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Novel Schiff Base Hydrogel Based on Crosslinked between Pectin Aldehyde and Amine-Terminated Cyclotriphosphazene for Drug Delivery

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Abstract

Drug delivery systems became of great importance in modern medicine, enhancing the therapeutic efficacy of pharmaceutical compounds while minimizing their side effects. Derived from Schiff base crosslinking, hydrogels exhibit tunable properties, biocompatibility, and controlled drug release capability. In this present study, the new paracetamol substituted cyclotriphosphazene compounds (**MSPCT** and **MSHY**) were designed and synthesized. Moreover, the controlled drug release profile of the hydrogel synthesized with **MSHY** and pectin aldehyde (**PECALD**) was investigated. **PECALD** was reacted with the novel diamine terminated cyclotriphosphazene crosslinker (**MSHY**) via a Schiff-base reaction that yielded crosslinked hydrogel. Single crystal X-ray crystallography (for **MSHY**), FT-IR, NMR (^{31}P , and ^1H), spectroscopies, SEM-EDX analysis (for **MSHY/PECALD**) and MALDI-TOF mass analysis were used to comprehensively characterize the structures of the novel compounds. The synthesized hydrogel exhibited favorable cytocompatibility toward BJ fibroblast cells and showed pH-dependent degradation behavior. Together with the controlled release results, these findings indicate the potential of the developed hydrogel system for drug delivery applications.

Keywords: Drug delivery; Phosphazene; Cyclotriphosphazene; Schiff base; Paracetamol; X-ray.

1. Introduction

Drug delivery is the method for the administration of pharmaceuticals with the aim of enhancing the efficacy and safety of therapeutic treatments. Therefore, it became an important area of interest in pharmaceutical science [1–3]. Among many materials used in drug delivery, Schiff base crosslinked polymers gained the interest of scientists [4, 5] due to their variable properties and biocompatibilities as promising candidates for drug delivery [6]. Hydrogels are a fascinating class of materials that can swell with water provided by their hydrophilic polymer networks [7, 8]. They are ideal materials for multiple applications in biomedical science, such as, tissue engineering, medication delivery methods, and treatment of wounds due to their exceptional physicochemical properties, softness and elasticity of their structure, nontoxic nature, controllable degradation, and providing high permeability for small molecules [9–11]. Cyclotriphosphazene compounds, which are the most popular among the phosphazene group, serve as basic cross-linkers in the system [12]. Pectin can be chemically modified via aldehyde functionalization, which makes it a perfect option for generating Schiff bases with amines, like phosphazene compounds [4].

Biocompatibility is an additional important factor that lowers the possibility of toxicity and immunogenic reactions [13]. Recent advances in polymer chemistry have facilitated the design of biodegradable, mechanically robust, and biocompatible hydrogels capable of delivering complex therapeutic agents, including proteins, peptides, and nucleic acids. Moreover, increasing attention is being paid to targeted delivery, especially for challenging sites such as brain tumors [14]. Nevertheless, issues remain regarding large-scale production, structural stability, and clinical translation [15].

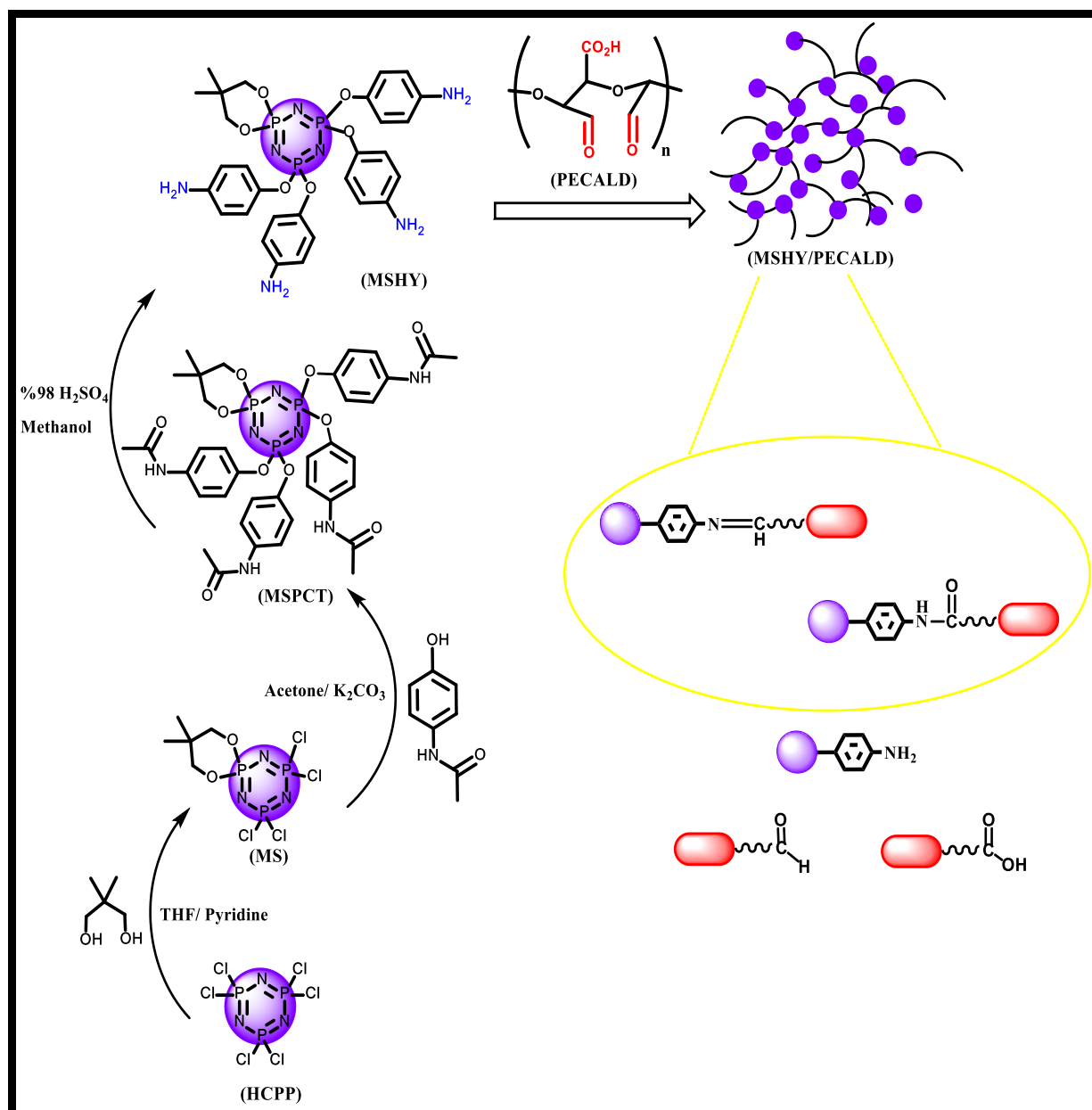
Pectin is a component of polysaccharides and exists in the plants' cell walls [16] and has many benefits when it comes to the delivery of pharmaceuticals [17]. Pectin is a natural polysaccharide widely recognized for its biocompatibility and biodegradability, making it an ideal candidate for biomedical applications [16]. Its gelling qualities [18], low toxicity, and biocompatibility [19] make it an excellent matrix for drug encapsulation. Pectin undergoes an oxidation reaction to form pectin aldehyde, and this provides manufacturing various pectin-based materials [19, 20]. When oxidized to introduce aldehyde groups, pectin can form dynamic covalent imine bonds (Schiff bases), which are essential for the development of injectable, self-healing, and pH-sensitive hydrogels [20]. Schiff's base reaction was frequently used to create conjugations between amino compounds such as adriamycin [21], chitosan [22], silk fibroin [23], and oxidized pectin. These hydrogels have demonstrated promising results in terms of

cytocompatibility and sustained drug release, particularly in cancer therapy contexts [24]. Cyclotriphosphazene derivatives have been used to tailor drug release capability in responsive drug delivery systems, due to their versatility in chemical modification [25]. By integrating pectin aldehyde with cyclotriphosphazene, the resulting hydrogels can demonstrate enhanced mechanical strength and drug release modulation that surpass those of traditional hydrogel systems.

Hexachlorocyclotriphosphazene is a six-membered ring that can be utilized to achieve altering its side groups to produce new chemical and physical characteristics [26-29]. The special rubbers [30], the flame retardants [31,32] LED (light-emitting diodes) [33, 34], probes for fluorescence [35], anti-cancer agents [36] and antibacterial therapies [37, 38] are some of the new uses for derivatives of cyclotriphosphazene. Phosphazene-based polymers also show high thermal stability and are resistant to a variety of environmental conditions, preserving the drug delivery system's integrity [39]. Aldehyde groups of pectin react with amine terminated of phosphazene and generate covalent Schiff base linkages. Hydrogels formed via the Schiff base reaction using phosphazene are highly stable and porous, suitable for controlled release and drug encapsulation [40]. They can be programmed to release pharmaceuticals as a response to certain stimuli such as pH [40, 41], temperature [42] or enzymes [43], resulting in regulated and controlled drug release. This precise control over drug release is essential for delivering focused therapy with fewer side effects. Furthermore, the crosslinked polymers can encapsulate a rich diversity of medicine forms, like small molecules, nucleic acids, and proteins, expanding their application in the pharmaceutical area [44]. These polymers may become increasingly important in improving medication delivery systems and patient outcomes as the field's research progresses. [45].

