtiyar Ajdar oglu Mammadov
doctor of chemical sciences, professor
Rasim Mirali oglu Alosmanov

Dissertation council ED1.15 of Supreme Attestation Comission under the President of the Republic of Azerbaijan operating at the Institute of Catalysis and Inorganic Chemistry named after acad. M.Naghiyev of Azerbaijan Republic Minstry of Science and Education

Vice chairman of dissertation council :
Corresponding member of
ANAS, doctor of chemical
sciences, professor
Mahammad Baba oglu Babanli

Scientific secretary of dissertation council:
PhD in Chemistry,
associated professor
Ulviyya Ahmed gizi Mammadova

Chairman of scientific seminar:
doctor of chemical sciences,
associate professor
Kazim Gafar oglu Guliyev

GENERAL DESCRIPTION OF WORK

Relevance of the topic and degree of development – It is known that the long-term intake of physiologically active substances such as antibiotics, enzymes, alkaloids, etc., in their free form, cannot maintain therapeutic concentrations in the body. As a result, inflammation or injury areas are not effectively treated, and to maintain the therapeutic effect, it becomes necessary to take the drug frequently or at doses 2-3 times higher than the therapeutic amount. This, in turn, negatively impacts the function of other organs, leading to adverse reactions, and in some cases, causing damage to those organs and the development of other chronic diseases. Over the past 20-30 years, to address this deficiency, nanostructured hydrogels have been developed to maintain therapeutic concentrations of drug substances in the bloodstream for extended periods.

Hydrogels – [1] hydrogels are three-dimensional network structured materials obtained through chemical reactions between small molecular compounds with bifunctional active groups in the composition of natural and synthetic polymers with a linear structure. Due to their ability to expand and contract depending on the medium, and their capacity to absorb a certain amount of water, hydrogels can behave like tissue, which provides their biocompatibility. This property ensures the controlled release of drug substances immobilized on their volume and surface.

Recently, the loading of drug formulations with metal nanoparticles in nanostructured hydrogel matrices, which hold the main active components, has led to the development of a new field in macromolecular chemistry to achieve a broad spectrum of therapeutic effects. Structures formed as polymer and metal nanoparticle complexes ultimately result in the formation of new, advanced, and targeted delivery capabilities, as well as antibacterial and controlled release properties within the matrix. The obtained nanostructured metal-polymer-drug formulation demonstrates significantly superior positive

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[1] Nikolaos A Peppas. Hydrogels and drug delivery // Current Opinion in Colloid & Interface Science, 1997,Vol. 2, Iss. 5, pp. 531-537.

indicators in terms of effect, targeted delivery, controlled release, potency, therapeutic distribution, and antibacterial effects compared to previous pharmacological formulations. Hydrogels that are sensitive to pH and temperature are particularly interesting for the targeted delivery and long-term controlled release of drug substances. The polymer-drug complex in liquid form, when injected into the injury part, undergoes structural changes at body temperature, transforming into a gel, and over time, as it is exposed to the surrounding pH and enzymatic degradation, mass loss occurs. As a result, the active drug begins to release[2].

Naturally, the kinetics of the process are regulated by the molar ratio of the components in the gel and the average molecular weight of the polymer. In this area, polyethyleneglycol and polypeptide systems are considered more suitable. Poly-ε-caprolactone is particularly chosen for the delivery and controlled release of drug substances. Its non-toxicity and biodegradability properties enhance its application potential. The traditional synthesis methods of poly-ε-caprolactone involved metal-organic catalysts, which required high purification, thus limiting its use. Therefore, the synthesis of a polymer with a low average molecular weight and the possibilities of drug delivery from it remain current issues.

Considering the above, the dissertation work is focused on the development of non-toxic, low molecular weight carriers based on poly-N-vinylpyrrolidone, polycaprolactone, polyacrylic acid, polyacrylamide, polyethylene glycol, and its methyl ether, as well as natural polysaccharides such as chitosan, gum arabic, and arabinogalactan. This includes the development of hydrogels and their combinations with silver and magnetite Fe_3O_4 nanoparticles, and the immobilization of doxorubicin, doxycycline, levofloxacin, and trypsin onto these biomaterials. The biological activity of the resulting bionanocomposites and the controlled release of the active ingredients are investigated both *in vitro* and *in vivo*, considering their potential use as a new drug combination.

The work was carried out as part of the scientific research plan of

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[2] Yuanhan T., Xin Zh., Xinyue L., Chiyue M., etc all. A review on recent advances of Protein-Polymer hydrogels, European Polymer Journal,2022, Vol.162, p. 110881.

the Institute of Catalysis and Inorganic Chemistry named after acad. M.Nağıyev of the Ministry of Science and Education of Azerbaijan (State Registration No. 0115 Az 2096).

Purpose and objectives of the research - The main aim of the research is the synthesis of pH- and temperature-sensitive nanostructured hydrogels based on synthetic and natural polymers, including their shape-memory forms, as well as gel-complexes with certain biologically important microelement ions and nanoparticles. Additionally, the study focuses on the immobilization, targeted delivery, and controlled release of doxorubicin, doxycycline, trypsin, and levofloxacin within these hydrogels.

To achieve this objective, the following tasks have been set:

- Synthesis of nanostructured hydrogels based on low molecular weight poly-N-vinylpyrrolidone (PVPr), polyacrylic acid (PAa), polyacrylamide, chitosan, arabinogalactan, and gum arabic; immobilization of trypsin and doxorubicin within these hydrogels, as well as investigation of their targeted delivery and release into the medium;
- Synthesis of temperature-sensitive nanostructured hydrogels via polycondensation of methyl either of polyethyleneglycol with N-carboxyanhydrides of L-alanine (Ala) and L-aspartic acid (Asp); study of the delivery capabilities for doxycycline and doxorubicin, and investigation of their release kinetics using the main kinetic models;
- Synthesis of nanostructured hydrogels containing silver and Fe_3O_4 magnetite nanoparticles stabilized with synthetic and natural polymers including polyethyleneglycol, poly-N-vinylpyrrolidone, chitosan, and gum arabic; determination of the optimal conditions for immobilizing model drugs (trypsin and doxorubicin) into the resulting polymer–metal nanoassemblies;
- Synthesis and investigation of non-toxic gel-complexes formed by low molecular weight poly-N-vinylpyrrolidone with copper, cobalt, and nickel ions, as well as the study of drug delivery potential through the sorption-based immobilization of doxorubicin and trypsin into these metal–gel complexes;
- Synthesis and study of low molecular weight polycaprolactone using microwave irradiation in the presence of acetic, benzoic, and

trifluoroacetic acids, followed by immobilization of doxorubicin and analysis of its release using kinetic equations.

- ❖ Development of nanostructured hydrogels based on the natural polysaccharides arabinogalactan, gum arabic, and chitosan; immobilization and investigation of doxorubicin, levofloxacin, trypsin, and doxycycline hydrochloride within these hydrogels;
- ❖ Immobilization and study of doxorubicin, trypsin, and doxycycline into hydrogels synthesized from graft copolymers of arabinogalactan and chitosan (derived from cherry gum) with poly-N-vinylpyrrolidone;
- ❖ Synthesis of pH-sensitive hydrogels, including both simple and magnetite-based nanostructured forms, using the graft copolymer of N-vinylpyrrolidone and 4-vinylpyridine; investigation of trypsin immobilization via adsorption method and the study isotherm and thermodynamic parameters;
- ❖ Development of shape-memory nanostructured gels and metal–gel complexes based on natural and synthetic polymers; investigation of their swelling behavior and immobilization of the mentioned biologically active compounds.

Research methods – The following physicochemical methods and approaches were conducted to carry out experimental studies:

- ✓ Modern scientifically grounded synthesis methods were used, considering medical-pharmacological requirements for the polymers and reagents applied in the preparation of hydrogels designed for targeted and controlled drug delivery.
- ✓ The chemical compositions and molecular structures of initial materials and all synthesized polymers, including the nanostructured carriers, were characterized using high-sensitivity, high-precision analytical techniques such as Scanning and Transmission Electron Microscopy (SEM, TEM), Nuclear Magnetic Resonance (NMR) spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, Thermogravimetric Analysis (TGA), Differential Thermal Analysis (DTA), X-ray Diffraction (XRD), and MALDI-TOF mass spectrometry, and others.
- ✓ The biological activity, antibacterial properties, and degradation behavior of the synthesized hydrogels and drug-loaded composites

were investigated. The sorption of selected drugs was analyzed using Freundlich, Langmuir, Temkin, and Dubinin–Radushkevich (D-R) isotherms, while kinetic and thermodynamic parameters were assessed using second-order kinetic models. Controlled release behavior was evaluated by fitting the experimental data to mathematical models including Zero- and First-order kinetics, Higuchi, Korsmeyer–Peppas, Hixson–Crowell models.

Key findings submitted for defense – The following specific conclusions were derived from the scientific analysis conducted in accordance with the dissertation topic, supported by classical and fundamental theoretical frameworks, and compared with existing studies by other researchers:

- Optimization of mild synthesis conditions, favorable swelling properties, and efficient therapeutic-dose-level immobilization methods for nanostructured hydrogels based on poly-N-vinylpyrrolidone, polyacrylic acid, arabinogalactan, gum arabic, and their graft copolymers;
- Spectroscopic characterization of the chemical interactions between enzymes/antibiotics and carriers synthesized with silver and Fe_3O_4 magnetite nanoparticles stabilized in chitosan, polyethyleneglycol, and gum arabic media;
- Microwave-assisted ring-opening polymerization for the synthesis of polycaprolactone, and effective immobilization of doxorubicin into this nanostructured hydrogel, with confirmation of controlled release behavior through kinetic models;
- Development of therapeutic metal–gel complexes using nanostructured hydrogels based on various natural and synthetic polymers combined with Cu(II), Co(II), Ni(II) ions, silver, and Fe_3O_4 nanoparticles; establishment of a simple and effective method for enzyme and antibiotic immobilization;
- Synthesis of memory-shape carrier matrices from nanostructured polymer hydrogels and nanoparticle-based metal–gel materials; determination of optimal drug loading capacities and controlled release profiles using pharmacokinetic modeling, leading to the proposal of matrix-based pharmaceutical dosage forms;
- Evaluation of the antibacterial activity of drug-loaded hydrogels

(prepared from both natural and synthetic polymers) against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* strains, demonstrating the pharmacological efficacy of the synthesized nanostructured carriers;

- Synthesis of an amphiphilic, pH-sensitive nanostructured hydrogel based on poly-N-vinylpyrrolidone-co-4-vinylpyridine incorporating Fe_3O_4 magnetite nanoparticles; effective immobilization of trypsin by sorption; application of Langmuir, Freundlich, First- and Second-order, Temkin, and Dubinin–Radushkevich isotherm models; and optimization of thermodynamic parameters such as Gibbs free energy, enthalpy, and entropy;
- Synthesis of temperature-sensitive nanostructured hydrogels via polycondensation of methoxy polyethylene glycol with activated N-carboxyanhydrides of selected amino acids; confirmation of polymer structure, study of sol–gel transitions and degradation behavior; and kinetic evaluation of doxycycline and doxorubicin immobilization and release processes for development of novel pharmaceutical formulations.

Scientific novelty of the research - Nanostructured, water-swelling hydrogels were synthesized by crosslinking low molecular weight poly-N-vinylpyrrolidone, polyacrylamide, and polyacrylic acid—as well as their graft copolymers with gum arabic and arabinogalactan—using *N,N′*-methylenebisacrylamide as a crosslinker. The immobilization of doxorubicin and trypsin into these hydrogels was systematically investigated. Additionally, a novel pH-sensitive nanostructured hydrogel containing magnetite (Fe_3O_4) nanoparticles was synthesized based on N-vinylpyrrolidone and 4-vinylpyridine. Its structure and physicochemical properties were characterized, and the effective immobilization of trypsin via adsorption was achieved. Thermodynamic and kinetic parameters of the adsorption process were determined. Furthermore, nanostructured hydrogel composites incorporating silver and Fe_3O_4 nanoparticles were developed using hydrogels based on poly-N-vinylpyrrolidone, polyacrylic acid, polyethylene glycol, and chitosan. These materials were studied as targeted delivery systems for immobilizing bioactive agents such as trypsin, doxycycline, and other pharmaceutical compounds.

In addition, shape-memory nanostructured hydrogel forms based on the above-mentioned synthetic and natural polymers were synthesized. These hydrogel carriers were used to prepare extended-release formulations by immobilizing model drugs, providing prolonged therapeutic effects. Comparative studies were also conducted on the immobilization of trypsin into poly-N-vinylpyrrolidone-based metal–gel complexes with Cu(II), Co(II), and Ni(II) ions. A novel method for synthesizing polycaprolactone—a widely used polymer in drug delivery—was proposed, involving microwave-assisted ring-opening polymerization in the presence of organic acids. The resulting polycaprolactone–acid complexes were used for doxorubicin immobilization, and their controlled release behavior was investigated through kinetic modeling. Additionally, temperature-sensitive hydrogels were synthesized by polycondensation of methoxy polyethylene glycol with *N*-carboxyanhydrides of L-alanine and L-benzylaspartic acid. These hydrogels were loaded with doxycycline (as a model drug), and their release behavior into the surrounding medium was studied.

Theoretical and practical significance of the research - Biocompatible hydrogels based on poly-N-vinylpyrrolidone, polyacrylic acid, polyacrylamide, polyethylene glycol, chitosan, gum arabic, and arabinogalactan—with the ability to swell at physiological pH values (7.0–8.0) were synthesized. Their nanocomposite forms incorporating silver and magnetite Fe_3O_4 nanoparticles were also developed. Effective methods were proposed for the immobilization of bioactive compounds such as doxycycline, levofloxacin, trypsin, and doxorubicin at neutral pH, maintaining their biological activity. A novel microwave-assisted synthesis method was introduced for the preparation of low average-molecular-weight poly(ε-caprolactone), using organic acids as initiators. Additionally, pH-sensitive nanostructured hydrogels based on N-vinylpyrrolidone and 4-vinylpyridine containing magnetite nanoparticles were shown to act as effective adsorbents for the separation of proteins from aqueous and biological media, and as potential matrices for protein delivery. The developed smart, memory-shape nanostructured hydrogels demonstrated selective sensitivity to specific antibiotics and proteins and are considered promising candidates for the design of novel drug formulations as advanced delivery systems.

Compared to synthetic polymers, the use of natural biopolymers such as chitosan, arabinogalactan, and gum arabic offers significant advantages. These include enhanced biocompatibility, immunostimulatory effects, and the ability to bind and neutralize toxic compounds in the body while also functioning as efficient carriers for drug delivery. Based on the scientific results of the dissertation and their citations in peer-reviewed journals, the research can be expanded further. Synthesized hydrogels may be used as carrier systems for other physiologically active agents in biomedical and pharmaceutical applications.

Personal contribution of the author. The personal participation of the author was a leading in conducting experimental studies, analyzing and discussing the obtained results, and writing of the dissertation work, publishing articles and discussing report thesis materials at international conferences.

Approbation and implementation: The main results of the dissertation have been presented and discussed at the following national and international scientific conferences: Proceedings of the Young Scientists Scientific Conference held under the EIF/GAM-1-2011-(4) grant project (Baku, 2012); *All-Russian Conference with International Participation of Young Scientists in Chemistry, Mendeleev-2014* (St. Petersburg, Russia, 2014); IV International Scientific Conference of Young Researchers (Baku, 2016); *Abstracts of the Cluster Conference on Organic Chemistry "OrgChem-2016"* (St. Petersburg, Russia, 2016); XXVI International Chugaev Conference on Coordination Chemistry (Kazan, Russia, 2014); 8th World Congress on Chemistry and Organic Chemistry & International Conference on Biomedicine and Pharmacotherapy (Frankfurt, Germany, 2018); 4th International Eurasian Conference on Science, Engineering and Technology (Ankara, Turkey, 2022); XI International European Conference on Interdisciplinary Scientific Research (January 20–22, 2025, Lisbon, Portugal); BİLTEK-X: 10th International Congress on Scientific Research and Current Developments (2025, Istanbul, Turkey); II International Multidisciplinary Ecology and Environmental Studies

Congress (2025, Paris, France); XII International Scientific and Practical Conference *World of Science* (2025, Moscow, Russia).

Name of the organization where the dissertation has been performed: The dissertation research was conducted at the laboratory of “Nanostructured Metal-Polymer Catalysts” of the Institute of Catalysis and Inorganic Chemistry named after academician M. Naghiyev, under the Ministry of Science and Education of the Republic of Azerbaijan.

In addition to the experimental work carried out at the home institution, several key experiments and structural-compositional analyses of the synthesized hydrogels were performed in collaboration with international research centers and universities, including: National Tsing Hua University (Taiwan); University of Hamburg (Germany); Azerbaijan Medical University (Azerbaijan); Marmara University (Turkey); Karlsruhe Institute of Technology (KIT) (Germany); Institute for Polymers, Composites and Biomaterials (Italy).

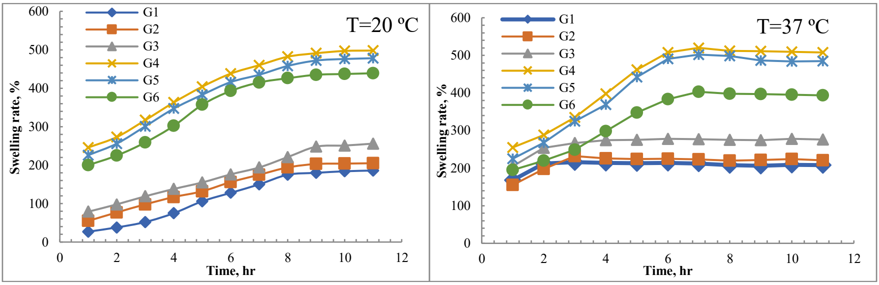
Publications: A total of 28 scientific works related to the content of the dissertation have been published. These include: 16 scientific articles published in national and international peer-reviewed journals, 12 abstracts and proceedings presented at reputable scientific conferences. Out of the 16 published articles: 9 are indexed in the Web of Science, including 6 in the Web of Science Core Collection (Expanded); 10 articles are indexed in Scopus, and have appeared in high-impact journals such as: *International Journal of Biological Macromolecules* (IF: 8.2), *Sensors and Actuators: A. Physical* (IF: 4.6), *Journal of Polymer Research* (IF: 2.8), *Macromolecular Research* (IF: 2.127), *Cellulose Chemistry and Technology* (IF: 1.467), *SOCAR Proceedings* (IF: 2.08), *Journal of Biomimetics, Biomaterials and Biomedical Engineering* (IF: 0.81), among others.

Volume, structure and the main content of the dissertation: Dissertation consists of 289 computer-formatted pages and includes the following sections: Introduction (27421 signs), Chapter I – 63191 signs, Chapter II – 28014 signs, Chapter III – 73412 signs, Chapter IV – 30049 signs, Chapter V – 88970 signs, Chapter VI – 47382 signs. The total volume of the main text is 358439 signs (excluding spaces). The dissertation is supplemented with 96 figures, 23 tables, and 326 references, including scientific sources and medical documentation.

Chapter I presents a literature review and comparative analysis of recent studies on temperature- and pH-sensitive hydrogels based on natural and synthetic polymers, particularly PVPr, PAa, PAA, poly-ε-caprolactone, and polysaccharides such as chitosan, GA, and AG. It also discusses the synthesis of these materials and the role of metal nanoparticles in drug delivery systems in combination with hydrogels, as well as the immobilization of biologically active compounds.

Chapter II includes the initial materials, methods of synthesis of hydrogels, methods of conducting research and determination of physicochemical properties of the obtained products, as well as the methods of applying the analytical and spectroscopic methods which used. Also, a schematic description of some synthesis processes were carried out and visual images of the products are shown.

Chapter III focuses on the synthesis of gels based on natural and synthetic polymers, the formation of gel-complexes with some of them using Cu(II), Co(II), Ni(II) ions and Fe_3O_4 nanoparticles, and the immobilization of selected drug compounds onto these systems. Initially, hydrogels were synthesized using natural polymers such as AG (arabinogalactane), chitosan, and GA (gummi arabic), and synthetic polymers such as PVPr, PAa, PAA, as well as graft copolymers of chitosan with PVPr and poly(4-vinylpyridine) (P4VP), and with PVPr. It was found that in both the grafting of homopolymers and natural polymers, increasing the amount of cross-linker led to a higher gel fraction yield and improved grafting efficiency. In grafting synthetic polymers, an increase in the amount of the cross-linking agent N,N-methylenbisacrylamide (MBAA) resulted in a decrease in the swelling degree. This is attributed to an increase in pore number accompanied by a reduction in pore size as the amount of cross-linker increases. Considering the intended biomedical application of the immobilized drug compounds, the swelling kinetics of the hydrogels were studied at 20 °C and 37 °C (Figure 1).



Şəkil 1. Swelling kinetics of gels synthesized from natural and synthetic polymers at 20 ºC and 37 ºC.*G1-PVPr10%* M_η*=40 kDa pH=6, G2-PAA10% pH=6, G3-PAA10% pH=7, G-4 AG-PVPr(5%) 1% pH=8, G-5 GA-PVPr(5%) 1% pH=8, G-6 Chitosan-PVPr-P4VP 1% pH=7.*

Gels prepared from natural polymers reach a stable swelling degree after approximately 6 hours and continue to swell with a slight decrease thereafter. This can be explained by the high average molecular weight of natural polymers, the effect of temperature on the ionization of functional groups, and the reduction in chemical interaction between the functional groups and water molecules. Trypsin was immobilized onto both natural and synthetic-based hydrogels, and some parameters were determined (Table 1).

Table 1.

Amount of trypsin ımmobilization onto natural and synthetic-based hydrogels and metal-gel complexes.

Carrier hydrogel	**Protein amount, mg/gr**	**DI, %**	**SAIT UD/mg**	**S, %**	**RA, %**
PVPr 10 kDa, 10% cr.	0,20	10,00	38	5,24	9,44
PVPr 40 kDa, 10% cr.	0,45	22,50	51	7,42	11,68
PVPr --,10%(t)-Cu(II)	3,60	72,35	92	12,8	20,60
PVPr--,10%(t)-Co(II)	3,30	66,70	76	9,40	15,36
PVPr--,10%(t)-Ni(II)	3,44	68,80	85	10,60	17,58
PAa 10 kDa, 10% cr.	0,24	12,37	43	6,12	10,4
PAA 80 kDa, 10% cr.	1,48	32,18	49	8,4	15,3
AG-PVPr(5), 1% cr.	3,73	74,96	94	14,7	21,8
GA-PVPr(5), 1% cr.	3,29	66,12	77	9,48	15,8
Chs-PVPr-P4VP, 1%	3,85	77,38	96	15,6	19,2

SAIT –is specific activity of immobilized trypsin and ***S*** – is releative activity, and ***cr***- is cross-linker. It has been determined that the metal ion not only increases the degree of enzyme immobilization but also enhances its biological activity. This suggests that the enzyme remains in continuous contact with the metal ion within the gel matrix, with the enzyme being more firmly bound to the polymer structure through the metal ion.

The process of immobilizing doxorubicin (Dox) onto hydrogels synthesized from different amounts of PAa and cross-linked with MBAA was carried out. It was found that as the pH of the environment increased, the immobilization degree in all gel samples increased to 60-70%. As the alkality medium the deprotonation of the hydrogel surface and its consequent negative charge led to the immobilization of a higher amount of positively charged Dox molecules. It was shown that for the PAa-based hydrogel the immobilization capacity for Dox at pH=8 was 14.12 mg/g, while for the polymer cross-linked with 20% MBAA, it was 12.64 mg/g. This is associated with the reduction in pore size in the hydrogel as the amount of cross-linker increases.

The dependence of the Dox loading capacity of hydrogels synthesized from PVPr with an average molecular weight of 360 kDa, cross-linked with 5–20% (by weight) MBAA, on the pH of the medium was investigated (Fig.2).

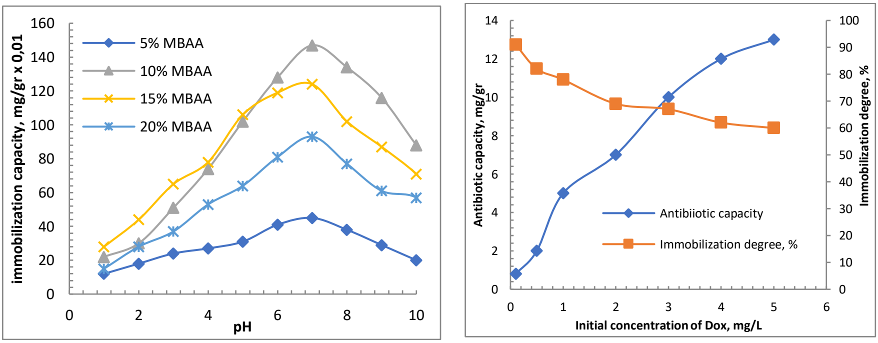


Figure 2. Dependence of the antibiotic loading capacity for Dox in PVPr-based hydrogels on environmental pH and initial drug concentration.

It was determined that as the cross-linker density in the gel increases, the antibiotic loading capacity begins to decrease, with maximum immobilization occurring at pH = 7.

Magnetite (Fe_3O_4) nanoparticles were synthesized in the presence of GA, AG, chitosan, and their graft copolymers with VPr, and the immobilization of trypsin and Dox onto these systems was investigated (Table 2).

Table 2.

Immobilization degree and capacity of trypsin and Dox onto natural and synthetic-based magnetite nanogels.

Fe_3O_4 nanoparticle based hydrogel	Size, nm	For trypsin		For doxorubicine	
		ID, %	PC, mg/gr	ID, %	AC, mg/gr
GA-nanoFe_3O_4	39.16	66.12	3.29	21.32	42.65
AG-nanoFe_3O_4	38.45	63.43	3.17	21.78	43.57
GA-PVPr-nFe_3O_4	33.27	58.80	2.94	23.20	46.39
GA-PVPr(5%)-tikici	---	74.96	3.73	25.46	50.92
AG-PVPr-nFe_3O_4	34.58	54.80	2.74	24.69	49.38
AG-PVPr(5%)-c/l	---	73.27	3.66	27.94	55.88
Chitosan-nanoFe_3O_4	32.46	75.52	3.77	26.87	53.74
Ch-PVPr-PVPFe_3O_4	29.14	63.60	3.18	32.18	64.37
Ch-PVPr-P4VP1%-t	---	77.38	3.85	33.52	67.04

ID-immobilized degree, PC-protein capacity

It has been determined that the incorporation of magnetite nanoparticles into the gel matrix reduces the gel’s capacity for both trypsin and doxorubicin. This is due to the protection of the functional groups that would otherwise form chemical bonds with the biologically active compound, as well as the lower swelling degree of the gel. The presence of magnetite nanoparticles in the structure enhances the potential for targeted delivery of the biocomposite. Additionally, it has been found that trypsin immobilized onto magnetite nanogels retains its biological activity for a longer period compared to samples without Fe_3O_4 nanoparticles. The gel formed with Dox, however, releases the antibiotic from the matrix more quickly.

A temperature-sensitive hydrogel composed of polyethylene glycol-polypeptide (L-alanine-co-benzyl aspartate) was synthesized, and its structure and morphology were characterized. The controlled release of the encapsulated doxycycline drug from the obtained thermoresponsive gel was studied in isotonic and enzymatic media. The synthesis of the mPEG-Ala-BenzAsp block copolymer was carried out via the ring-opening polymerization of the N-carboxyanhydrides of L-alanine and benzyl aspartic acid with aminated mPEG.

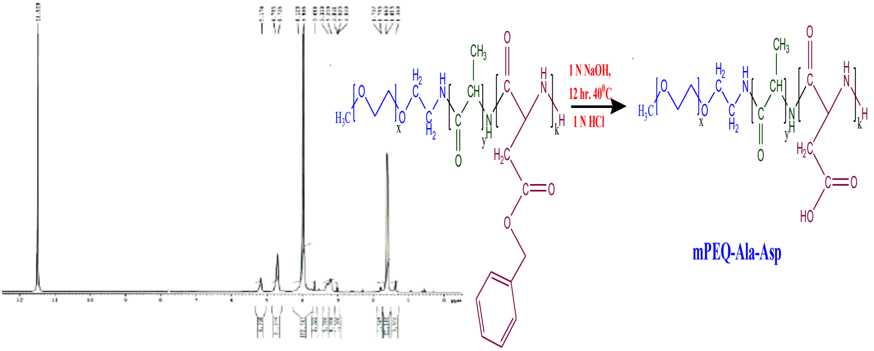


Figure 3. The removal of the benzyl protecting group from the polymer chain and the characterization of the mPEG-Ala-Asp block copolymer by ^1H NMR spectroscopy.

In the final stage, the defragmentation of the benzyl moiety associated with the benzyl aspartate amino acid was carried out to remove it from the structure of the obtained product. Based on the analysis of the ^1H NMR results, the signals observed around δ 1.38 ppm, 4.72 ppm, and 7.8 ppm (weak) correspond to the $-CH_3$, –CH–, and –NH– protons of alanine residues in the block copolymer. [3] The signals at δ 2.6 ppm (weak), 11.53 ppm (strong), 1.8 ppm (weak), and 4.0 ppm (strong) are characteristic of the $-CH_2-$, –COOH, –NH–, and –CH– protons of the other amino acid residue, respectively.

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[3] Tapdigov Sh.Z. Encapsulation and in vitro controlled release of doxycycline in temperature-sensitive hydrogel composed of polyethyleneglycol–polypeptide (L-alanine–co–L-aspartate) // J. Biomim., Biomat.Biomed. Eng, 2021, vol 49, p.119-129.

The peaks at 3.28 ppm and 3.51 ppm correspond to the –CH_3 and methylene protons of the main mPEG backbone. Additionally, signals observed around 3.62 ppm (or 3.71 ppm) and 8.0 ppm are attributed to the methylene and secondary amine protons introduced via aminated mPEG. Based on the relative proton integration and quantitative analysis, the number-average molecular weight of the obtained block copolymer was calculated to be approximately 3642 Da. The average block lengths of the L-alanine and the other amino acid residues were estimated to be around 37 and 9.4 units, respectively. The hydrodynamic radius and surface charge of the micelles formed in solution were investigated using dynamic light scattering (Figure 4).