In this study, the novel hydrogel with amine terminated cyclotriphosphazene (**MSHY**) and pectin aldehyde was synthesized to investigate their characteristics. Several studies are reported in the literature on pectin aldehyde, yet there is not any published study on hydrogels consisting of pectin aldehyde and crosslinking using a phosphazene derivative agent with two active molecular groups that provide the creation of bridges in polymer networks. Using an acid catalyst to hydrolyze the resulting paracetamol compound, the novel diamine terminated phosphazane (**MSHY**) was successfully synthesized and defined by single crystal X-ray crystallography. All of the compounds (Scheme 1) were identified by NMR (^1H , ^{31}P), FT-IR, and MALDI-TOF MS spectroscopies. The **MSHY/PECALD** hydrogel was prepared via the Schiff-base reaction. To determine the structural properties of **MSHY/PECALD** hydrogel,

SEM-EDX and FT-IR spectroscopy were employed as analytical tools. The impact of different synthesis component quantities on swelling ratio and yield was optimized and assessed. As a model drug molecule, paracetamol was used to investigate the potential of the newly synthesized hydrogel as a controlled mechanism for delivering drugs. The use of pectin aldehyde and amine terminated cyclophosphazene in the synthesis of a Schiff base crosslink hydrogel offers considerable promise for medication delivery.



Scheme 1. Synthesis Pathway of MS, MSPCT, MSHY of Cyclotriphosphazene and MSHY/PECALD Hydrogel.

2. Results and discussion

2.1. Synthesis and characterization of MS, MSPCT, MSHY, and MSHY/PECALD

According to the literature, the synthesis of the mono-spiro cyclotriphosphazene derivative (**MS**) bearing a 2,2-dimethyl-1,3-propanedioxy substitution was achieved via the reaction between hexachlorocyclotriphosphazene ($\text{N}_3\text{P}_3\text{Cl}_6$) (**HCPP**) and 2,2-dimethyl-1,3-propanediol [46, 47]. The new molecular structure **MSPCT** was obtained by the reaction compound **MS** and paracetamol in tetrahydrofuran under reflux in the presence of K_2CO_3 . Acid-catalyzed hydrolysis of **MSPCT** was then carried out to successfully synthesize the amino-functionalized monomer (**MSHY**) (**Scheme 1**). The reaction mixtures were monitored by NMR spectroscopy and thin layer chromatography analysis. Single crystal X-ray crystallography, MALDI-TOF (^{31}P , ^1H) NMR, and FT-IR spectroscopies were used to purify and characterize each of the final compounds (**MSPCT** and **MSHY**). Shown in Figure 1b is the ^{31}P NMR spectrum of **MSHY**. The proton-decoupled ^{31}P NMR spectrum of **MSHY** displayed an AB_2 spin system with two signals: a triplet at $\delta = 10.1$ ppm corresponding to the PR_2 groups ($\text{R} = \text{paracetamol}$) and a doublet at $\delta = 13.8$ ppm for the PN spiro group (**Figure 1b**). According to MALDI-TOF MS measurements, the molecular ion peaks (m/z $[\text{M}]^+$) of molecules **MSPCT** and **MSHY** were 836.85 and 670.10, respectively (**Figure 2**), all observations are consistent with the suggested structures. The ^1H NMR spectra showed aliphatic proton signals from the paracetamol NCH_3 and the 2,2-dimethyl-1,3-propanedioxy group in the range $\delta = 4.0\text{--}1.1$ ppm, while paracetamol's aromatic protons were observed between $\delta = 7.5\text{--}6.0$ ppm, as reported in scientific sources (**Figure 3**) [48]. The FT-IR analysis of **MSHY** revealed characteristic aromatic C–H stretching bands at $3135\text{--}3050\text{ cm}^{-1}$, N–H stretching vibrations at $3473\text{--}3373\text{ cm}^{-1}$, and a C=O stretching band at 1666 cm^{-1} . In contrast, the secondary N–H bending band at 1608 cm^{-1} , observed in **MSPCT**, was absent in **MSHY**. Stretching vibrations of P=N bonds were detected between $1176\text{--}1050\text{ cm}^{-1}$, while P–O–C stretching bands appeared within the range of $927\text{--}968\text{ cm}^{-1}$ (**Figure 4**).

The peaks observed at 3366 and 2882 cm^{-1} are the characteristic O–H and C–H stretching vibration peaks and the peaks at 1688 cm^{-1} and 1602 cm^{-1} depict the carboxymethyl ($-\text{COOCH}_3$) and C=O groups of pectin. the C–O stretch of the secondary alcohol groups are presented with the peak at 1013 cm^{-1} [49]. The FT-IR spectrum of **PECALD** shows that oxidation of pectin yielding **PECALD** increased the intensity of peaks.

The FT-IR spectrum of the crosslinked **MSHY/PECALD** hydrogel has strong absorption bands at 1617 and 1736 cm^{-1} are related to the band of --HC=N-- and --C=O stretching that indicates the crosslink between **PECALD** and **MSHY**. The broad band from 3100 to 3600 cm^{-1} depicts the presence of --OH and --NH_2 groups in hydrogel. The successful synthesis of **MSHY/PECALD** hydrogel via a Schiff-base reaction between **PECALD** and **MSHY** is proven by the FTIR spectrum [12].

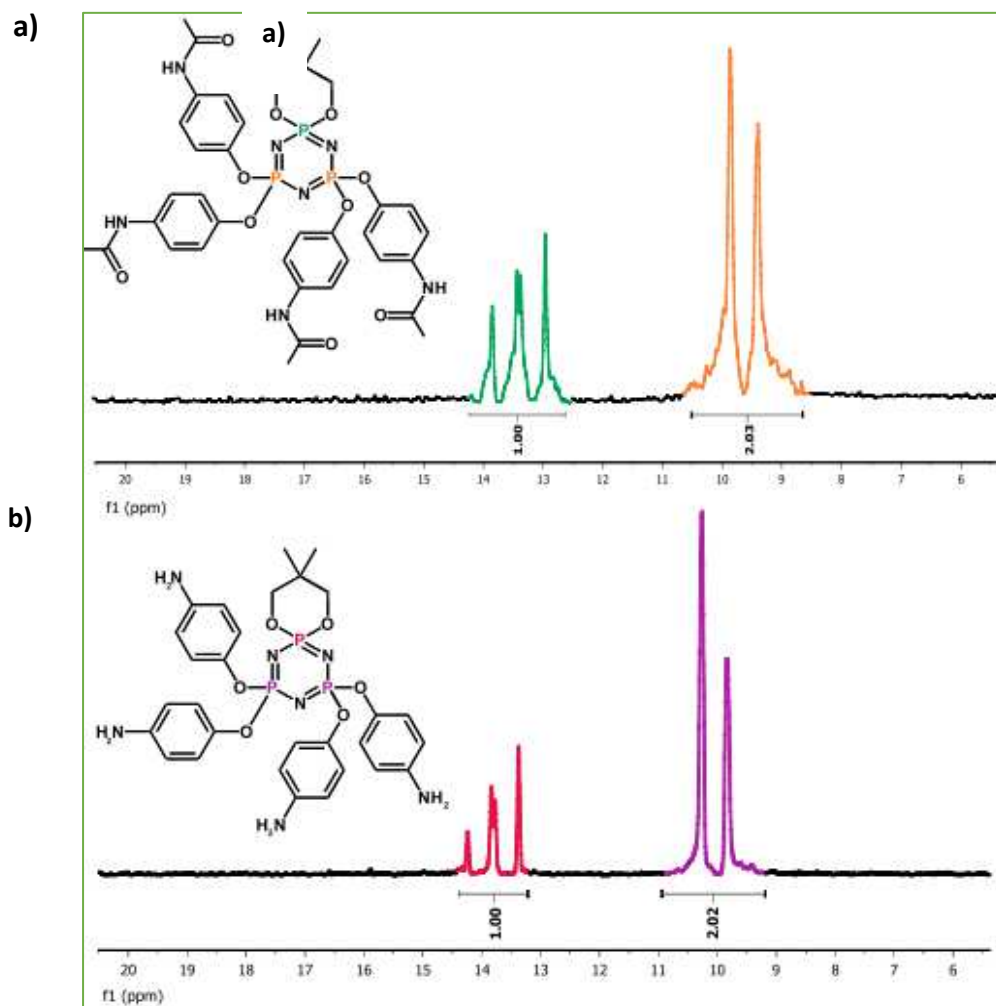


Figure 1. Proton-decoupled ^{31}P NMR spectra in CDCl_3 of compounds [a] **MSPCT** [b] **MSHY**.