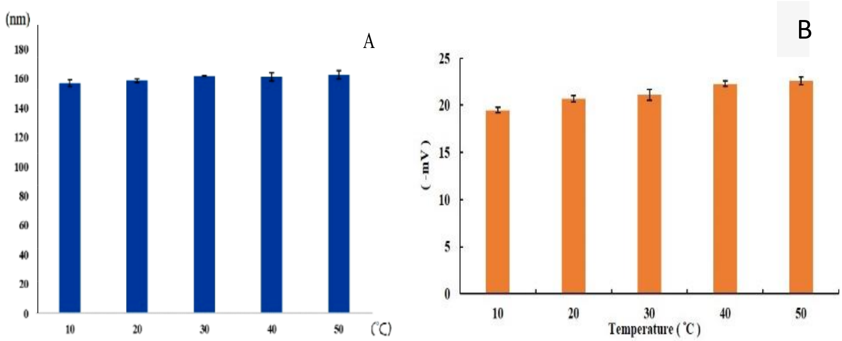

Figure 4. Temperature-dependent profiles of (A) the average size and (B) the zeta potential of aggregates formed by the mPEG-Ala-Asp block copolymer in a 0.01% aqueous solution over the temperature range of 10 °C to 50 °C.

As shown in Figure 4, the micelle size at 10 °C is 158 ± 3 nm, and aggregation occurs with increasing temperature, reaching a maximum of approximately 160 nm at 40 °C. The temperature-dependent profile of the micelle surface charge indicates that there is no significant difference between physiological temperature and 10 °C, with the zeta potential remaining around 20 ± 1.5 mV. In addition to DLS analysis, TEM micrographs of the mPEG-Ala-Asp micelles were also examined (Figure 5), revealing particle sizes in the range of 45–64 nm.

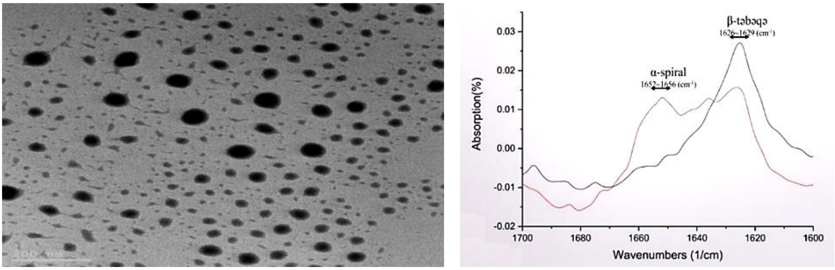

Figure 5. TEM image of a dried sample obtained from a 0.01% aqueous solution of mPEG-Ala-Asp micelles and the corresponding FT-IR spectra.

The spectra illustrate the α-helical conformation of mPEG-Ala and the β-sheet structure of mPEG–Ala–Asp. Compared to the DLS results, the particle size observed in the TEM images is characterized as smaller. The relatively larger size of the micelle in the solution is associated with the surrounding water molecules, gel formation, and internal diffusion. During drying, the macromolecule undergoes compaction, which leads to a reduction in the micelle size.

Although the micelle size is approximately 158 nm, considering that the size of the immobilized agent to be incorporated is only 1.0–1.5 nm, there are no restrictions on the complexes either in crossing cell membranes or in their movement through the bloodstream. Indeed, the size of particles in blood varies from a few nanometers up to 7–8 micrometers. For example, the size of vitamin B9, which can be absorbed through the intestinal villi, is about 140 nm, and the size of vitamin B12 after encapsulation can increase up to 650 nm. This demonstrates that such sizes do not pose any obstacle to their passage.

The polycondensation of mPEG with alanine N-carboxyanhydride was monitored by FT-IR spectroscopy (Figure 5). The incorporation of amino groups into the macromolecular chain results in the formation of a broad band at around 1650 cm^{-1}, characteristic of the protein secondary structure II (amide I, α-helix). Subsequent attachment of the next amino acid fragment to the chain via polycondensation induces internal granular hydrogen bonding and electrostatic interactions corresponding to the β-sheet structure, observed at approximately 1630 cm⁻¹.

The temperature dependence of the sol-gel transition of 3–10% solutions of the mPEG–Ala–Asp block copolymer was investigated. Considering physiological body temperature, the 3% concentration solution of the block copolymer and its corresponding viscosity were found to be optimal. The 3–4% solution of the block copolymer was accepted as the optimal concentration for injection at 37 °C.

Microwave-assisted ring-opening polymerization and investigation of polycaprolactone. For the first time, the ring-opening polymerization of ε-caprolactone was carried out in the presence of three different acids, and the optimal reaction time for each case was determined. Additionally, analogous reactions were performed in the presence of a catalyst, where the monomer conversion and melting temperatures of the products were measured for each reaction (Table 3).

Table 3.

Reaction conditions, conversion, and thermal data for the synthesis of PCL-acid derivatives

Samples	Radiotion time, (min)	Conversation (mass%)	T_{melt} (ºC)	Hm (J/gr)	Xc* (%)
PCL-AA	9	96.95	52.7	99.4	71.5
PCL-BA	9.5	77.85	62.3	106.5	76.6
PCL-TFAA	3	97.19	60.6	129.9	93.4
PCL-AA(ct)	4.5	96.52	55.2	120.8	86.9
PCL-BA(ct)	3	99.37	46.7	88.1	63.4

* *The crystallinity of the products was calculated based on the melting enthalpy of 100% crystalline PCL, which is 139 J/gr for PCL.*

FTIR characterization of the synthesized PCL samples was also performed. From the FTIR analysis of the PCL-AA sample (Figure 6), it was determined that a broad peak appears at around 3436.47 cm⁻¹, characteristic of –OH groups and internal hydrogen bonding[4].

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[4] I. M.Ahmadova, Sh.Z.Tapdigov, M.B.Sennaroğlu, M.S.Eroğlu. Microwave-assisted ring-opening polymerization of Ɛ-caprolactone4th International Eurasian Conference on Science, Engineering and Technology, Turkey. 2022, p.119.

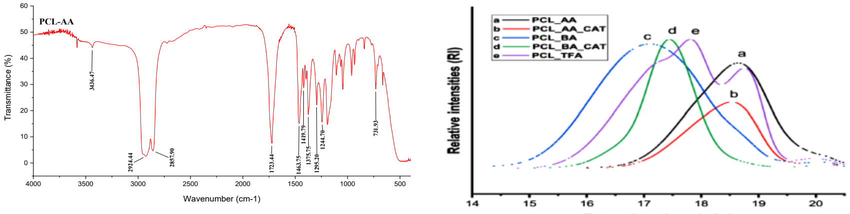

Figure 6. FTIR spectrum of the PCL-AA sample and GPC refractive index chromatograms of the PCL-acid samples

A characteristic absorption peak was identified at 1723.44 cm^{-1}, corresponding to the symmetric stretching vibration of the carbonyl (C=O) group present in PCL. Deformation vibrations characteristic of the C–H bonds in the CH_3–CH_2– groups were observed with weak intensity at 1463 cm^{-1}, 1375 cm^{-1}, and 731 cm^{-1} in the spectrum. The peaks at 2924 cm^{-1} and 2857 cm^{-1} are attributed to the stretching vibrations of C–H bonds in the methyl and methylene groups. Strong absorption peaks at 1419 cm^{-1}, 1295 cm^{-1}, and 1244 cm^{-1} characterize the absorption related to the carbonyl group in the carboxyl moiety. FTIR analyses were also conducted for the PCL-BA and PCL-TFAA samples. Unlike the others, the PCL-TFAA sample exhibited a deformation vibration peak characteristic of the C–F bond at 731 cm^{-1}, confirming the incorporation of TFAA into the main polymer chain during the polymerization reaction. Gel permeation chromatographic (GPC) analysis was performed on the products obtained from the ring-opening polymerization of PCL in the presence of organic acids (Table 4).

Table 4.

Average molecular weights and polydispersity of the PCL samples

Samples	**M_n ^{1}H-NMR**	**M_n GPC-LS**	**M_w (gr/mol) GPC-LS**	**PDI M_w/M_n**	**n Monomer**	**Terminal COOH/ OH**
PCL-AA	914	1355	1830	1.351	8	0.86/0.14
PCL-BA	1085	1590	2000	1.26	9	0.85/0.15
PCL-TFAA	5365	4900	6192	1.256	46	0.36/0.65
PCL-AA(ct)	1489	3970	5298	1.330	12	0.62/0.38
PCL-BA(ct)	1025	1507	2300	1.544	8	0.50/0.50

Using GPC, the number-average molecular weight (M_n), weight-average molecular weight (M_w), and the polydispersity index (M_w/M_n) were determined (Table 4). The number-average molecular weight of the polymer obtained during PCL synthesis depends on the pK_a value of the organic acid. This can be attributed to the increase in the concentration of the active center-forming component in the medium as the acid dissociation constantly increases. Specifically, the pK_a of AA is 4.756, while that of TFFA is 0.52. The refractive index increments (dn/dc) of the PCL-based polymer samples were studied in THF at 25 °C at a concentration of 0.07 ml/g. For five synthesized PCL polymer samples, the molecular weight ranged from 1.761×10^3 to 6.375×10^3 g/mol, and the polydispersity index (PDI) ranged from 1.26 to 1.54, indicating moderate polydispersity across all samples. Since the macromolecular chains in our system contain carbon atoms adjacent to hydroxyl and carboxyl groups, ^{1}H NMR spectroscopy was employed for characterization.

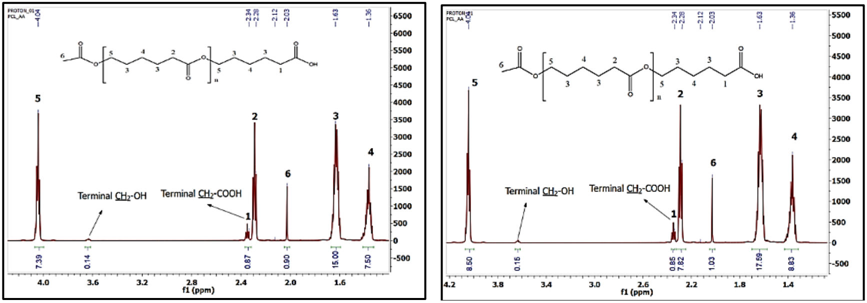

Figure 7. ^{1}H NMR spectra of synthesized PCL-AA and PCL-AA in the presence of $Sn(Oct)_2$, sol. D_2O.

^{1}H NMR spectra of the synthesized PCL-AA, PCL-BA, and PCL-TFAA samples were recorded for both catalyst-free and catalyst-assisted polymerizations, and the characteristic proton signals were identified. The ^{1}H NMR spectrum of the PCL-AA sample is presented in Figure 7. The specific ^{1}H NMR peaks observed for PCL (δ, ppm) include: 4.04 (methylene protons of H5, $–CH_2–$), 2.03 ppm (methylene protons in the $–O–CH_2–$ groups), 2.03 ppm (terminal $–CH_3$), 2.28 ppm (methylene protons in the $–CH_2–C=O$ group), 2.34 ppm (methylene protons in the terminal $–CH_2–COOH$), and 1.63 ppm and 1.36 ppm (protons in methylene groups H3 and H4, respectively), along with H6 ($-CH_3$). In the PCL-AA sample, two low-intensity proton signals were observed, indicating that the polymerization reaction proceeds in two directions, forming terminal $-CH_2-COOH$ and $-CH_2OH$ groups. These were identified by the signals at 2.34 ppm and 3.63 ppm, respectively. Integration of the proton signals corresponding to the terminal groups showed their ratio to be 87:14[5].

When the reaction was carried out in the presence of a catalyst, no sharp peaks were observed in the ^{1}H NMR spectrum of the resulting product.

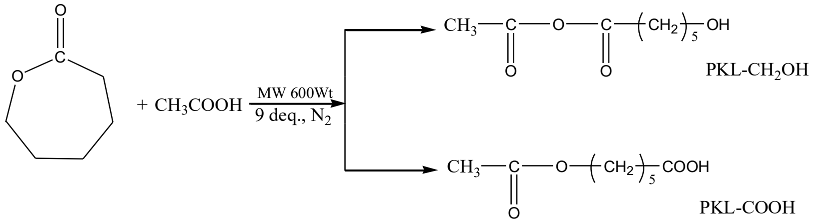

Figure 8. Microwave-assisted ROP (ring-opening polymerization) of caprolactone in the presence of AA

The occurrence of the reaction was confirmed by both NMR and FTIR analyses, under conditions with and without the use of a catalyst. Based on the ^{1}H NMR spectrum, the structure of the product obtained from the polymerization of CL in the presence of AA can be represented as shown in Figure 8. When examining the ^{1}H NMR spectra, the signal corresponding to protons of $PCL-CH_2OH$ macromolecules is observed with very low intensity. In contrast, the sample containing terminal carboxylic acid groups shows a relatively higher intensity. This indicates that the PCL–COOH polymer predominates in the mixture in terms of quantity.

In Chapter IV, the structure of the nanostructured hydrogels synthesized and their complexes with trypsin and doxorubicin were analyzed using UV-Vis, IR, and NMR spectroscopic methods to

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[5] Inara Ahmadova, Shamo Tapdigov, Müge Sennaroğlu Bostan, Mehmet S. Eroğlu. "Microwave Assisted Ring- Opening Polymerization of Ɛ-Caprolactone using Organic Acids", Journal of Polymer Research. 2023, V.30, p.291-296.

determine the nature of the interaction between the active reagent and the hydrogel. It is known that the characteristic functional group in PVPr is the >C=O group, which exhibits a maximum absorption at 205.5 nm. The ability of PVPr to form complexes with various organic and inorganic compounds is attributed to weak intermolecular interactions, including hydrogen bonding and basically electrostatic attractions around the >C=O group in the pyrrolidone ring, typically observed as layered structures.

Trypsin is a polypeptide, and its spectrum exhibits absorption bands characteristic of proteins in the 3000–3800 cm^{-1} region. Additionally, absorption bands at 1521 cm^{-1} (α-structure), 1534 cm^{-1} (β-structure), and 1548 cm^{-1} (disordered conformation) are also possible. During immobilization, these structural elements may participate in weak interactions and undergo slight chemical shifts.

To investigate the chemical shifts occurring during complex formation, the ^{1}H NMR spectrum of a homogeneous PVPr–trypsin mixture at a 10:1 mass ratio in D_2O was recorded (Figure 9). Based on this analysis, it was confirmed which functional group of the polymer is primarily involved in the complexation process during immobilization.

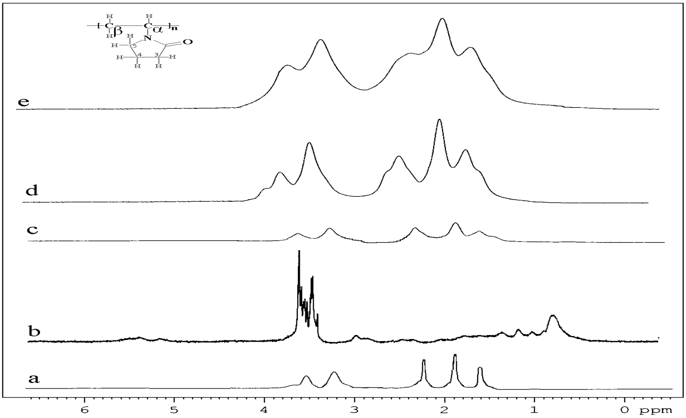

Figure 9. Comparative ^{1}H NMR spectra of PVPr (a), trypsin (b), PVPr–trypsin complex (c), PVPr–Cu(II) complex (d), and PVPr–Cu(II)–trypsin complex (e) in D_2O medium.

The interactions of Cu(II), Co(II), and Ni(II) complexes in aqueous PVPr solution, as well as their interaction with trypsin, were studied in model systems using UV-Vis spectroscopic methods. It was determined that the formation of metal ion complexes with PVPr is accompanied by a decrease in absorption intensity (hypochromic effect) and a shift toward longer wavelengths (bathochromic shift). In contrast, the formation of complexes between the metal–polymer conjugates and trypsin is characterized by an increase in absorption intensity (hyperchromic effect) and a shift toward shorter wavelengths (hypsochromic shift).

Furthermore, the spectrum shows that complexation also occurs in the range of 5000–4200 cm^{-1} and at wavelengths of 200–230 nm. To investigate the mechanism of immobilization in the presence of metal ions, a model system was used.

During the interaction of PVPr with trypsin, chemical shifts were observed primarily in the protons located at the 3rd and 5th carbon atoms near the >C=O group of the pyrrolidone ring.[6] In other words, the electrostatic nature of the interaction between the polymer and trypsin affects the nearby atoms or atomic groups during complex formation. However, the chemical shifts observed in the protons at the 2nd and 3rd carbon atoms of the polymer chain are not significantly different.

Following the synthesis of low molecular weight polycaprolactone (PCL), research was conducted to prepare nanocapsules based on it. Using Dox as a model drug, encapsulation efficiency, drug loading, and release kinetics were studied. The nanocapsule obtained exhibited a high encapsulation efficiency for doxycycline, reaching 71.8%. The drug loading capacity was determined to be 15.69%, which corresponds to approximately 0.157 mg of Dox per 1 mg of nanocapsule. The release behavior of doxycycline from the PCL-based nanocapsules was investigated over time at two different pH values (4.8 and 7.4), and the results are

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[6] Tapdigov Sh.Z. The bonding nature of the chemical interaction between trypsin and chitosan based carriers in immobilization process depend on entrapped method: A review // International Journal of Biological Macromolecules, 2021, vol. 183, pp.1676-1696.

presented in Table 5. As shown, the release profile varies significantly depending on the medium. Under mildly acidic conditions (pH 4.8), about 50% of the drug was released within 12 hours, and the release plateaued at around 90% after 24 hours.

Table 5.

Time-dependent release values of the antibiotic from the PCL-Dox-based nanocapsule at different pH medium. T=37 °C, V=5 ml, $m_{kaps\text{-}Dox}$=13.725 mg.

Time, hour	**pH=4.8**			**pH=7.4**		
	C_{rel}, mkm/ml	m_{rel}. mg	Release, %	C_{rel}, mkm/ml	m_{rel}. mg	Release, %
0.5	7.34	0.011	6.51	2.67	0.004	2.78
1.0	14.0	0.021	13.25	4.66	0.007	4.56
2.0	27.34	0.041	25.84	9.34	0.014	9.15
3.0	32.67	0.049	31.43	16.0	0.024	15.43
6	38.66	0.058	37.17	20.68	0.031	19.51
12	50.00	0.075	48.26	25.34	0.038	24.84
24	78.00	0.117	74.56	30.00	0.045	28.31
48	92.00	0.138	88.34	32.67	0.049	31.48
72	96.00	0.144	91.73	34.00	0.051	32.63

In contrast, under neutral conditions (pH 7.4), a much slower release was observed, with only 32% of the immobilized Dox being released into the medium over 72 hours. To gain a better understanding of the release mechanism, the kinetic data were fitted to zero-order, first-order, Higuchi, Korsmeyer–Peppas, and Hixson–Crowell models, and the corresponding correlation coefficients were compared.

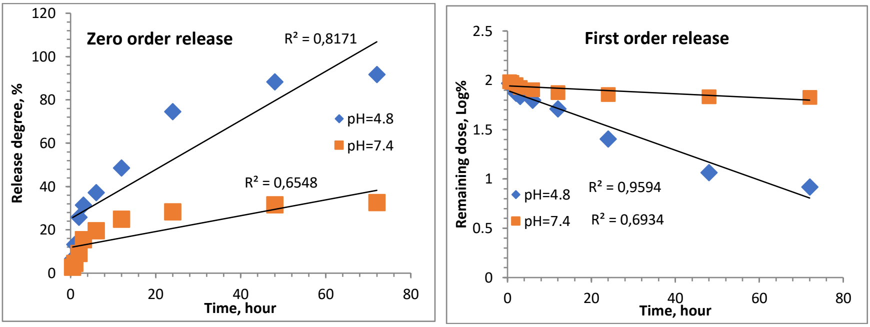

Figure 10. The zero-order kinetic model for the release of doxorubicin from PCL nanocapsule

As can be seen at pH = 4.8, the drug release corresponds relatively well to diffusion-controlled release with an R^2=0.8171. However, this model does not fit in a neutral medium. The first-order release mechanism, on the other hand, does not match the studied system in any case (Figure 10). Additionally, since the investigated PCL nanocapsules do not have a porous matrix structure and the R^2 values are less than 0.55, it can be concluded that the release does not follow this mechanism[7]. The amount of release was also evaluated using other kinetic models, and the results are presented in the following figures.

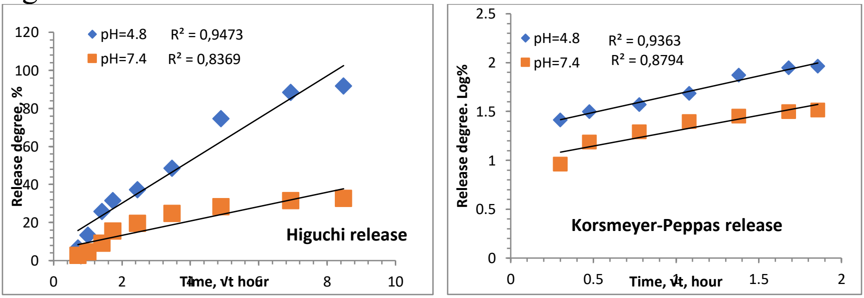

Figure 11. Kinetic models of doxorubicin release from PCL nanocapsules according to Higuchi, Korsmeyer-Peppas, and Hixson-Crowell models.

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[7] Tapdıgov, Sh., Taghıyev, D. Immobılızatıon of doxorubıcın onto poly-ε-caprolactone based carrıer and apply kınetıc model for release // XI International European Conference on Interdisciplinary Scientific Research, - Lisbon: - 2025.- p.121-122.

Since PCL, PVPr, and PEG are incorporated into the studied system, it cannot be considered as a solid matrix. The fit, based on the R^2 values, is observed mainly at pH = 4.8, which corresponds more to proportional release during the initial 24 hours.[8] However, this model does not hold true in a neutral environment.

In **Chapter V**, the kinetics of the release of model enzymes and antibiotics from natural and synthetic nanostructured hydrogels was investigated. The sorption isotherms and thermodynamic parameters of trypsin sorption using pH-sensitive poly(N-vinylpyrrolidone-co-4-vinylpyridine) hydrogels and their magnetite-based derivatives were studied.

Furthermore, the immobilization of doxorubicin into memory-type nanostructured hydrogels of natural and synthetic origin was carried out. The loading of doxorubicin into temperature-sensitive mPEG-Ala-Asp hydrogels and the in vitro investigation of its controlled release behavior were also presented.

During the studies, the activity of trypsin immobilized onto synthetic polymer-based gels, as well as onto metal complexes of PVPr (with an average molecular weight of 40 kDa) with copper, cobalt, and nickel ions, was investigated under different pH conditions based on its release into the solution. The investigation of trypsin release from polymer gels and metal-gel complexes into the medium within a pH range of 3 to 11 under static conditions showed that, depending on the nature of the polymer and the ionization degree of its functional groups, the enzyme is released into the medium at different pH values over 24 hours (Figure 12). In contrast, release from metal-gel complexes is characterized by significantly higher release rates in distinctly different environments. While the release from synthetic polymer-based gels is observed mostly in neutral or near-neutral conditions, in metal-gel complexes, the release is significantly enhanced in acidic mediums.

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[8] Ş.Z.Tapdıqov, M.M.Mustafayev, İ.M.Əhmədova. Poli-ε-kaprolkton əsaslı daşıyıcıya immobilizə olunmuş doksorubisinin mühitə ayrilmasinin kinetik modellərdə tədqiqi. Sumqayıt Dövlət Universitetinin Xəbərləri, 2023, Cild 23, No 1, s.30-36.

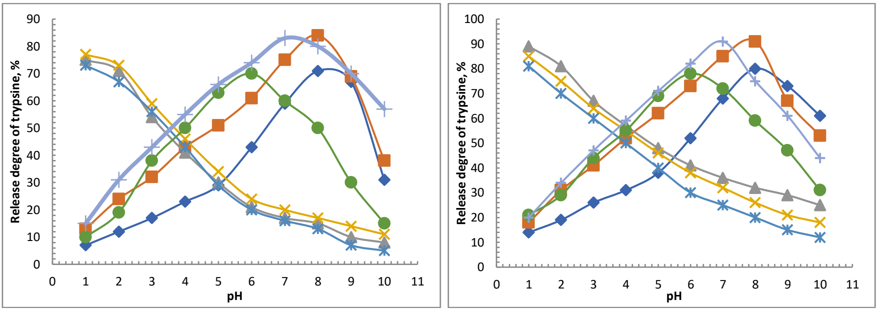

Figure 12. The pH-dependent release of trypsin into the solution from synthetic polymer-based and PVPr metal-gel complexes at 24 °C (left) and 36.5 °C (right). ♦-PVPr 10 kDa, ■-PVPr 40 kDa, •-PAa, +-PAA, ×-PVPr 40 kDa/Co(II), ∗- PVPr 40 kDa/Ni(II), Δ-PVPr 40 kDa/Cu(II). t=24 hr.

As shown in Figure 12, the release of trypsin immobilized on PVPr, PAa, and PAA-based hydrogel samples into the solution is minimal starting from pH 1 and reaches its maximum at pH 8, pH 6, and pH 7, respectively. [9]At pH 9 and above, the release of trypsin into the solution decreases sharply. This can be explained by the fact that with increasing hydroxyl ion concentration in the medium, the swelling degree of the hydrogel gradually decreases, and the enzyme molecules become trapped within the pores of the gel. Furthermore, at pH values above 9, denaturation of the enzyme macromolecule occurs, resulting in the loss of its biological activity. The onset of gel collapse also hinders the release of trypsin from the carrier. It was found that increasing the temperature to 36–37 °C accelerates the release of trypsin from all hydrogel samples, characterized by a higher release rate. In the case of metal-gel complexes, the relatively linear release behavior at pH values between 5 and 8 indicates a controlled release process. It is evident that increasing the temperature enhances the mobility and flexibility of macromolecules, which leads to the unfolding or loosening of coiled macromolecular

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[9] Sh.Z.Tapdiqov, D.B.Taghiyev, N.A.Zeynalov. Study of trypsın release in kınetıc models from poly-N-vınylpyrrolıdone based pH-sensıtıve hydrogel / // Azerbaijan Chemical Journal, - Baku: - 2025. No1, - p. 75-82.

chains. As a result, trypsin molecules located on the surface and inside the gel are released. In addition, elevated temperature affects the chemical bonding or electrostatic interactions responsible for binding the enzyme to the macromolecule, causing weak interactions to break and stronger ones to weaken.

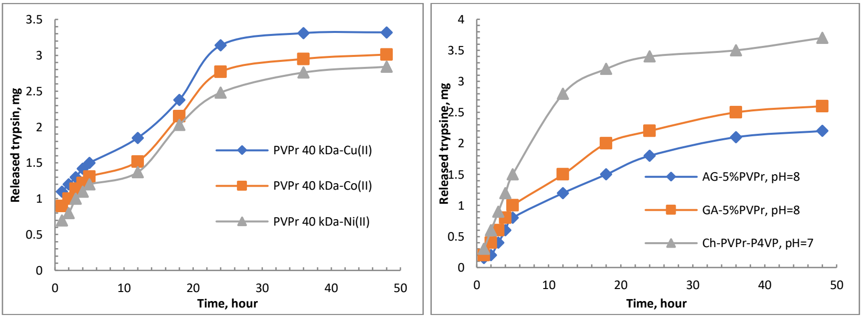

Figure 13. Kinetics of trypsin release into solution from natural and synthetic hydrogels and PVPr-based gel complexes with copper, cobalt, and nickel ions at 36.5 °C

When examining the time dependence of trypsin release at 36 °C under the optimal pH conditions corresponding to the maximum swelling degree of each gel sample (Figure 13), it is observed that the majority of the enzyme is released within the first 3 hours of the 24-hour period. As seen from the figures, in all gel samples, after the carrier reaches its maximum swelling capacity, a stabilization in the amount of trypsin released into the solution is observed. The reason for the incomplete release of the total immobilized enzyme is the establishment of an equilibrium between the immobilized and the released trypsin in the solution. In synthetic polymer-based gels, the release becomes linear after 2 hours, whereas in natural polymer-based gels, this linear release begins after 10 hr.

In the next stage of the research, the immobilization of the model drug compound Dox onto the synthesized nanostructured hydrogels via sorption, as well as its release into the medium, was investigated (Table 6).

Table 6

Values of doxorubicin immobilization via sorption onto nanostructured hydrogels based on natural and synthetic polymers and their corresponding metal-hydrogel complexes, T=20 ºC, V=20 ml, m_{gel}=50 mq, kontakt müddəti 24 saat, $C_{0doks.}$=500 mq/l.

Gel carriers	Equil. Concent., *mg/l*	Immobilization degree, *%*	Gel capacity, *mg/gr*
PVPr 10 kDa-10% c/l	452,4	9,52	19,04
PVPr 40 kDa-10% c/l	432,5	13,50	27,00
VPr/3% MBAA	418,4	16,32	32,64
PAa 10 kDA-10% c/l	465,8	6,84	13,68
PAA 80 kDA-10% c/l	405,6	18,88	37,76
PVPr 40 kDa-Cu(II)	387,5	22,50	45,00
PVPr 40 kDa-Co(II)	392,2	21,56	43,12
PVPr 40 kDa-Ni(II)	390,8	21,84	43,68
AG-PVPr (5%) 1% c/l	360,3	27,94	55,88
GA-PVPr (5%) 1% c/l	372,7	25,46	50,92
Ch-PVPr-P4VP 1% tikici	332,4	33,52	67,04

The use of Dox as a model drug compound is due to its colored nature, which makes its monitoring more convenient via visual observation. The synthesized nanostructured hydrogels and hydrogel–metal complexes were loaded with Dox, and the drug-loading capacities of the carriers were calculated. As observed, the hydrogels structured from low-molecular-weight polymers exhibit lower drug-loading capacities and immobilization efficiency compared to others. In contrast, the drug-loading capacity of natural polymer-based hydrogels and metal–hydrogel complexes increases significantly.