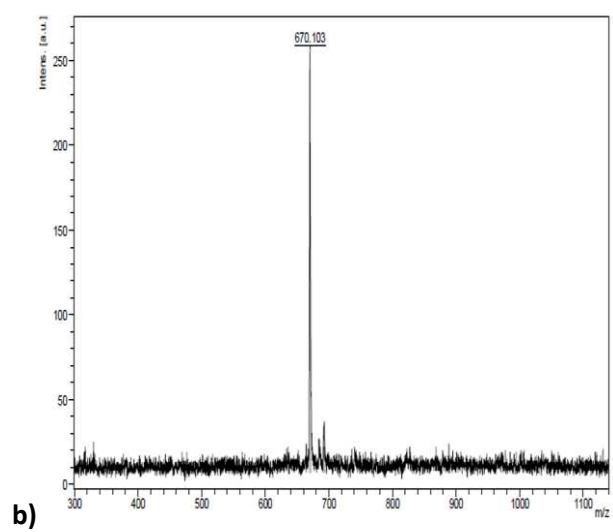
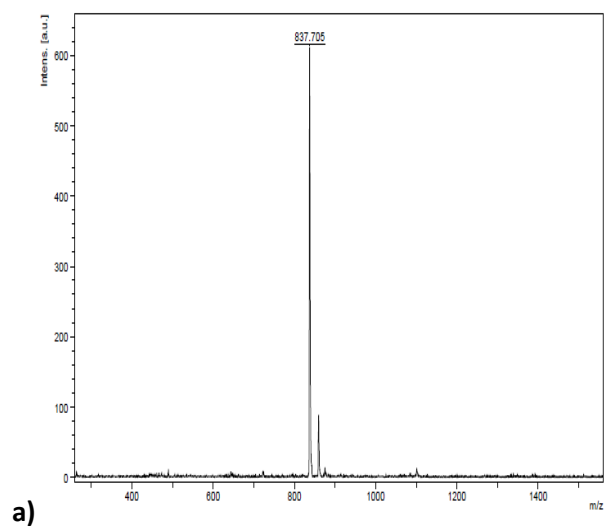


Figure 2. MALDI-MS spectra of compounds [a] MSPCT and [b] MSHY

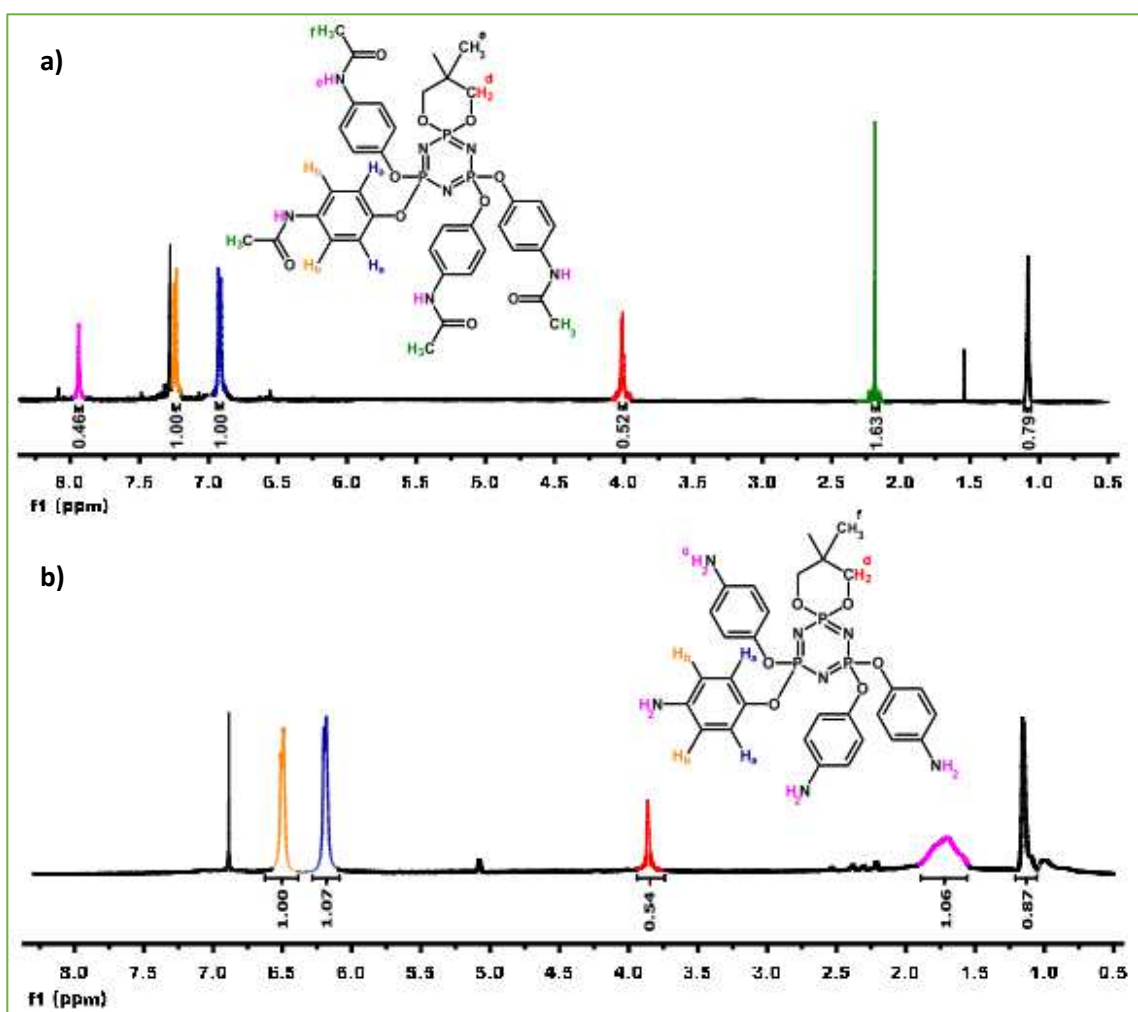


Figure 3. ^1H NMR spectra in CDCl_3 of compounds [a] MSPCT [b] MSHY.

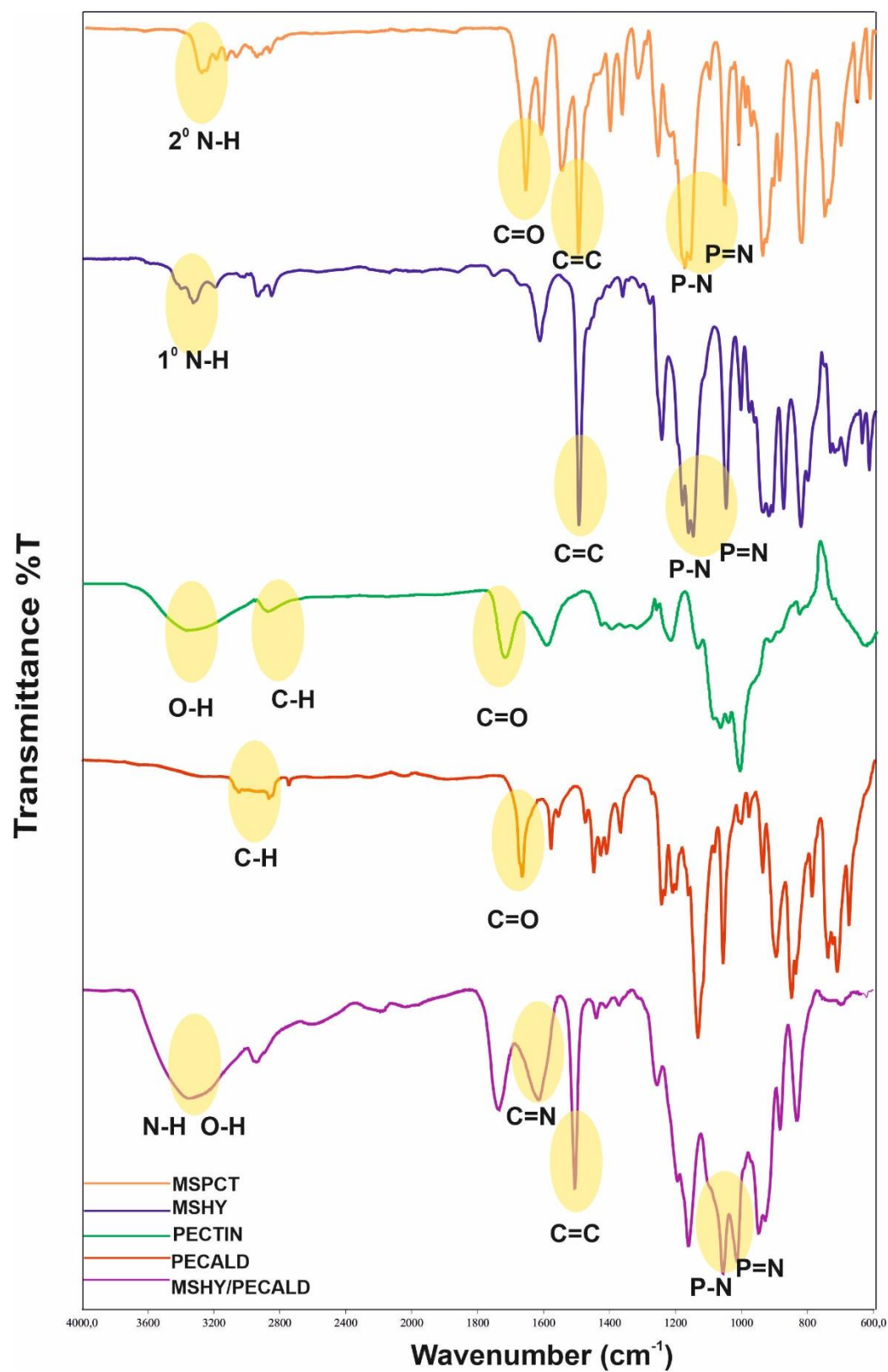
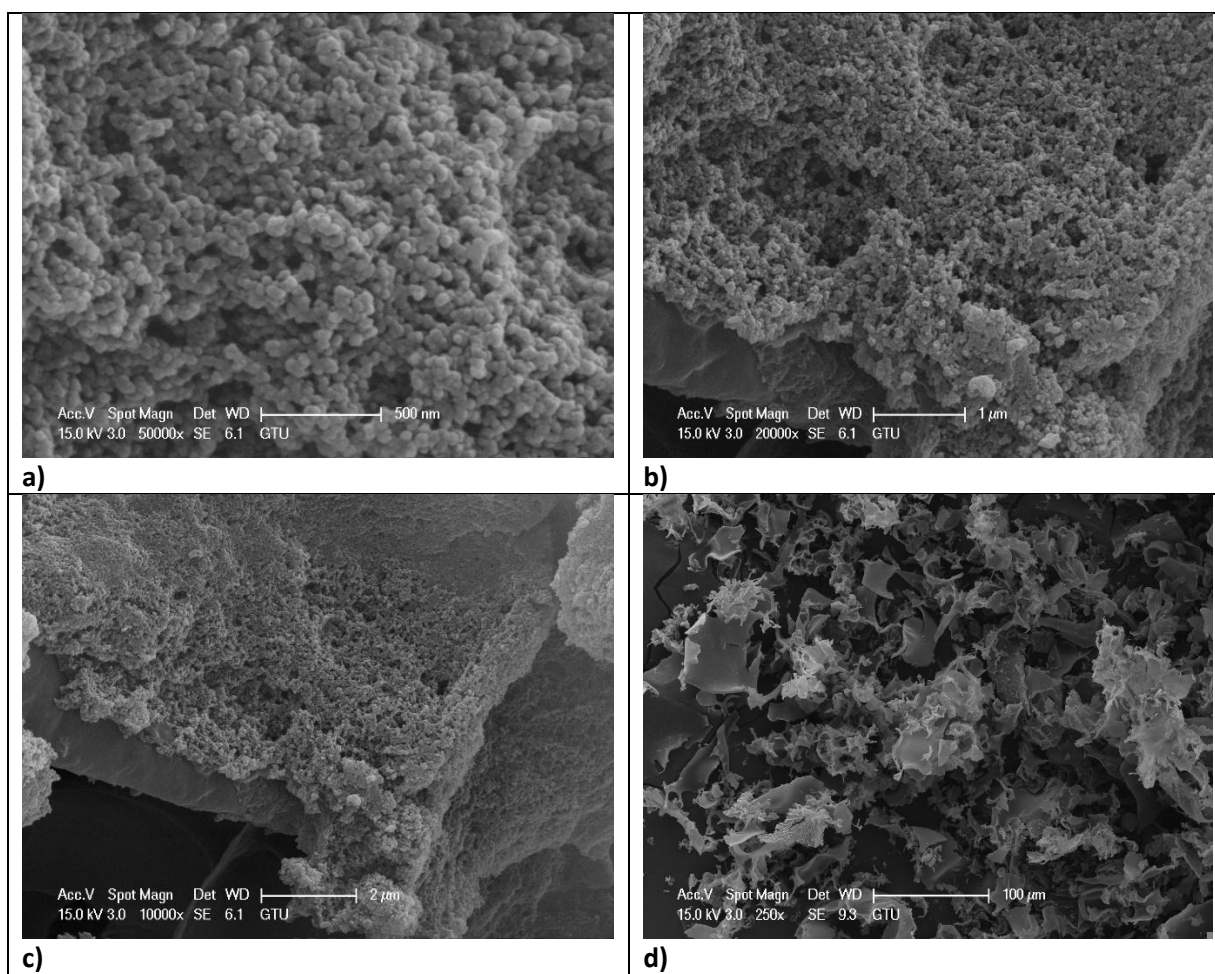