Furthermore, compared to trypsin, the capacity of the hydrogels for doxorubicin is considerably higher. This can be explained by the chemical composition of the doxorubicin molecule and the greater spatial reactivity of its functional groups.

It is well known that once drug compounds are immobilized in polymer matrices, a key focus of investigation becomes the effect of medium pH on the maximum release of the antibiotic from the carrier.[10] For this purpose, the pH-dependent release of Dox from natural and synthetic polymer-based hydrogels and metal–hydrogel complexes containing various amounts of the drug was studied. The results are presented in the figures below.

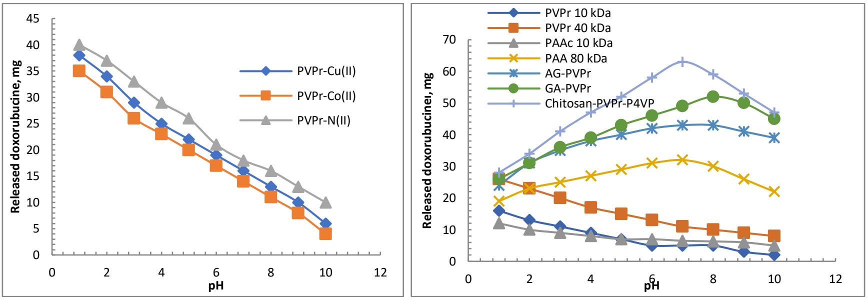

Figure 14. The pH-dependent release of doxorubicin from natural and synthetic polymer-based gels and metal–gel complexes, T=37 ºC, t=24 hr, V_{buffer}=20 ml, $m_{car+dox}$=1-1,05 gr.

As seen, there are certain differences in the pH-dependent release behavior of doxorubicin compared to that of trypsin from the carriers. These differences are more pronounced in synthetic polymer-based gels. In metal–gel complexes, the release is characterized by higher values in acidic environments, and up to 50% of the antibiotic can be released at neutral pH levels, which is considered satisfactor[11]. This also confirms that doxorubicin binds more strongly to the carrier in the presence of metal ions, and in acidic environments, the breaking of coordination bonds characteristic of metal ions ensures a higher release of the antibiotic into the medium.

During the studies, the release kinetics of doxorubicin from gel carriers based on PVPr with an average molecular weight of 40 kDa, PAa with 80 kDa, metal–gel complexes of PVPr, as well as chitosan,

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[10] Sh.Z. Tapdiqov, D.B. Taghiyev. Apply of kınetıc results of doxorubıcın release from polyacrylıc acıd-based hydrogel to Hıguchı, Korsmeyer-Peppas, Hıxon-Crowell equatıons. SOCAR Proceedings, 2025, No. 1 , p. 130-135.

arabinogalactan, and gum arabic, were investigated in media where the gels exhibited maximum swelling. Considering the absorption of the doxorubicin antibiotic in the intestinal environment, the release kinetics from the gel samples were studied at pH 6–8 over a period of 0–72 hours. The results are presented in the following figures.

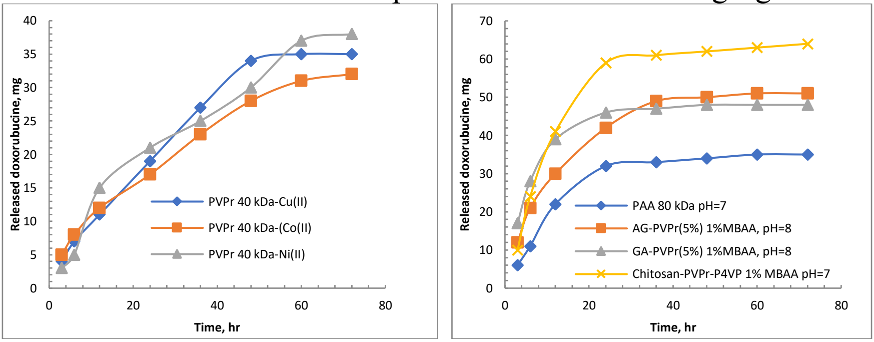

Figure 15. The time-dependent release of doxorubicin from PVPr-based metal gel complexes and natural polymer hydrogels.

As can be seen from the figure, the release of the antibiotic from the metal-gel complexes occurs proportionally over a long period of time, ensuring controlled release. However, in other gel samples, this process is observed within 20–25 hours. After 25 hours, the release virtually ceases, and a dynamic equilibrium persists for a prolonged period. Naturally, this is related to the involvement of metal ions, and it appears that the retention of metal ions within the gel matrix in neutral environments, as well as the stability of the complex, directly influences this process.

Chapter VI presents the kinetic investigation of doxorubicin release from hydrogels based on poly(N-vinylpyrrolidone) (PVPr) and poly(acrylic acid) (PAa), the study of a hydrogel grafted from chitosan and poly(4-vinylpyridine-co-N-vinylpyrrolidone), as well as the immobilization behavior of doxorubicin. Additionally, the electrostatic and hydrogen-bond-assisted immobilization of trypsin onto a pH-sensitive hydrogel composed of chitosan, poly(N-vinylpyrrolidone), and poly(4-vinylpyridine) radical-graft copolymer was investigated.

The adsorption capacity of the synthesized hydrogels for trypsin was evaluated at pH values ranging from 3 to 9. The hydrogel lacking Fe_3O_4 nanoparticles exhibited higher protein uptake (40–45 $mg \cdot g^{-1}$) in neutral media. The presence of magnetite particles occupied active sites, resulting in up to a two-fold decrease in trypsin adsorption under the same conditions. At pH ≤ 7, protonation of the active sites contributes to reduced protein binding, whereas under alkaline conditions, shrinkage of the hydrogel network hinders trypsin diffusion due to decreased swelling. In both gel types, increasing the amount of adsorbent leads to higher sorption capacity, reaching equilibrium at around 75–80 mg. The optimal sorption yields were determined as 78–80% for the PVPr-co-P4VP hydrogel at 75 mg adsorbent and 91–93% for the PVPr-co-P4VP/Fe_3O_4 hydrogel at 100 mg adsorbent. Clearly, increasing the hydrogel mass enhances the number of available binding sites; however, since adsorption equilibrium is achieved at these respective amounts, 75 mg and 100 mg were considered optimal doses.

The effects of temperature and contact time on the adsorption of trypsin were investigated. It was found that increasing the temperature up to 45 °C enhanced the adsorption efficiency of trypsin on both hydrogels, reaching 80–91%. This increase can be attributed to the enhanced mobility of the macromolecules and active binding sites in the solution at elevated temperatures. Experimental data indicated that trypsin adsorption proceeds rapidly within the first 50–60 minutes and reaches equilibrium within 70–80 minutes. In the case of the magnetite-containing PVPr-co-P4VP/Fe_3O_4 hydrogel, equilibrium is achieved more quickly, within approximately 60 minutes, while for the crosslinked PVPr-co-P4VP hydrogel, equilibrium is attained around 80 minutes.

The adsorption behavior of trypsin on both hydrogels was analyzed using Langmuir, Freundlich, and Temkin isotherm models. The fitting was performed using specialized software, and the corresponding parameters are summarized in Table 7. It was determined that trypsin adsorption onto the PVPr-co-P4VP hydrogel fits well with the Freundlich and Langmuir models, and partially with the Dubinin–Radushkevich (D-R) isotherm ($R^2 \geq 0.98$). Adsorption on the PVPr-co-P4VP/Fe_3O_4 magnetite hydrogel showed a strong correlation with the Langmuir ($R^2 = 0.9961$) and Freundlich ($R^2 \geq 0.9893$) isotherm models. According to the Langmuir isotherm, the adsorption of trypsin onto both hydrogel samples occurs in an energetically equivalent manner across the entire surface of the hydrogel. In the presented system, the Langmuir constant K_L was found to be greater than 1 for both gels, with values of 1.218 and 5.880, respectively. Furthermore, the values of 1/n for both hydrogels were less than 1 (0.60–0.63), indicating that the adsorption of trypsin is favorable. According to the Temkin isotherm model, if the parameter B_T has a positive value, the adsorption process is considered endothermic, and a correlation coefficient $R^2>0.99$ suggests strong interaction between the sorbate and sorbent.

Table 7.

Adsorption parameters of trypsin onto the crosslinked PVPr-co-P4VP and PVPr-co-P4VP/Fe_3O_4 magnetite hydrogels at 25 °C according to various isotherm models.

Isotherm models	**Parameters**	**Cross-linked PVPr-so-P4VP**	**Cross-linked PVPr-so-P4VP/Fe_3O_4**
Langmuir	K_L, $L \times qr^{-1}$	1.218	5.880
	q_{max}, $mq \times qr^{-1}$	205.76	145.34
	R^2	0.9832	0.9961
Freundlich	K_F	101.73	178.46
	n (1/n)	1.65 (0.606)	1.57 (0.636)
	R^2	0.9839	0.9893
Temkin	B_T, $J \times mol^{-1}$	27.38	30.92
	K_T, $L \times mq^{-1}$	48.12	96.69
	R^2	0.9249	0.8674
Dubinin-Radushkevich (D-R)	q_m, $mq \times qr^{-1}$	133.46	142.67
	$\beta \times 10^{-7}$	0.559	0.264
	E, $kC \times mol^{-1}$	2.988	1.376
	R^2	0.9815	0.8797

For the PVPr-co-P4VP and PVPr-co-P4VP/Fe_3O_4 hydrogels studied, the obtained B_T values were positive—27.38 and 30.92

J·mol^{-1}, respectively—indicating the presence of electrostatic interactions between trypsin and the gels.

According to the D-R isotherm model, if the free energy of adsorption is below 8 kJ·mol^{-1}, the process is governed by Van der Waals interactions.[11] In both hydrogel systems, the calculated free energy ranged between 1–3 kJ·mol^{-1}, which is typical for polymer-based hydrogel systems interacting with proteins via weak chemical bonding, include electrostatic attractions and Van der Waals forces.

Adsorption isotherm studies conducted at different temperatures confirmed that trypsin adsorption is more effective at relatively higher temperatures, reflecting the endothermic nature of the process (Table 8).

Table 8.

Thermodynamic parameters of trypsin adsorption onto crosslinked PVPr-co-P4VP and PVPr-co-P4VP/Fe_3O_4-based magnetite hydrogels

Thermo-dynamic parameters	**Temperatur, K / °C**					
	298 / 25		**308 / 35**		**318 / 45**	
	PVPr-so-P4VP	**PVPr-so-P4VP/Fe_3O_4**	**PVPr-so-P4VP**	**PVPr-so-P4VP/Fe_3O_4**	**PVPr-so-P4VP**	**PVPr-so-P4VP/Fe_3O_4**
ΔG°, kJ×mol^{-1}	-13.145	-14.263	-14.05	-15.569	-14.81	-16.584
K_d	201.48	316.36	241.4	437.01	270.88	530.08
ΔH°, kJ×mol^{-1}	11.684	20.381				
ΔS°, J×mol^{-1}K^{-1}	83.39	116.41				
TΔS°, kJ×mol^{-1}	24.85	34.69	25.68	35.85	26.52	37.02
R^2	0.9976	0.9981				

For the crosslinked PVPr-co-P4VP hydrogel, increasing the temperature up to 45 °C does not result in a significant change in the

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[11] Sh.Z.Tapdiqov, L.Ambrasio, D.B.Taghiyev, M.G.Raucci, N.A.Zeynalov. Adsorption of trypsin onto pH sensitive poly-N-vinylpyyrolidone-co-poly-4-vinylpyridine and its magnetic based hydrogel: Sorption isotherms and thermodynamic parameters. Sensors and Actuators: A Physical, 2023, Vol.356 p.114371.

ΔG° values, suggesting that the spontaneity of the process remains relatively constant. However, for the magnetite-containing hydrogel, the ΔG° value is –14.263 kJ·mol^{-1} at 35 °C and becomes slightly more negative, –16.584 kJ·mol^{-1}, at 45 °C. Based on spectroscopic data and isotherm analysis, in weak interactions such as Van der Waals forces and, to a lesser extent, hydrogen bonding with the trypsin molecule (Figure 16).

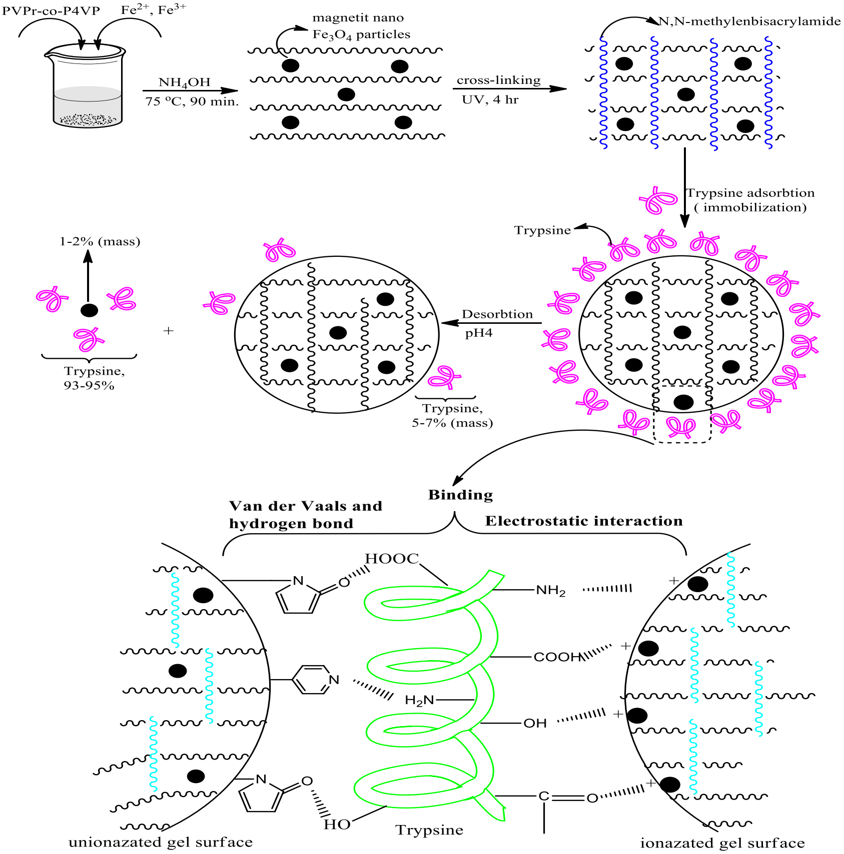

Figure 16. Schematic illustration of the synthesis of the PVPr-co-P4VP/Fe_3O_4 magnetite hydrogel and the interaction of trypsin molecules with the active sites of the hydrogel and magnetite nanoparticles

Such interactions are more prevalent in the gel sample that does not contain magnetite nanoparticles [12].

During the study, the surface morphology of the synthesized graft copolymer—the precursor of the final product—and the material after trypsin immobilization was examined by SEM at various positions (Figure 17).