Figure 4. FT-IR spectra of compounds **MSPCT**, **MSHY**, **PECTIN**, **PECALD** and **MSHY/PECALD**.



e)

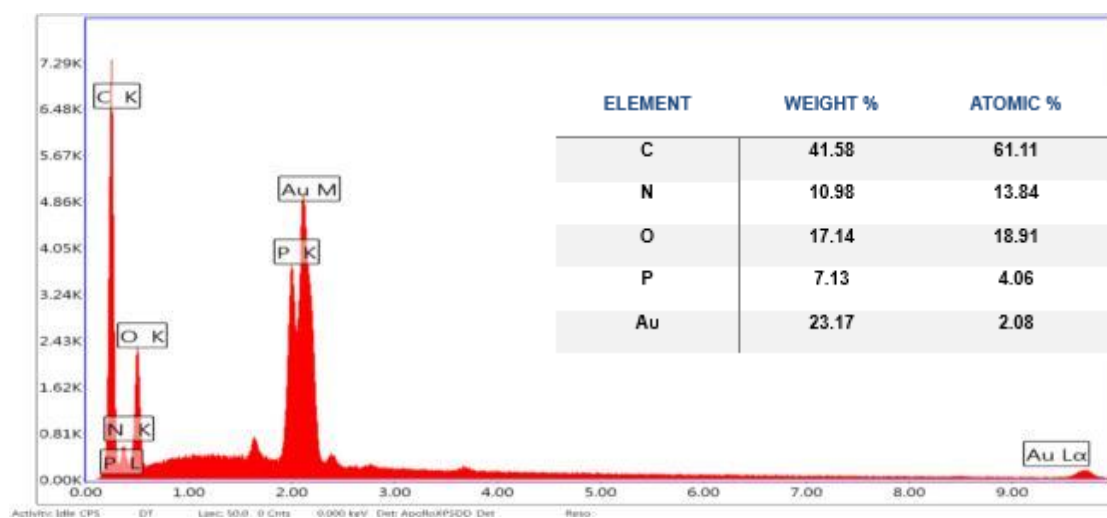


Figure 5. SEM images of hydrogel (MSHY/PECALD) a) 50000x, b) 20000x, c) 10000x, d) 250x and e) EDX spectrum of hydrogel (MSHY/PECALD).

The **MSHY/PECALD** morphology was observed by SEM technique (**Figure 5a-d**). **PECALD** are evenly dispersed throughout the polymer to create a crosslinked 3D network, as seen in **Figure 5**. As one can see from the SEM images, the hydrogel has a porous structure, and the pores are homogenously distributed over the hydrogel. This porous structure is in good agreement with swelling studies. The unique three-dimensional network structure of hydrogels may provide them to be suitable material to be used as drug delivery systems. The EDX maps showed intense signals for N, P, and O. Observation of the existence of P on the whole hydrogel surface can be interpreted as the confirmation of the crosslinking between MSHY and PECALD once more [12].

In vitro swelling studies

The swelling behavior of the **MSHY/PECALD** hydrogel at different pH values was investigated. The swelling percentages of **MSHY/PECALD** hydrogel were found to be 540% for pH 7.4, whereas that of pH 1.2 was found to be 650% (**Figure 6**). The hydrogel showed superabsorbent properties in both pH values.

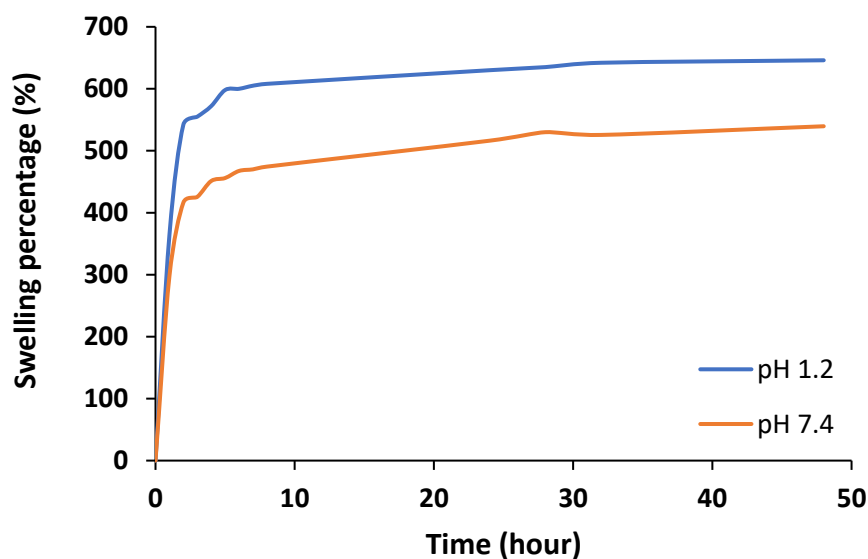


Figure 6. Swelling percentages of **MSHY/PECALD** hydrogel at different pH values.

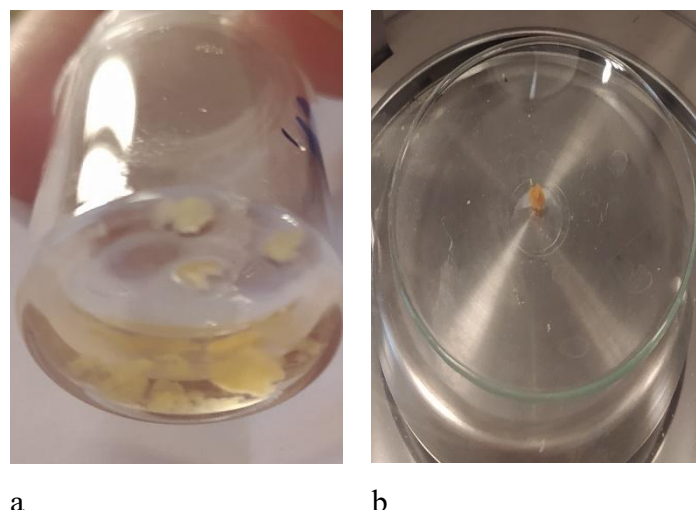
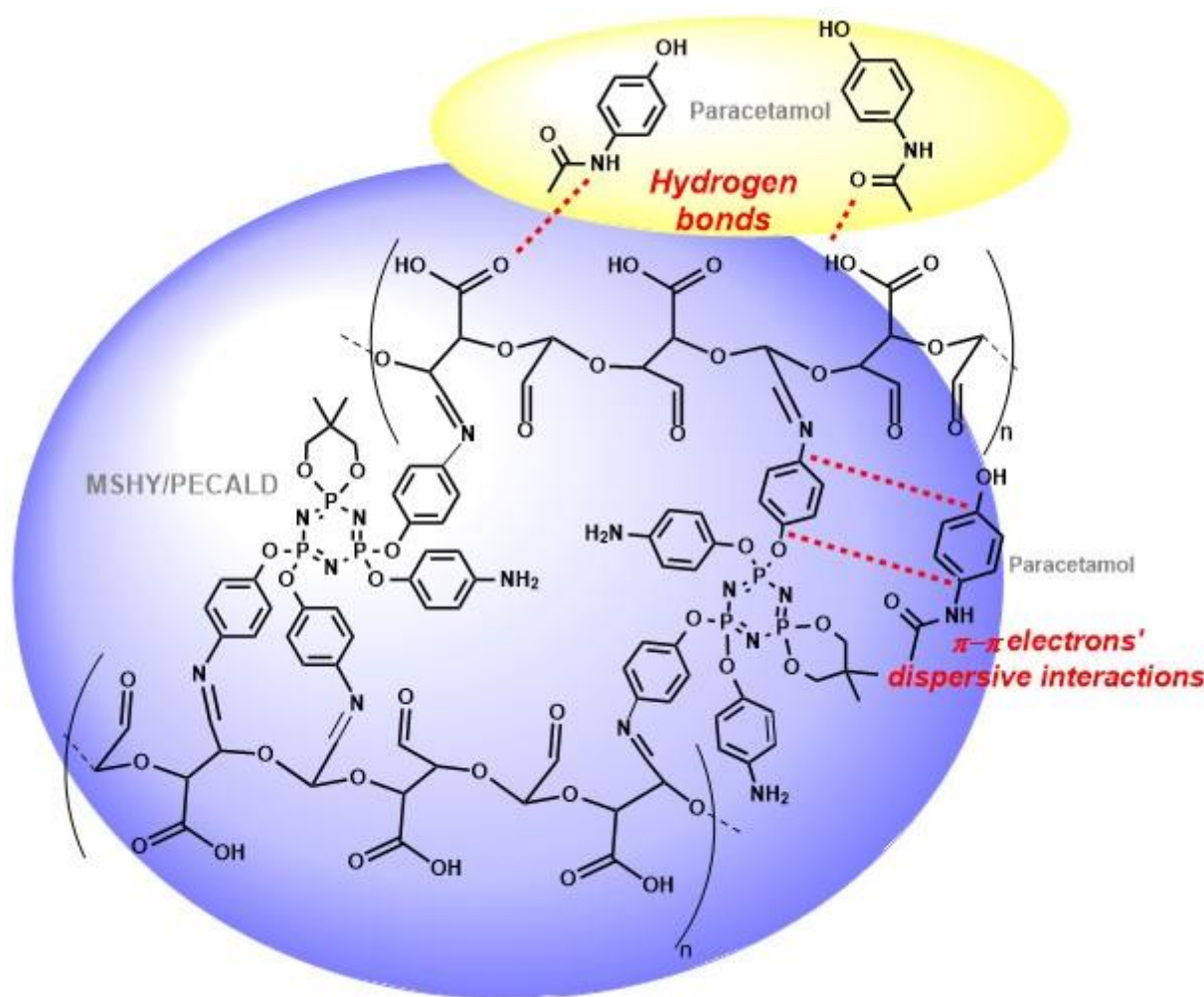


Figure 7. Images of swollen (a) and freeze dried (b) MSHY/PECALD hydrogel

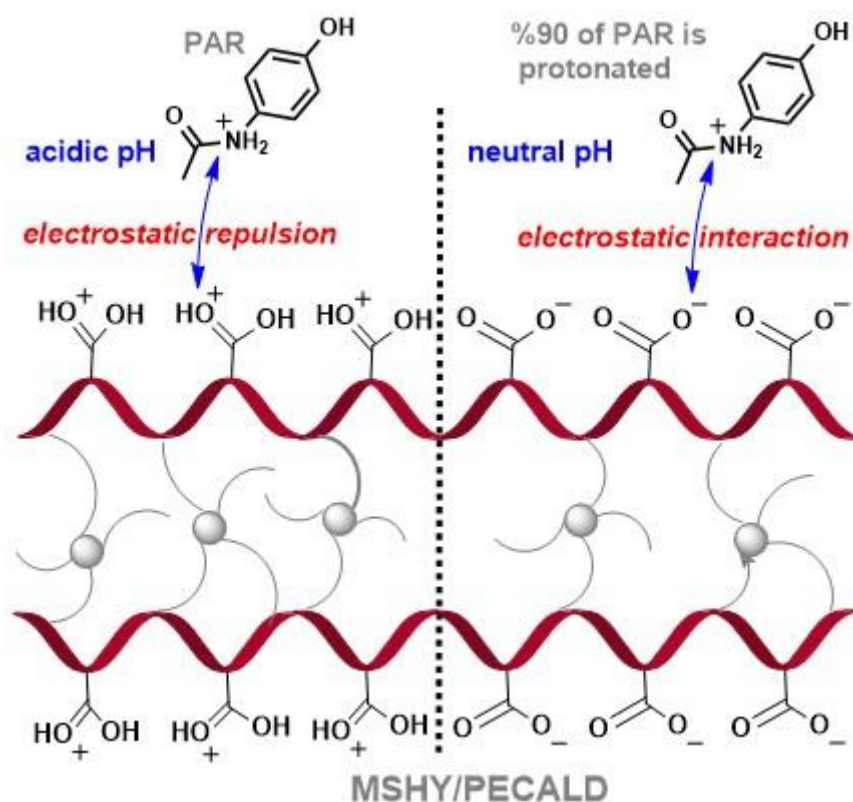
In vitro drug release studies

pH-controlled release of model drug **PAR** from **MSHY/PECALD** hydrogel (**Figure 7**) was investigated in both pH: 1.2 (gastric fluid) and pH: 7.4 (PBS saline). The amount of released **PAR** was determined by measuring sample solutions taken at different times with a UV-Vis spectrometer, and the results were presented as cumulative release given as a percentage of the overall quantity of PAR loaded into the hydrogels. As can be seen in **Figure 8**, the pH controlled **PAR** release was mainly observed in a strong acidic medium, which is provided as a medium that mimics gastric fluid. When this result was compared with the cumulative percentage release result at pH 7.4, it was seen that the cumulative percentage release of **PAR** in an acidic medium was 7 times higher than that at pH 7.4. The possible interactions between the MSHY/PECALD hydrogel and PAR can be summarized as follows: i) The carbonyl groups, displayed on the surface of the **MSHY/PECALD** hydrogel, are in interaction with the aromatic ring of paracetamol [50], as a result of this interaction, adsorption of the drug molecule on the hydrogel is increased. ii) π - π interaction between aromatic structures of paracetamol and the MSHY/PECALD hydrogel occurs. iii) Hydrogen bonds between the hydrogel and PAR (Scheme 2). iv) Electrostatic interactions at different pH values (Scheme 3). While under the influence of these interactions, the release of paracetamol at acidic pH is not surprising. Paracetamol's pKa value (9.38) and its pH dependent conjugated forms mean that paracetamol exists only as its protonated form in an acidic medium [51]. In addition, functional groups of **MSHY/PECALD** hydrogel are also protonated at acidic pH, and due to the electrostatic interactions, paracetamol is released from the hydrogel matrix. At pH 7.4 almost 90% of paracetamol is still protonated and carboxylic acid groups of the hydrogel are in carboxylate form [51]. Due to the electrostatic interactions between protonated paracetamol and carboxylate

groups of the hydrogel, the drug molecule PAR is not released from the hydrogel (Scheme 3). On the other hand, PBS saline (pH 7.4) includes additional ions come from KCl and NaCl, and causes osmotic pressure difference resulting in decrease of PAR release of pH 7.4 [11]. This knowledge, it can be said that in vitro swelling study results are in good agreement with this phenomenon. At acidic pH, the hydrogel swells more than that of pH 7.4 and provides more cumulative percentage release at acidic pH.



Scheme 2. Representation of possible interactions between the MSHY/PECALD hydrogel and PAR.



Scheme 3. Representation of the MSHY/PECALD hydrogel and PAR at different pH values.

With this study, we demonstrated that the **MSHY/PECALD** hydrogel obtained from the Schiff base reaction of **PECALD** and **MSPCTHY** can be used for pH-controlled release. While these prototypical example of **PAR** loaded **MSHY/PECALD** hydrogel doesn't release all the loaded drug, this study is the first example of Schiff base reaction between -NH_2 group of phosphazene derivatives and aldehyde group of **PECALD**. In other reported studies aldehyde groups of the phosphazene reacts with -NH_2 groups of a biopolymer [12]. We believe future iterations with other drugs will be capable of doing so.