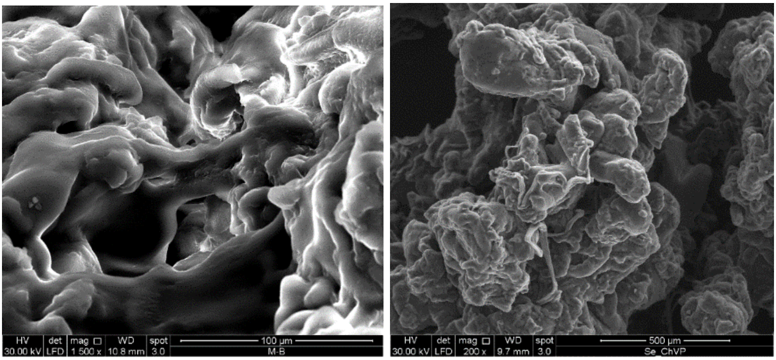

Figure 17. SEM micrographs of the PVPr-co-P4VP graft copolymer-based hydrogel before and after trypsin immobilization.

It was determined that after trypsin immobilization, the surface structure of the hydrogel became smoother compared to its previous state. The nearly complete coverage of the surface smoothness indicates that the immobilization is predominantly localized on the hydrogel surface. Considering that trypsin is a protein molecule with molecular dimensions significantly larger than those of simple small organic molecules, it is inferred that the immobilization process occurs as a coating over the entire hydrogel surface.

It is well known that the selective sorption of both pharmaceutical substances and metal ions, as well as the development of sorbents or hydrogels with higher loading capacities, is a subject of intensive research, particularly focusing on memory-based hydrogel systems. Such studies can be found in the works of the late academician Ayaz Efendiev of the Azerbaijan National

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[12] Shamo Tapdıgov, Dilgam Taghıyev. Sorption isotherms for trypsin by pH-sensitive poly(N-vinylpyrrolidone-co-4-vinylpyridine) and its magnetite-based hydrogel. BİLTEK-X, X Uluslararası Bilimsel Araştırmalar ve Güncel Gelişmeler Kongresi, 2025, Istanbul, Turkey, p.233.

Academy of Sciences (ANAS). In his works, the academician developed quaternized poly-4-vinylpyridine-based swellable sorbents selective for individual metal cations such as Cu(II), Co(II), and Ni(II), for the selective removal of these cations from wastewater.

He demonstrated that initially forming homogeneous complexes of metal cations with the polymer or its monomer, followed by macromolecular crosslinking and structuring into hydrogel form, effectively "shape" the hydrogel with memory. The desorption of metal ions from metal–hydrogel complexes by acid treatment creates pores characteristic of the ionic radii of each metal cation within the hydrogel structure, leaving these pores vacant. Consequently, the hydrogel imprinted for a specific metal ion exhibits enhanced selectivity and faster sorption of that metal cation from subsequent solutions.

The creation of such memory-based polymer hydrogel systems has also been successfully applied for the immobilization of low molecular weight pharmaceutical compounds as well as enzymes, resulting in effective outcomes[13].

The immobilization of the enzyme trypsin and the antibiotic doxycycline onto synthesized nanostructured hydrogels was studied in memory-imprinted hydrogel samples. It was demonstrated that the preparation of memory-based nanostructured hydrogel systems with trypsin does not yield successful results. Specifically, during the thermal crosslinking of polymer-trypsin homogeneous complexes with MBAA, whether by ultraviolet irradiation or at 110–120 °C, the enzyme cannot remain fully stable. The trypsin molecules undergo hydrolysis and structural alterations, preventing the formation of pores characteristic of the molecular dimensions of intact trypsin. Consequently, the loading capacity of the hydrogel upon re-immobilization with trypsin does not significantly differ. Since the difference in loading is approximately 2–3%, detailed analysis of these values is considered unnecessary.

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[13] Sh.Z.Tapdiqov, E.F.Nasiyyati, D.T.Babayeva Immobilization of doxorubicin in with shape-memory natural and synthetic based hydrogel and metalgel complexes. Proc. Azerbaijan High Technical Educ.Inst. 2023, Vol. 24, p.83-91.

Studies conducted with doxorubicin demonstrated a memory effect, whereby water-soluble biocomplexes with polymers, upon subsequent treatment with a crosslinking reagent, retain their initial chemical structure within the internal architecture of the hydrogel. The immobilization of the doxorubicin antibiotic was carried out on these nanostructured hydrogels after washing and drying them with hydrochloric acid solution and distilled water. Finally, for each nanostructured hydrogel sample, the degree of antibiotic immobilization and the loading capacity of the hydrogel were calculated (Table 9).

Table 9.

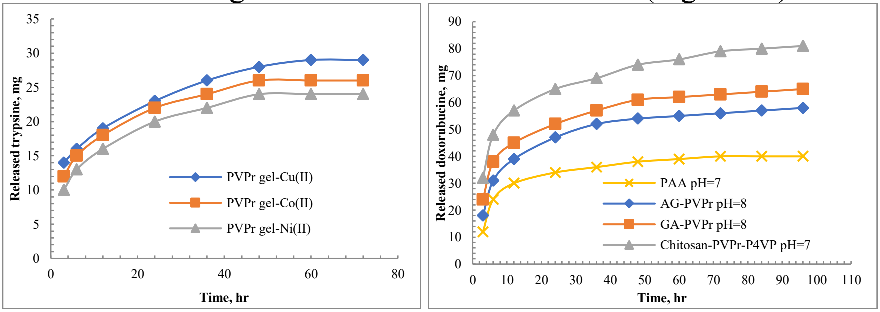
Immobilization of doxorubicin onto memory-shape based natural and synthetic polymer-based hydrogels.

Hydrogel	**Memory-shape based**							**Unmemory**	
	Eq. concentr mg/l, pH=7-	Immobilization degree, *İD*, %			Gel capacity, *GC*, mg/gr			pH=7-8 medium	
		pH 7-8	0.9% NaCl	0.1% ql-za	pH 7-8	0.9% NaCl	0.1% ql-za	*ID*, %	*GC*, mq/qr
PVPr 10 kDa	446.2	10.7	8.24	9.3	21.5	16.4	18.6	9.52	19.04
PVPr 40 kDa	417.6	16.4	9.63	10.7	32.9	19.2	21.4	13.5	27.00
VPr / 3% t	402.2	19.5	11.4	13.5	39.1	22.9	27.0	12.4	24.94
PAa 10 kDa	457.3	8.5	6.12	7.2	17.0	12.2	14.5	6.84	13.68
PAA 80 kDa	376.7	24.6	18.6	20.1	49.3	37.2	40.2	18.8	37.76
PVPr - Cu^{2+}	404.1	19.1	10.4	13.3	38.3	20.8	26.7	22.5	45.00
PVPr - Co^{2+}	407.4	18.5	10.7	12.8	37.0	21.5	25.6	21.5	43.12
PVPr - Ni^{2+}	411.3	17.7	11.5	13.0	35.4	23.0	26.0	21.8	43.68
AG-PVPr-1%	302.2	39.5	28.6	31.3	79.1	57.2	62.6	27.9	55.88
GA-PVPr-1%	310.8	37.8	26.4	29.1	75.6	52.8	58.2	25.4	50.92
Ch-PVPrP4VP1%	256.3	48.7	32.0	35.5	97.4	64.1	71.0	33.5	67.04

As shown in the table, when memory-imprinted nanostructured hydrogel samples are pre-formed with pores complementary to the doxorubicin antibiotic molecules, these hydrogels exhibit increased selectivity towards the drug and possess higher sorption capacities. For example, before imprinting, the chitosan-based hydrogel absorbed 33.5% of doxorubicin and had a loading capacity of 67 mg/g. After imprinting, the immobilization degree increased to 49%, and the hydrogel's loading capacity for the antibiotic rose to 97 mg/g.

Additionally, the results indicate that when memory-based systems are prepared in the presence of metal ions, the immobilization degree and loading capacity of the metal–gel complexes towards the antibiotic decrease. Specifically, the copper ion-imprinted gel complex exhibited a loading capacity of 45 mg/g and an immobilization degree of 22.5%, whereas after imprinting, these values decreased to 38.3 mg/g and 19.1%, respectively.

Kinetic studies of antibiotic release show that the antibiotic immobilized on the surface of the metal–gel complex is released into the medium in larger amounts over a shorter time (Figure 18).



Şəkil 18. Time-dependent release of doxorubicin from memory-shaped PVPr, metal, and other natural polymer-based hydrogels

The release of doxorubicin from memory-shape carriers formed both with metal ions and other polymers occurs via different mechanisms and in a more controlled manner compared to non-imprinted systems. It was found that during the initial 3–5 hours, 36% of the immobilized antibiotic is released from the Cu(II)-containing memory-imprinted metal–gel complex, while from other polymer hydrogels, such as chitosan-based gels, approximately 31% is released into the medium. In contrast, the release from non-imprinted hydrogel samples was only 8.9% and 15%, respectively.

Furthermore, the release of doxorubicin from memory-imprinted nanostructured hydrogel samples continues gradually over an extended period. Examination of the time-dependent release curve shows that after 12 hours, the antibiotic release exhibits a linear behavior and reaches a steady state.[14] This behavior is favorable for the targeted delivery and prolonged therapeutic effect of drugs, especially antibiotics, from memory-imprinted natural and synthetic nanostructured hydrogels.

The surface morphology of all, or at least some, of the synthesized memory-shape samples was investigated by SEM.

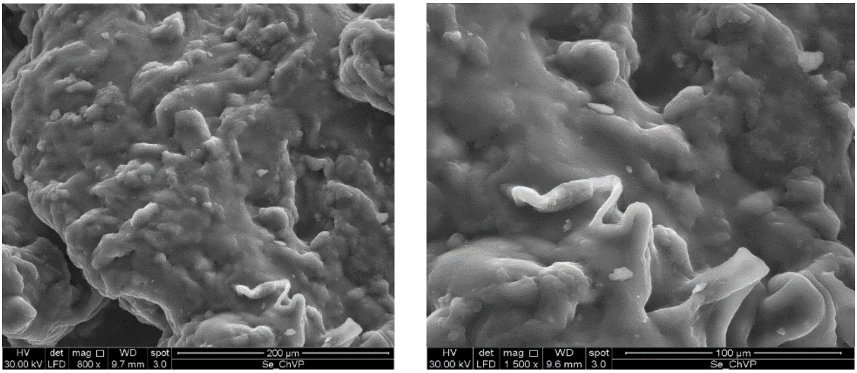


Figure 19. SEM microimages of memory-shaped PVPr-copper complex after immobilization of trypsin.

The SEM images of the obtained products reveal both surface smoothness resulting from protein immobilization and the presence of smaller-sized structures characteristic of copper ions. When examining the surface at 1500× magnification, the crystallinity typical of salts is clearly visible. Morphological analysis of the surface indicates that after the immobilization of trypsin on PVPr-metal gel complexes, the product retains the main surface structure characteristic of the initial gel with only slight modifications. This suggests its potential use as a carrier matrix for the controlled delivery and release of certain antibiotics and proteins derived from such complexes.

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[14] Sh.Z.Tapdiqov, D.B.Taghiyev, N.A.Zeynalov. Immobılızatıon and release kınetıc of doxorubıcıne from the shape-memory poly-N-vınylpyrrolıdone based metal-gel complexes. XII Международной научно-практической конференции WORLD OF SCIENCE, 2025, Москва, p.23-24.

Considering the chemical composition, physical properties, mode of action, and application area, the next study focused on loading a small amount of the widely used anticancer antibiotic doxorubicin onto a temperature-sensitive gel based on mPEG-Ala-Asp and investigating its release under specific conditions.[15] After obtaining the initial gel–drug complex, the samples were dried at 30 ˚C for 48 hours to reach a constant weight, then incubated in 10 mL buffer solutions at different pH values (pH = 2, 7.4, and 8.6) at 37 ˚C. The release kinetics of doxorubicin from the hydrogels were studied over time intervals ranging from 0 to 300 minutes (Figure 20).

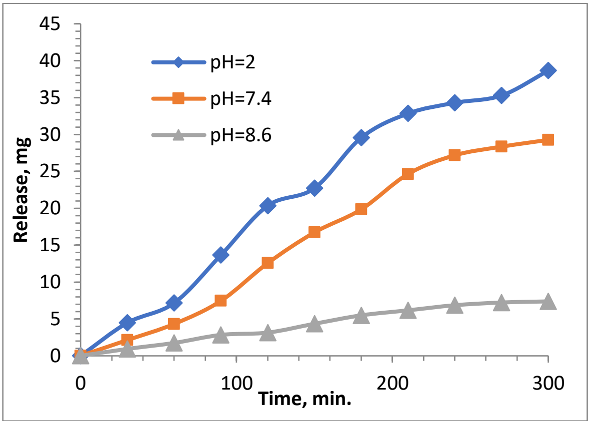

Figure 20. Time-dependent release of doxorubicin from PEQ-Ala-Asp gel in acidic, neutral, and alkaline medium, T=37 ˚C, V=10 ml.

In acidic media, the release initially reaches a steady value at a certain time point, followed by a linear release phase. This type of release corresponds to degradation-assisted release mechanisms. If the drug release from the matrix exhibits a linear time dependence, it characterizes diffusional steady (controlled) release. When the release shows an initial burst followed by an increase and stabilization, it indicates release associated with matrix degradation.

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[15] Sh. Z. Tapdiqov, D.B. Taghiyev. Immobilization and *in vitro* kinetic controlled release study of doxorubicin from temperature-sensitive mPeg-Ala-Asp polypeptide hydrogel. SOCAR Proceedings, 2024, Special Issue No. 1, p. 001-008.

The last graph demonstrates that the release correlates with the swelling degree of the polymer system, indicating a more controlled release profile. In neutral media, the release of doxorubicin exhibits relatively controlled release behavior, whereas in alkaline media, the release mechanism is more characteristic of diffusion-controlled release.

The kinetic data of drug release in different media were fitted to various mathematical models, including zero-order, first-order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas models, by analyzing time-dependent release profiles. Initially, the results were applied to the zero-order kinetic model, and the release constants were calculated (Figure 21). In zero-order release kinetics, the drug is released freely from the carrier, independent of the drug concentration in the medium. Its simplest form is expressed by the following equation:

$$Q = Q_0 + K_0 t$$

Here, **Q** is the amount of drug released or dissolved, and **Q_0** is the initial amount of drug in the medium, which is usually zero. **K_0** represents the zero-order release kinetic constant. When applying the model, the time dependence of the released drug amount is expressed either as a percentage or in milligrams.

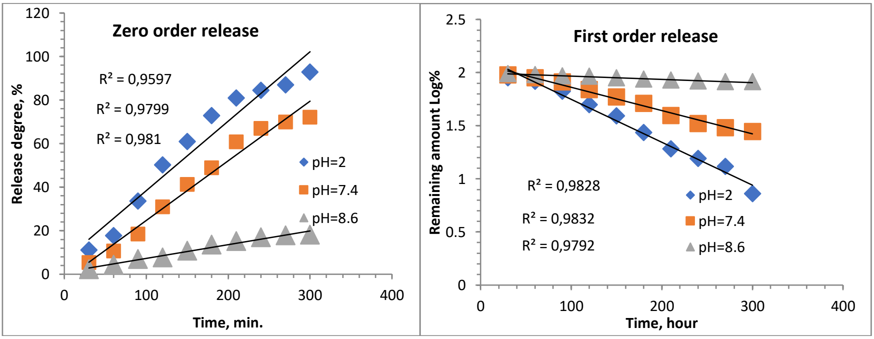

Figure 21. Zero and first-order kinetic dependence of doxorubicin release from mPEG-Ala-Asp gel in different pH medium, T=37 °C.