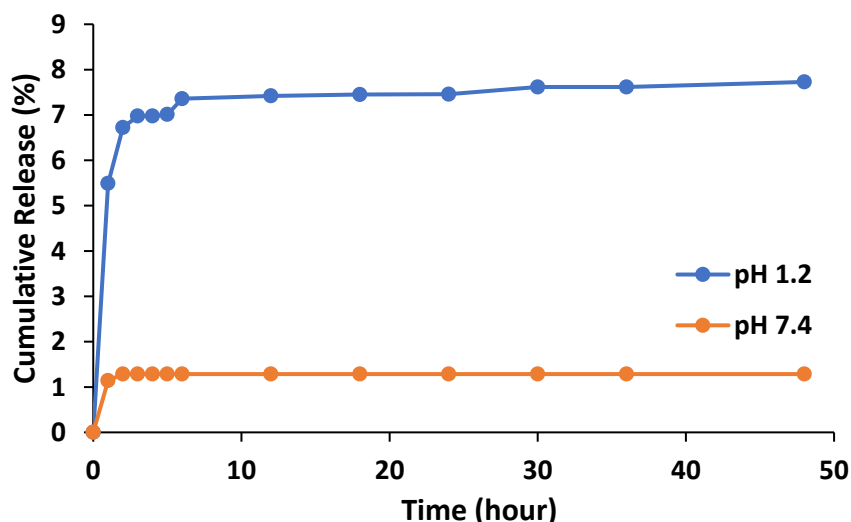


Figure 8. pH controlled delivery of **PAR** from **MSHY/PECALD** hydrogel at pH 1.2 and 7.4.

2.2. X-ray crystallographic characterization

The MSHY compound's crystal form was discovered by single-crystal X-ray crystallography, and **Table 1** presents a selection of the collection and refinement details. As shown in **Figure 9**, the molecule is spiro substituted cyclotriphosphazene (P_3N_3) derivatives in which one phosphorus atom is replaced with 2,2-dimethyl 1,3-propanedioxy- units forming six membered spiro- rings (PO_2C_3) while other two phosphorus atoms are geminally substituted with two 4-aminophenoxy- groups. The P3 atom exhibits the greatest departure from a planarity, whereas the six-membered cyclotriphosphazene ring has a subtly flattened boat shape [0.1045(10) Å]. Meanwhile, the six-membered spiro ring has chair conformations. Further conformational parameters, including related torsion angles and total puckering amplitudes (Q_T), are provided in **Table 2**.

Crystal structure investigation has demonstrated that there is no substitution effect on bond lengths and angles of P_3N_3 ring of **MSHY**. The P–N bond lengths, N–P–N, and P–N–P bond angles are comparable for the spiro (P1 atom) and geminal (P2 and P3 atoms) substituted phosphorus atoms. However, compared to geminal substituted P2 and P3 phosphorous atoms, spiro substitution on the P1 phosphorous atom produced a broader O–P–O bond angle (**Table 2**). All of these values are consistent with those previously reported for comparable cyclotriphosphazene compounds [52].

Due to the existence of four amine groups, the molecule has several intermolecular interactions in the crystal packing of **MSHY**. Moreover, one water molecule found as a solvent in the unit cell also contributed to intermolecular interactions (**Table 3**). The angles between two phenyl rings of two paracetamol units that are coupled to the same phosphorus atom have

been found to be 87.61° and 68.60°, respectively, demonstrating how effective these connections occur in the paracetamol units' directions. This value is wider than that previous observation [36]. **Table 2** gives the associated torsion angles.

Table 1. Compound MSHY's X-ray crystallographic information and refinement parameters.

Compound	MSHY
Empirical formula	C ₂₉ H ₃₄ N ₇ O ₆ P ₃ ·H ₂ O
Formula weight	687.56
Temperature (K)	273
Crystal system	Triclinic
Space group	<i>P</i> -1
a (Å)	10.7928 (10)
b (Å)	11.5181 (10)
c (Å)	16.0333 (14)
α (°)	81.789 (2)
β (°)	79.147 (1)
γ (°)	62.299 (1)
Volume (Å³)	1729.6 (3)
Z	2
Density (calc, Mg/m³)	1.320
Absorption coeff. (mm⁻¹)	0.23
F(000)	720
θ_{max} (°)	25
Reflections collected	19243
Independent reflections	6067
R_{int} (merging R value)	0.040
Parameters	449
R (<i>F</i>²>2σ<i>F</i>²)	0.046
wR (all data)	0.123
Goodness-of-fit on <i>F</i>²	1.02

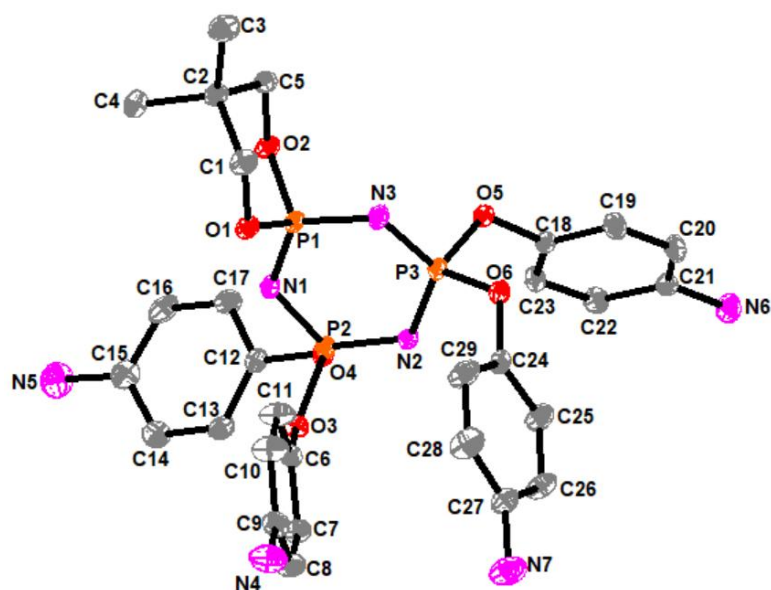


Figure 9. Crystal structure of compound MSHY with the atom-numbering scheme. Displacement ellipsoids are drawn at the 30% probability level. The hydrogen atoms and solvent (H₂O) molecule have been omitted for clarity.

Table 2. The selected bond and conformational parameters of compound **MSHY** [(Å) for length and (°) for angle].

Cyclotriphosphazene ring			
P1—N1	1.572 (2)	N1—P1—N3	116.79 (11)
P1—N3	1.590 (2)	N1—P2—N2	117.33 (11)
P2—N1	1.587 (2)	N3—P3—N2	117.49 (11)
P2—N2	1.574 (2)	P2—N1—P1	122.96 (13)
P3—N2	1.578 (2)	P2—N2—P3	121.63 (13)
P3—N3	1.580 (2)	P3—N3—P1	121.68 (13)
total puckering amplitude(Q_T)	0.1524(18)	θ	68.3(7)
ϕ	245.3(7)	Maximum deviation from planarity	0.1045(10) (P3)
Spiro unit			
P1—O1	1.5712 (18)	O2—P1—O1	103.08 (9)
P1—O2	1.5709 (18)		
Total puckering amplitude(Q_T)	0.541(3)	θ	4.6(3)
ϕ	196(3)		

Geminal unit			
P2—O3	1.5727 (18)	N1—P2—O3—C6	−69.0 (2)
P2—O4	1.5785 (18)	N1—P2—O4—C12	−50.2 (2)
P3—O5	1.5859 (19)	N2—P2—O3—C6	62.9 (2)
P3—O6	1.5751 (18)	N2—P2—O4—C12	−178.51 (19)
O3—P2—O4	99.87 (10)	N2—P3—O5—C18	−55.3 (2)
O5—P3—O6	100.32 (10)	N2—P3—O6—C24	−28.5 (2)
		N3—P3—O5—C18	175.99 (18)
		N3—P3—O6—C24	102.7 (2)

Table 3. Selected inter-molecular interactions for compound **MSHY**.

D—H⋯A	D—H	H⋯A	D⋯A	DHA	Symmetry code
N4—H4⋯N6	0.90	2.34(2)	3.198 (4)	159 (4)	−x+2, −y+1, −z+1
N6—H6⋯N1	0.88	2.32 (1)	3.192 (3)	169 (3)	x+1, y−1, z
N7—H7A⋯N2	0.89	2.68 (2)	3.557 (4)	167 (4)	−x+2, −y+1, −z+1
N7—H7B⋯O7	0.89	2.25 (2)	3.123 (5)	164 (5)	−x+1, −y+1, −z+1
C22—H22⋯O7	0.93	2.63	3.362 (5)	136	x+1, y, z
O7—H7C⋯N4	0.86	1.94 (1)	2.791 (5)	172 (5)	−x+1, −y+1, −z+1
O7—H7D⋯N3	0.85	2.30 (3)	3.044 (4)	146 (5)	

2.3. In Vitro Cytocompatibility of MSHY/PECALD Hydrogels

The in vitro cytocompatibility of **MSHY/PECALD** hydrogels was evaluated against BJ fibroblast cells using the MTT assay after 1, 3, 5, and 7 days of incubation at different hydrogel extract concentrations (**Figure 10**). The obtained results demonstrated that the hydrogel extracts exhibited concentration- and time-dependent effects on cellular viability.

As shown in **Figure 10**, all hydrogel groups maintained high cell viability values during the initial incubation period. Particularly, after 1 day of treatment, all tested concentrations exhibited viability values above 90%, indicating that the hydrogel system did not induce significant acute cytotoxic effects toward fibroblast cells. The favorable cytocompatibility may be attributed to the biocompatible nature of the polysaccharide-based hydrogel network and the relatively stable Schiff-base crosslinked structure formed between the polymeric components. Similarly, dialdehyde polysaccharide-crosslinked hydrogel systems have also been reported to

exhibit high cytocompatibility toward different cell types, including MC3T3 cells, in biomedical applications [53].

With increasing incubation time, a gradual reduction in cell viability was observed, particularly at higher hydrogel extract concentrations (40–90 $\mu\text{g/mL}$). After 7 days of incubation, the viability values decreased to approximately 65% at the highest concentrations. This cellular response may be associated with residual components released from the polymeric matrix during prolonged incubation [54]. Similar cellular stress mechanisms have been reported for injectable hydrogel systems developed for biomedical and controlled drug delivery applications [55].

Despite the observed decrease at prolonged incubation periods, lower and moderate hydrogel concentrations preserved acceptable cellular viability throughout the experimental period. The obtained results indicate acceptable cytocompatibility of the hydrogel system, particularly at lower and moderate concentrations, according to ISO 10993-5 guidelines [56].

Furthermore, the porous morphology observed in SEM analysis may have positively contributed to the biological performance of the hydrogels by facilitating nutrient diffusion and mass transfer within the cellular microenvironment. Similar observations have been reported for porous hydrogel scaffolds developed for tissue engineering and biomedical applications [49]. The favorable cytocompatibility observed in this study is also consistent with previous reports on PEC-ALD-based Schiff-base hydrogels, which demonstrated negligible cytotoxicity toward fibroblast and mesenchymal stem cells [57].

The obtained results suggest that the **MSHY/PECALD** hydrogel system exhibits favorable cytocompatibility and potential applicability for biomedical and controlled drug delivery applications.

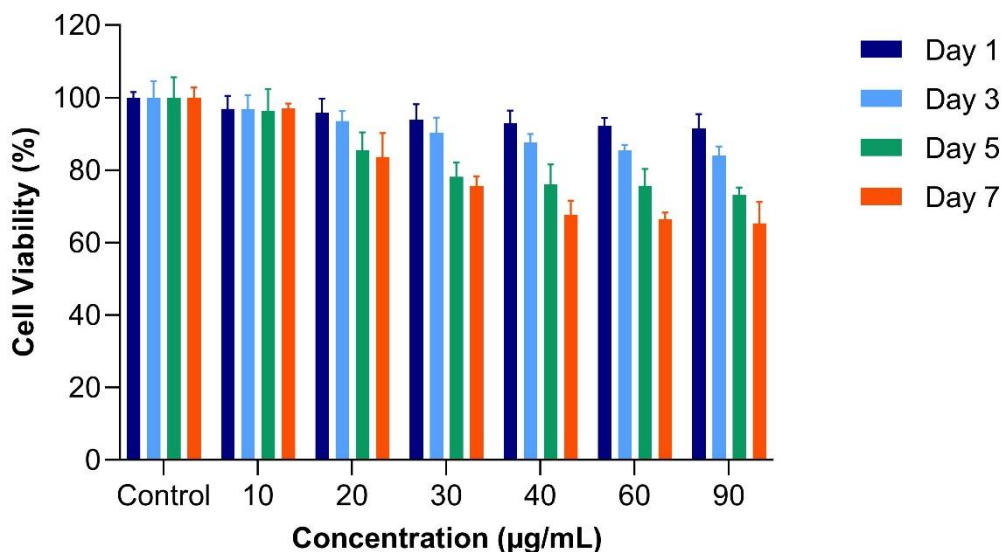


Figure 10. Cell viability of BJ fibroblast cells treated with hydrogel extracts at different concentrations for 1, 3, 5, and 7 days evaluated by MTT assay. Data are expressed as mean $\pm$ SD ($n = 3$).

2.4. In Vitro Degradation Behavior of MSHY/PECALD Hydrogels

The in vitro degradation behavior of **MSHY/PECALD** hydrogels was investigated under physiological (pH 7.4) and acidic (pH 1.2) conditions over 72 h (**Figure 11**). The remaining weight profiles demonstrated that the degradation behavior of the hydrogels was strongly dependent on the pH of the surrounding medium.

As shown in **Figure 11**, the hydrogels exhibited a gradual decrease in remaining weight over time in both degradation media, indicating progressive hydrolytic degradation of the polymeric network. However, the degradation rate was more pronounced under physiological conditions (pH 7.4) compared to acidic conditions (pH 1.2). After 72 h of incubation, the remaining weight of the hydrogels decreased to approximately 62% at pH 7.4, whereas higher weight retention was observed under acidic conditions.

The relatively slower degradation observed at pH 1.2 may be attributed to protonation within the polymeric network, which may promote stronger intermolecular interactions and a more compact hydrogel structure. In contrast, under physiological conditions, the hydrogel matrix may become more susceptible to hydrolytic cleavage and diffusion of degradation products into the surrounding medium. Similar pH-dependent degradation behavior has been reported for Schiff-base and oxidized polysaccharide-based hydrogels developed for biomedical and controlled drug delivery applications [58].

Furthermore, the degradation behavior of hydrogel systems is closely associated with the crosslinking density and structural stability of the polymeric matrix. The obtained degradation profile suggests controlled degradation of the hydrogel network rather than rapid structural collapse. Similar observations have been reported for dialdehyde polysaccharide-crosslinked hydrogels, where the extent of crosslinking significantly influenced hydrogel stability and degradation kinetics [53].

Controlled degradation behavior is particularly important for hydrogel-based biomaterials, as it directly affects structural stability, release characteristics, and long-term biological performance [59]. The gradual mass loss observed in this study indicates that the hydrogel network retained its structural integrity during the degradation process without exhibiting rapid disintegration. These findings suggest that the **MSHY/PECALD** hydrogel system exhibits controlled and pH-responsive degradation behavior, which may be advantageous for future biomedical and controlled drug delivery applications.

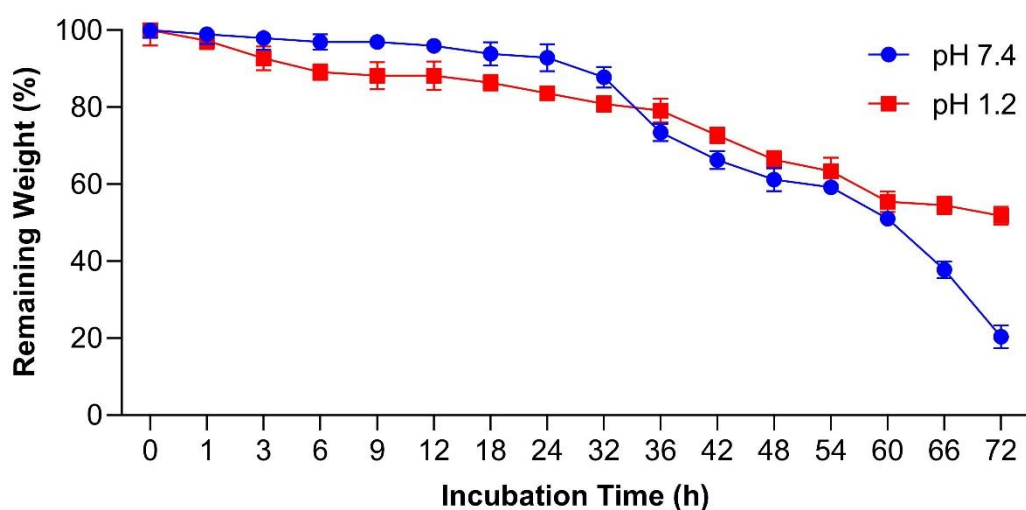


Figure 11. pH-dependent degradation behavior of **MSHY/PECALD** hydrogels at pH 7.4 and pH 1.2 conditions over 72 h. The remaining weight (%) of the hydrogels was determined at different incubation times. Data are expressed as mean $\pm$ SD ($n = 3$).

3. Experimental

3.1. Materials and methods

Hexachlorocyclotriphosphazene (Otsuka Chemical Co., Ltd.) was obtained from n-hexane by fractional crystallization. 99% 2,2-dimethyl 1,3-propanediol was provided from Sigma-Aldrich. 4-Acetamidophenol (98.0%) was obtained from Thermo Scientific. Pectin (poly-D-galacturonic acid methyl ester, MW 21–70 kDa), n-hexane (95.0%), tetrahydrofuran (99.0%), sodium periodate, acetone ($\geq 99.5\%$), dichloromethane (99.0%), %25 ammonia

solution, thionyl chloride (97.0%), sulfuric acid (98.0%), CDCl_3 , Silica gel 60 (230–400 mesh), and sodium hydride (60%) were purchased from Merck. All performed reactions were monitored by using thin-layer chromatography on Merck silica gel plates (Merck, Kieselgel 60, 0.25 mm thickness) with F_{254} indicator. Purification of the products was performed on silica gel (Merck, Kieselgel 60, 230–400 mesh; for each 2 g crude mixture, 120 g silica gel was filled in a column with 3 cm in diameter and 110 cm in length) with column chromatography. ^1H and ^{31}P NMR spectra of the synthesized products were recorded in CDCl_3 solution on a Varian INOVA 500 MHz spectrometer. Mass spectra of the products were recorded by a Bruker MALDI-TOF (Rheinstetten, Germany) spectrometer using DIT (1,8,9-anthrasenetriol) and DHB (2,5-Dihydroxybenzoic acid) as a matrix. FT-IR spectra of both the products and the hydrogel were recorded by Perkin Elmer Spectrum 100 FT-IR spectrometer. X-ray crystallography verified the solid-state structures of the compounds. The SEM analysis of the composites was performed using a Philips XL 30 SFEG.