As shown in the figure, the regression coefficients of the lines in all media are close to unity, and the release rate constants (K_0) for pH 2, 7.4, and 8.6 are 0.319, 0.274, and 0.063, respectively. Based on the results, the high R^2 value (0.981) and the K_0 value indicate that doxorubicin immobilized in the mPEG-Ala-Asp gel exhibits more controlled and stable release at pH 8.6, making this environment and temperature condition favorable for its application.

The results were also evaluated using the first-order kinetic release model, and the rate constants of release were calculated. The first-order kinetic equation describes the dependence of the drug release rate on its concentration. This dependence reflects the concentration gradient between the stagnant liquid layer adjacent to the solid surface and the bulk liquid, which predicts a first-order release behavior. The dependence graph is plotted as the logarithm of the percentage of drug remaining in the matrix versus time (Figure 21). As observed, the R^2 values are closer to unity in acidic and neutral media compared to the zero-order model, confirming the applicability of this release type at pH 2 and 7.4.

The simplified Higuchi model describes drug release from insoluble matrices based on Fickian diffusion and expresses the release dependence on the square root of time (Figure 23).

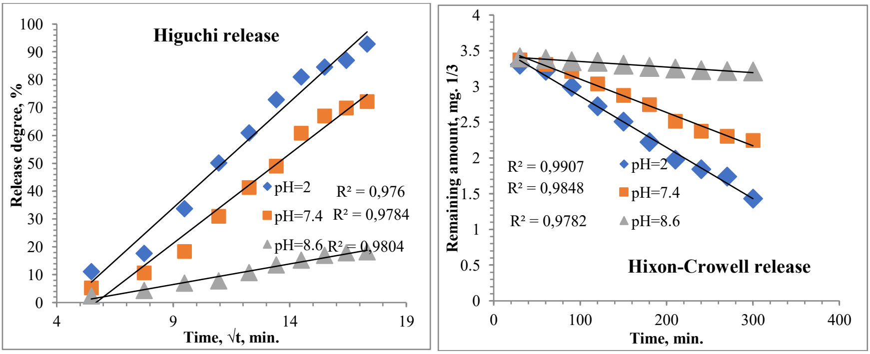

Figure 22. Higuchi and Hixon-Crowell kinetic models for doxorubicin release from mPEG-Ala-Asp gel in various pH medium, T=37 °C, V=10 ml.

It was determined that the regression coefficient in the alkaline medium is relatively close to unity, indicating that this model fits better in this environment. The corresponding values for pH 2, 7.4, and 8.6 are 7.592, 6.418, and 1.479, respectively. The release characterized by a high regression coefficient and a low value of K can be applied in transdermal adhesive or oral drug delivery systems.

The Korsmeyer-Peppas cube root law describes release from systems where changes occur in the surface area or diameter of particles or tablets. Applying the sink conditions, the cube root law can be expressed as follows:

$$Q_t^{\frac{1}{3}} = Q_0^{\frac{1}{3}} - K_{HC}t$$

Q_t is the amount of drug remaining at time t, in mg. Q_0 is the initial mass of the solid phase at $t = 0$, in mg. K_{HC} is the dissolution (release) rate constant, reflecting the surface-to-volume incorporation. Under the specified conditions, the release profile of Dox from mPEG-Ala-Asp gel is shown in Figure 26. According to this model, the release rate is likely limited not by diffusion, but by the dissolution rate of the drug substance. As observed, the kinetic results of Dox release from mPEG-Ala-Asp gel correspond more closely to the Higuchi-Korsmeyer kinetic model in acidic and neutral media. Specifically, in acidic medium, the gel undergoes erosion, leading to simultaneous release of both the matrix and the drug.

Ritger-Peppas, Korsmeyer, and Peppas developed an empirical equation analyzing drug release from both swelling and non-swelling gels, describing both Fickian and non-Fickian release mechanisms:

$$\frac{M_t}{M_\alpha} = Kt^n$$

Here, M_t/M_α represents the fraction of drug released at time t. K is the release rate constant, which reflects the structural and geometrical characteristics of the delivery system. n is the release exponent indicating the mechanism of drug transport through the polymer matrix. To verify the conformity of the results with this model, the logarithm of the percentage of drug released was plotted against the logarithm of time in various media. The results show linearity across all media, with the coefficient of determination R^2 values closer to unity in neutral and alkaline environments.

According to the Ritger-Peppas model, drug release from non-swelling cylindrical matrices follows a non-Fickian mechanism with the release exponent $0.45<n<0.890$. It has been determined that the release of Dox from the mPEG-Ala-Asp gel matrix occurs due to the unfolding or detachment of proteinaceous groups at the terminal chains of the polymer network. Consequently, it can be concluded that performing release studies in alkaline medium allows the use of Dox immobilized mPEG-Ala-Asp gel for the formulation of cylindrical, spherical, or film-type drug delivery systems.

The surface morphology of the sample after immobilization of Dox onto the mPEG-Ala-Asp gel confirms the distinct loading of crystalline Dox particles on the surface (Figure 23).

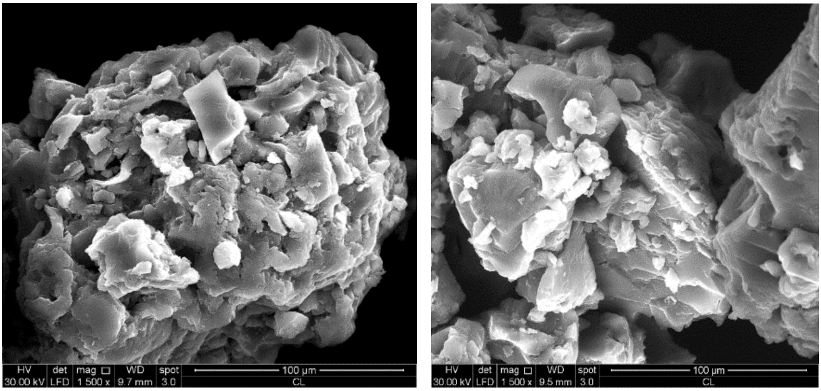

Figure 23. Doxorubicin-immobilized mPEG-Ala-Asp sample`s SEM micro image

After immobilization of drugs, the key parameter is the mechanism of their release from the hydrogel, as this ensures a controlled and long-term release of the drug in the body. In this regard, in the research work, kinetic studies of the release of Dox and trypsin immobilized on a hydrogel obtained from the cross-linking of PVPr with an average molecular weight of 40 kDa from the carrier at pH 2.2 and 7.4 were also conducted (Table 10).

Table 10.

Kinetic interpretation using different models of of Doxorubicine and Trypsine release from the PVPr (10 kDa, 10% MBAA) based hydrogel

Release medium		Zero order $[R^2]$, k_0 (min^{-1})	First order $[R^2]$, k_1 (min^{-1})	Higuichi $[R^2]$, k_H $(min^{-1/2})$	Korsmeyer -Peppas $[R^2]$, k_{KP} (min^{-n})	Hixson-Crovell $[R^2]$, k_{HK} $(min^{-1/3})$
Doxorubicine	**pH=2.2**	0.9658	0.9824	0.9946	0.9958	0.9943
	Des.water	0.9724	0.9946	0.9956	0.9932	0.9983
	pH=7.4	0.9817	0.9712	0.9972	0.9947	0.9981
Trypsine	**pH=2.2**	0.9631	0.9642	0.9831	0.9605	0.9747
	Des.water	0.9587	0.9705	0.9823	0.9647	0.9837
	pH=7.4	0.9613	0.9671	0.9856	0.9586	0.9816

According to kinetic results, 10% (w/w) cross-linked of PVPr based hydrogel can be used in the targeted delivery of antibiotics and protein and as well as the treatment of local infections.

The kinetic data of doxorubicin release from a pH-sensitive gel, obtained by crosslinking low molecular weight PAa and loaded with doxorubicin, at 37 ˚C in a pH 7 medium, were also studied by applying various mathematical models to analyze the release behavior (Figure 24,25).[16]

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[16] Tapdigov Sh.Z. Designing poly-N-vinylpyrrolidone based hydrogel and applied Higuchi, Korsmeyer-Peppas, Hixson-Crowell kinetic models for controlled release of doxorubicine // Chemical Problems, 2020, 17 (2), pp. 207-213.

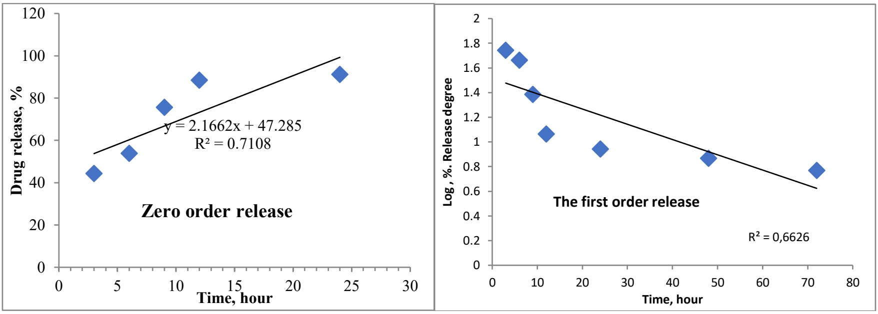

Figure 24. Linear dependencies in Zero- and First-order kinetic models for the release of Dox from the PAa-based hydrogel,T=37 ˚C, V=15 mL.

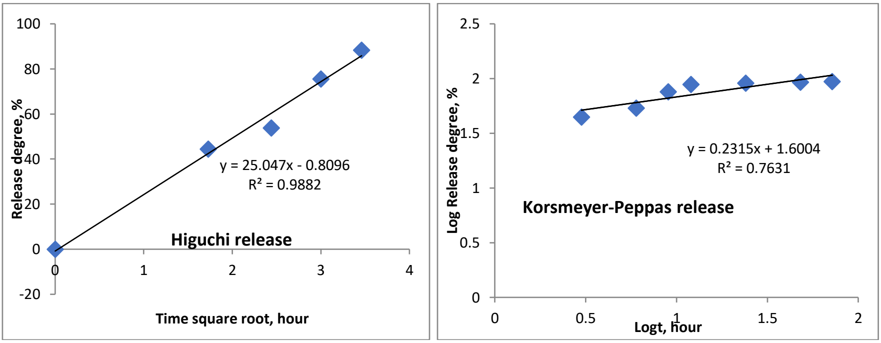

Figure 25. Linear correlations of Dox release from the pH-sensitive PAa-based hydrogel in neutral medium within the Higuchi and Korsmeyer–Peppas kinetic models, T=37 ˚C, V=15 mL.

According to the R^2 values, the highest linearity was observed for the Higuchi release model. By applying the equation $Q = K \cdot t^{1/2}$, the release constant (K) was determined based on the slope of the linear fit, and a value of K = 6.472 was obtained. The relatively high value of K suggests that the developed system may be more suitable for the formulation of orally administered pharmaceutical forms.

In the subsequent study, a novel pH-sensitive hydrogel was synthesized by grafting chitosan with VPr and 4VP and crosslinking with 10% and 14% (w/w) MBAA. The immobilization of doxycycline onto the hydrogel matrix was investigated. Drug loading occurred through hydrogen bonding between the $-OCOCH_3$ and –

COOH groups of doxycycline and the –OH and –NH– groups of the chitosan backbone. Additionally, electrostatic interactions between anionic COO^- groups and cationic NH_3^+ moieties contributed to drug retention. The swelling behavior and drug release kinetics of the hydrogel were studied at 37 °C for both crosslinker concentrations (Figure 26).

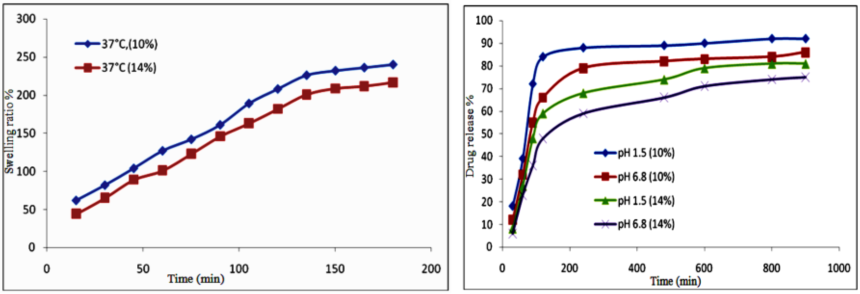

Figure 26. Swelling behavior and Dox release kinetics in acidic and neutral media as a function of temperature for chitosan-grafted copolymer-based hydrogels crosslinked with 10% and 14% MBAA

It was found that increasing the temperature up to 37 °C enhances water uptake, which is attributed to both the acceleration of diffusion in the solution and the increased absorption capacity of the hydrogel. The relatively higher release observed in acidic medium is associated with the greater swelling degree of the hydrogel and the improved solubility of the drug at pH = 1.5.

Kinetic parameters indicate that the initial release corresponds to the desorption of weakly bound drug molecules located near the surface of the hydrogel matrix.[17] In the subsequent stage, the active substance that has diffused into and been immobilized within the internal pores of the hydrogel begins to be released into the surrounding medium.

In the conducted studies, trypsin was immobilized onto a pH-sensitive hydrogel based on chitosan-grafted-PVPr-co-P4VP crosslinked with 10% MBAA, and the conductivity changes of the

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[17] Tapdigov Sh.Z. A Drug-Loaded Gel Based on Graft Radical co-Polymerization of N-Vinylpyrrolidone and 4-Vinylpyridine with Chitosan // Cellulose Chemistry and Technology, 2020, vol.54, Iss. (5-6), pp. 429-438.

hydrogel suspension were investigated within the pH range of 5 to 11. [18]It was found that the conductivity of the hydrogel suspension varied only slightly, indicating minimal influence on the surface dimensions of the hydrogel. The zeta potential of the hydrogel stabilized beyond pH 7, fluctuating around -0.35 mV. The pKa values of PVPr and P4VP lie within the range of 4 to 5.5, implying that the carbonyl group in the pyrrolidone ring and the nitrogen atom in the pyridine ring undergo ionization in the pH range of 5 to 11. When the pH of the medium is below the isoelectric point of trypsin, the enzyme molecule becomes positively charged.

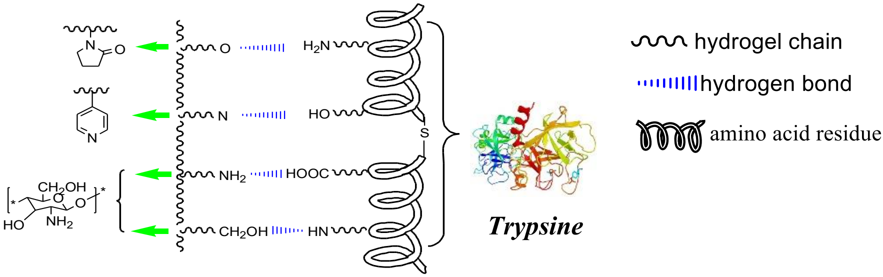

Figure 27. Schematic representation of hydrogen bond-mediated immobilization between chitosan-crosslinked PVPr-so-P4VP-10% MBAA hydrogel and trypsin

Protein loading decreases with the subsequent increase in pH, reaching its maximum activity at pH 7.0–8.0, and then declines. The pH range of 4.0–6.0 activates the functional groups of PVPr and P4VP solutions. It has been determined that in this case, immobilization primarily occurs due to hydrogen bonding (Figure 27) and electrostatic interaction forces. Hydrogen bonding forms between the functional groups in the hydrogel structure — such as –NH_2, –CH_2OH, >C=O, the nitrogen atom in the pyridine ring, –OH, and –NH– and those in the trypsin molecule, including –NH_2, –COOH, –OH, >C=O, –NH–, disulfide bridges, and complex ether

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[18] Tapdigov Sh.Z. Electrostatic and hydrogen bond immobilization trypsine onto pH-sensitive N-vinylpyrrolidone and 4-vinylpyridine co-grafted chitosan hydrogel // Macromolecular Research, 2021, vol. 29, pp. 120-128.

groups. Additionally, at pH 5–8, the functional groups in the gel undergo partial ionization, converting into $-NH_3^+$, $-OH_2^+$, and $>COH^+$ forms. The trypsin molecule similarly ionizes to the same extent, leading to electrostatic interactions with the carrier. It has been shown that Van der Waals forces, interactions between positively and negatively charged particles, and hydrogen bonding occur simultaneously between the carrier and trypsin. Figure 28 illustrates the dependence of immobilized trypsin activity output and protein loading amount on various buffer concentrations and immobilization times. Within the buffer concentration range of 20 to 40 mmol/L, the amount of protein loading increases.

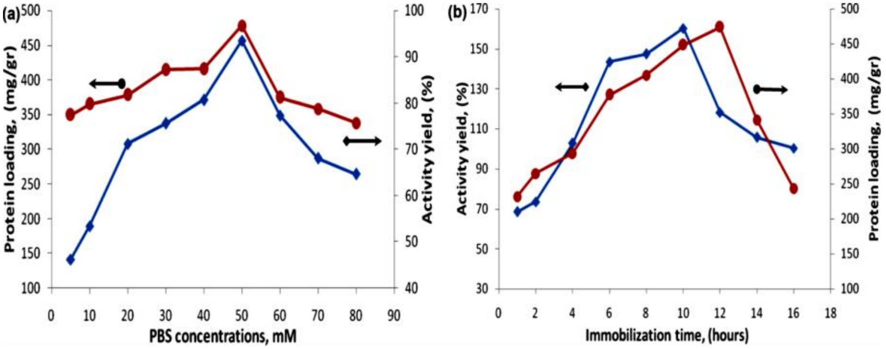

Figure 28. Dependence of trypsin loading amount and activity yield on buffer concentration and immobilization time for chitosan-crosslinked PVPr-so-P4VP-10% MBAA hydrogel

The dependence of trypsin activity yield and loading amount on immobilization time ranging from 1 to 16 hours was investigated at pH 8 and 37 °C. It was determined that trypsin exhibits high activity after 11–12 hours of immobilization, after which its activity begins to decrease with further extension of the immobilization time. On the other hand, as the immobilization time increases, multiple interactions—including hydrophobic and electrostatic interactions, Van der Waals forces, and hydrogen bonds—occur between the enzyme and the chitosan-crosslinked PVPr-so-P4VP-10% MBAA hydrogel, which alter the globular structure of the protein and inactivate its biological active sites. The initial activity of immobilized trypsin was studied by incubation at 37 °C for 210 minutes (Figure 29).

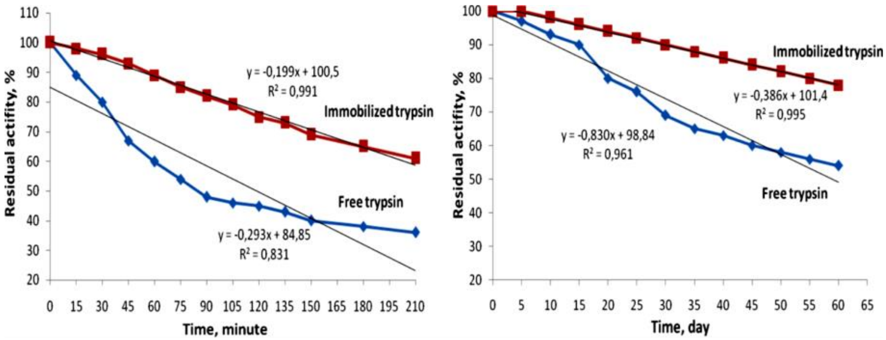

Figure 29. Thermal stability of free and immobilized trypsin at 37±3 °C for 210 minutes and at 4 °C for 60 days

It has been showed that immobilized trypsin retains 91% of its activity after 60 minutes of incubation, whereas free trypsin retains only 55–60%. After 210 minutes, immobilized trypsin exhibits 70% residual activity. The increased stability is attributed to multiple fixation interactions between trypsin and the chitosan-crosslinked PVPr-so-P4VP-10% hydrogel, which restrict the conformational mobility of the enzyme. The long-term stability of both free and immobilized trypsin at 4 °C was also investigated. It was found that immobilized trypsin maintains 85% of its activity over 60 days, compared to less than 55% for free trypsin. Based on the results, trypsin bound to the chitosan-crosslinked PVPr-so-P4VP-10% hydrogel through hydrogen bonding and electrostatic interactions retains its hydrolytic activity even after 30 days at 4 °C.[18,19] The thermal stability of free and immobilized trypsin was investigated over a temperature range of 20 °C to 80 °C. Free trypsin maintains its activity up to 38 °C (Figure 30), however, its activity sharply decreases above 40 °C. Enzymes generally undergo denaturation at

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[19] Tapdiqov, Sh.Z., Taghiyev, D.B.. Study of doxorubıcın release from the polyacrylıc acıd-based hydrogel apply some kınetıc equatıons // II International Multidisciplinary Ecology and Environmental Studies Congress, - Paris: - Feb.5-9, -2025, -p.19.

temperatures exceeding 60 °C, whereas electrostatic interactions and hydrogen bonding contribute to resistance against thermal denaturation.

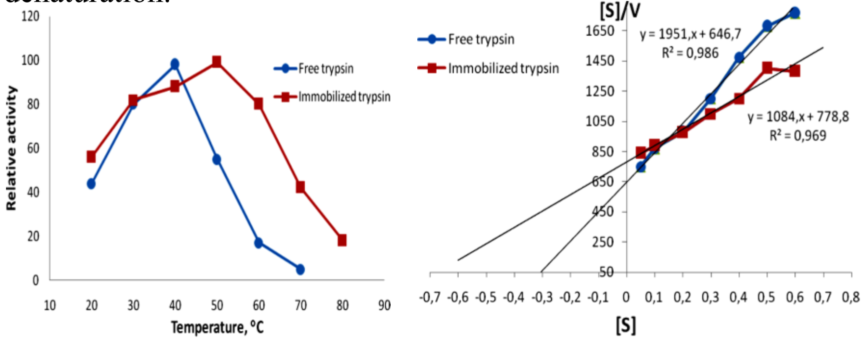

Figure 30. Effect of temperature on the catalytic activity of free and chitosan-crosslinked PVPr-so-P4VP-10% MBAA hydrogel-immobilized trypsin, and Hanes–Woolf plots for trypsin. The activity (F.I.P.u mg^{-1}), activity yield, Km (mM), and V_{max} ($\mu M \cdot s^{-1}$) values for free trypsin were found to be 10,000; 100%; 0.34; and 0.51, respectively.

Kinetic parameters, V_{max} and Km, for free and chitosan-crosslinked PVPr-so-P4VP-10% MBAA hydrogel-immobilized trypsin were calculated from the Hanes-Woolf plot (Figure 30). The increase in Km value indicates that immobilization alters the molecular conformational flexibility of trypsin and increases diffusion limitations. According to kinetic studies, trypsin immobilization primarily occurs through Van der Waals forces. Based on SEM analyses of the surface structures of chitosan as the starting material and the trypsin-immobilized samples as the final product, it was confirmed that protein macromolecule loading occurs (Figure 31). Based on the SEM micrographs, the increased surface smoothness and the formation of irregular wavy zones on the surface after immobilization indicate that the carrier's surface undergoes some form of transformation.

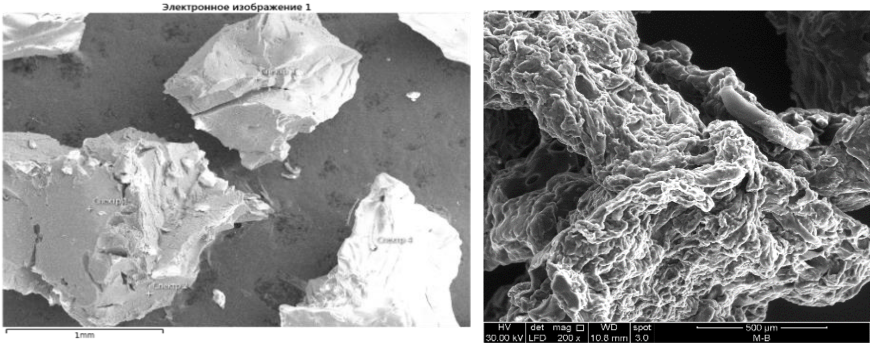

Figure 31. SEM micrographs of chitosan (left) and trypsin-immobilized chitosan-crosslinked PVPr-so-P4VP-10% MBAA hydrogel (right)

The protein molecule, possessing a helical and twisted structure, penetrates and embeds into the gel surface, restricting its mobility, which leads to changes in the surface morphology of the carrier.

During the study, a gel was synthesized by modifying chitosan with the N-benzyl N-methyl derivative, and the immobilization capacity for trypsin was evaluated. It was found that the increased hydrophilicity of the chitosan macromolecule resulted in relatively enhanced immobilization capability of the carrier.

THE MAIN RESULTS

1. Nanostructured hydrogels sensitive to pH were synthesized based on copolymers of poly-N-vinylpyrrolidone, polyacrylic acid, as well as arabinogalactan, chitosan, and gum arabic vinyl monomers (N-vinylpyrrolidone and 4-vinylpyridine), which swelling in water. The swelling kinetics of the obtained hydrogels in water and buffer systems were studied, and their suitability as carriers for targeted delivery of enzymes and antibiotics over a wide pH range was demonstrated **[1,4-6,15]**.

2. Trypsin and doxorubicin were immobilized onto nanostructured hydrogels based on arabinogalactan and its poly-N-vinylpyrrolidone copolymers. It was shown that the release of immobilized enzyme and antibiotic into solution occurs over a long period while retaining their biological activity. It was established that the interaction between the arabinogalactan-poly-N-vinylpyrrolidone chain and trypsin and doxorubicin is mainly due to hydrogen bonding and electrostatic forces **[3,7,9,13]**.

3. Metal-gel complexes of poly-N-vinylpyrrolidone with Cu(II), Co(II), and Ni(II) ions were synthesized, with metal ion load capacities of 3.3–3.6 mg/g as pre-designed. Immobilization of trypsin and doxorubicin on the obtained polymer-metal complexes was carried out and their targeted delivery was studied. It was observed that the metal gel complexes increased the trypsin loading capacity by 7–8 times, immobilization degree by 2 times, and specific enzymatic activity from 38 to 92 ED/mg **[7,15,17]**.

4. New nanostructured memory hydrogels based on polyacrylic acid and poly-N-vinylpyrrolidone were synthesized for doxorubicin immobilization and their release characteristics were compared with a system containing silver nanoparticles. Drug release from hydrogels was tested at various pH values, as well as in 0.9% NaCl and 0.1–10% glucose solutions. It was shown that by regulating ionic strength and non-electrolyte concentration in the medium, long-term release of the active substance can be controlled **[2,27]**.

5. The release mechanism of doxorubicin and trypsin from poly-N-vinylpyrrolidone and polyacrylic acid-based gels was studied using mathematical kinetic models including zero-order, first-order, Higuchi square root law, Korsmeyer-Peppas, and Hixson-Crowell models. According to kinetic parameters, doxorubicin release from poly-N-vinylpyrrolidone-based hydrogel fits all kinetic models ($R^2 > 0.96$), with the best fit observed for the Higuchi square root and Korsmeyer-Peppas models. The release of trypsin from the gel fits only the Higuchi square root model **[12,24,26]**.

6. Doxycycline was immobilized on a new pH-sensitive hydrogel synthesized by crosslinking chitosan copolymers with N-vinylpyrrolidone and 4-vinylpyridine at 10% and 14% weight crosslinker amounts. It was shown that doxycycline immobilization occurs via hydrogen bonding, and its release follows a non-Fickian mechanism. Most of the release occurs in an initial burst phase **[10,11,13]**.

7. Trypsin was immobilized on a new pH-sensitive gel based on chitosan copolymers with poly-N-vinylpyrrolidone and poly-4-vinylpyridine. The activity of immobilized trypsin was optimized over pH 5.0–11.0, trypsin concentrations of 0.5–7 mg/ml, and buffer concentrations of 5–80 mmol/l over 1–16 hours. Immobilized trypsin retained 85% activity over 60 days **[13,23]**.

8. Adsorption of trypsin from aqueous solution on pH-sensitive magnetite Fe_3O_4 nanoparticle-containing hydrogels based on N-vinylpyrrolidone and 4-vinylpyridine was studied. Kinetic data of trypsin adsorption were analyzed using Langmuir, Freundlich, Temkin, and Dubinin-Radushkevich isotherm models, with maximum monolayer protein capacity determined as 71–72 mg/g at 45 °C. Both hydrogels exhibited positive entropy change (ΔS°), equal to 83.39 and 116.41 $J \cdot mol^{-1} \cdot K^{-1}$ respectively. These results suggest PVPr-co-P4VP and PVPr-co-P4VP/Fe_3O_4 hydrogels can serve as matrices for protein and drug delivery with prolonged effect **[20,28]**.

9. Immobilization of trypsin and doxorubicin on synthesized natural and synthetic-based hydrogels with memory effect was investigated. It was found that doxorubicin immobilizes at 33.5% with a capacity of 67 mg/g on chitosan-based gels, while after imprinting, immobilization degree increases to 49% and capacity to 97 mg/g. The presence of metal ions during memory system formation decreases immobilization degree and capacity **[17,25]**.

10. Loading and characterization of doxycycline and doxorubicin drugs into a new temperature-sensitive hydrogel based on PEG-polypeptide were carried out. Release of doxycycline was monitored in PBS with zero and varying ionic strengths and in 0.15 M NaCl, showing higher release (90–95%) in PBS medium **[8,14,21]**.

11. New synthesis of poly-ε-caprolactone by microwave irradiation in the presence of organic acids was performed, and doxorubicin was immobilized by a double emulsion method. Drug release from the carrier was studied at pH 4.8 characteristic of cancer cells and physiological pH 7.4 of healthy cells. Release of doxorubicin from nanocapsules at pH 4.8 follows the Higuchi mechanism, while at pH 7.4 it fits the Hixson-Crowell kinetic model. The results suggest that poly-ε-caprolactone-based matrices can be used to prepare cylindrical pharmaceutical dosage forms **[16,18,19,22]**.

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