3.2. X-ray crystallography

A Bruker APEX II CCD diffractometer was used to record crystallographic data for two substances utilizing monochromatized $\text{Mo-K}\alpha$ ($\lambda = 0.71073 \text{ \AA}$) X-radiation. Absorption corrections were applied using the multi-scan method available in SADABS [60], and space group determinations were carried out with XPREP, as implemented in the APEX2 software suite [61]. Direct approaches (SHELXS-97) [62] and full-matrix least squares on F^2 with SHELXL [63] in the Olex2 software package [64] were used to solve the crystal structures. Anisotropic displacement parameters were utilized to improve every non-hydrogen atom, while C–H hydrogen atoms were positioned at calculated geometries and constrained to ride on their respective parent atoms during refinement. Additionally, the unit cell of **MSHY** has one water and disordered solvent molecule, which couldn't be simulated. The using of the SQUEEZE command of PLATON [65] for removing solvent molecule and the resting of the molecule and refining without solvent resulted with formed cavity with $160.1 \text{ \AA}^3$ volume and a 48 void electron count. The PLATON [65], MERCURY [66], and DIAMOND [67] programs were used to do the last geometrical computations and the molecular drawings. For compound **MSHY**, the structural assessment was provided to the Cambridge Crystallographic Data Centre with reference CCDC-2335718.

3.3. Synthesis

According to the literature, the mono-spiro cyclotriphosphazene derivative (**MS**) containing a 2,2-dimethyl-1,3-propanedioxy substitution was synthesized via the reaction between **HCPP** ($\text{N}_3\text{P}_3\text{Cl}_6$) and 2,2-dimethyl-1,3-propanediol [41, 42]. A dry argon atmosphere was maintained during all reactions.

3.3.1. Synthesis of MSPCT

For the synthesis of compound **MSPCT**, a three-neck round bottom flask was used as a reaction flask. Compound **MS** (0.2 g, 0.53 mmol) was transferred in the reaction flask, including 20 mL of dry acetone, and the mixture was stirred with a magnetic stirrer. Then, K_2CO_3 (0.292 g, 2.12 mmol) was put into the reaction flask. **PAR** (0.38g, 2.12 mmol) was mixed in dry acetone (40 mL), which was then introduced drop by drop into the reaction flask. The control of the progress of the reaction was performed by TLC and the reaction mixture was stirred at 70 °C for 4 days until the product formation was observed. After filtration of the reaction mixture, the solvent (acetone) was evaporized under pressure. The purification of the white solid compound (**MSPCT**) was achieved by column chromatography using the THF solvent system ($R_f=0.56$ THF) (yield: 45%) (Scheme 1).

Anal. Calc. For $\text{C}_{37}\text{H}_{42}\text{N}_7\text{O}_{10}\text{P}_3$; MALDI TOF (m/z) Calc. 837.70, Found: 837.705 $[\text{M}]^+$ (**Figure 2a**).

^{31}P NMR (CDCl_3 , 298 K, δ ppm AB_2 spin system): 13.41 ([PO(Spiro)], 1P), 9.61 ([PR₂], 2P), (**Figure 1a**). ^1H NMR (500 MHz, 298 K, CDCl_3 , δ , ppm): 1.18 (s, 6H, H_e); 2.20 (s, 12H, H_f); 4.41 (m, 4H, H_d); 6.91 (m, 8H, H_b); 7.25 (m, 8H H_a); 7.93 (s, 4H, NH_c) (**Figure 3a**).

FT-IR (ν , cm^{-1}): 943.1 ($\nu_{\text{P-O-C}}$); 1058.9 -1016.0. ($\nu_{\text{P=N}}$); 1502.8 ($\nu_{\text{(C=C) aromatic}}$); 1663.1 cm^{-1} ($\nu_{\text{(C=O)}}$); 3146.1-3092.6 ($\nu_{\text{(C-H) aromatic}}$); 3289.0($\nu_{\text{N-H}}$) (**Figure 4**).

3.3.2. Synthesis of MSHY

0.2 g of **MSPCT** (0.3 mmol) was dissolved in the two-neck round bottom flask in 8 ml of dry methanol, and 1.2 ml of 98% H_2SO_4 solution were added and then were refluxed at 80 °C while being stirred magnetically for 4 hours. After completion, the 25% NH_4OH solution was used to neutralize the mixture when it was back to room temperature. The resulting solution was filtered and dipped in deionized water. The **MSHY** product was crystallized as light pink crystals from a 1:1 n-hexan:acetone solvent system. (yield: 68%) ($R_f=0.60$ THF) (Scheme 1).

Anal. Calc. For $C_{29}H_{34}N_7O_6P_3$; MALDI TOF (m/z) Calc. 669.56, Found: 670.103 $[M+H]^+$ (**Figure 2b**).

^{31}P NMR ($CDCl_3$, 298 K, δ ppm AB_2 spin system): 13.8 (t, 1P), 10.1 (d, 2P) (**Figure 1b**). ^1H NMR (500 MHz, 298 K, $CDCl_3$, 1.04 (s, 6H, H_e); 3.99 (m, 4H, H_d); 6.53 (m, 8H, H_b); 6.87 (m, 8H H_a); 1.66 (m, 8H, NH_c); (**Figure 3b**).

FT-IR (ν , cm^{-1}): 925-943 (ν_{P-O-C}); 1050-1176 cm^{-1} ($\nu_{P=N}$); 1503.2 ($\nu_{C=C}$ aromatic); 2879-2963 cm^{-1} (ν_{C-H} aromatic); 3351-3432 cm^{-1} (ν_{N-H}) (**Figure 4**).

3.3.3. Synthesis of Pectin Displaying Aldehydes (PECALD)

Pectin bearing aldehyde groups was prepared by adaptation of the literature [4,57,68,69]. 100 mL of a 1% (w/v) solution of pectin was in ultrapure water. Then, sodium periodate (0.535 g, 2.5 mM) was added into the reaction flask as an oxidation agent, and the reaction mixture was stirred at room temperature in the dark for 24 h. Finally, in order to eliminate unreacted periodate, ethylene glycol (140 μ L, 2.5 mM) was introduced into the reaction flask, and the reaction mixture was stirred at room temperature in the dark for 1 h. For the removal of low molecular weight contaminants, dialysis tubing cellulose membrane (MWCO 3500) was used to dialyze the reaction mixture by using ultrapure water. Every 2 hours during the daytime the water was exchanged, and the dialysis process was continued for four days. Finally, the synthesized **PECALD** was freeze-dried (80.54% yield).

Synthesis of MSHY/PECALD hydrogels for drug delivery studies

The preparation of hydrogel was a typical Schiff base reaction. For preparation of hydrogels, firstly 2 w % solutions of (40 mg) **PECALD** and (40 mg) **MSHY** were dissolved in 2 ml DMSO. Then the **PECALD** solution and **MSHY** solution were mixed and stirred to obtain the hydrogels. Then the hydrogels were produced by combining these stock solutions in varied volume ratios as shown in **Table 4**, and precipitated hydrogels were removed from DMSO. The hydrogel formation occurred due to the spontaneous crosslinking undergone by the materials during the Schiff-base reaction, which was visually detected every hour during the daytime for 3 days at different temperatures (R.T. and 35°C). The optimal volume ratio, temperature, and reaction time were found to be 1:1, room temperature and 24 hours, respectively. The synthesized yellow hydrogel was washed with distilled water until clear washing water was obtained. Then the hydrogel was lyophilized. Hydrogel was obtained with a yield of 25.5%.

Table 4. The feed compositions of the hydrogels

Code of formulation	Volume ratio (MSHY/PECALD)
F1	1:1
F2	1:2
F3	2:1
F4	4:1
F5	1:4

In vitro Swelling studies

The swelling profile of the hydrogel was investigated at different pH values as a function of time. Firstly, in a vacuum oven at 40°C, the hydrogels were dried overnight. Then, the known dry masses of hydrogels (W_0) were immersed in buffer solutions at pH 1.2 and 7.4 at ambient temperature. Hydrogels were removed from the solutions, and the excess solution on their surfaces was wiped with a piece of filter paper at different time intervals and weighed (W_t). This process was continued until the hydrogels reached constant mass. The following equation was used for the calculation of the swelling percentage (SP):

$$SP (\%) = ((W_t - W_0)/W_0) \times 100 \quad \text{Eq. 1}$$

where W_0 and W_t are the weights of the hydrogels in the dry state and swollen state, respectively.

Drug loading and in vitro release studies

In order to investigate pH-dependent drug release profile of the obtained **MSHY/PECALD** hydrogel, paracetamol (**PAR**) was chosen as a model drug molecule for drug loading and release studies.

The drug loading process was performed as follows: approximately 5 mg of **MSHY/PECALD** hydrogels were maintained in 4 mL of 10 mg.mL⁻¹ drug solutions separately at room temperature for 24 hours. The amount of absorbed drug by obtained **MSHY/PECALD** hydrogels was determined from UV-Vis measurements (λ_{max} =243 nm) using a calibration curve plotted with standard **PAR** solutions. Then, the **PAR** loaded **MSHY/PECALD** hydrogel was dried in a vacuum oven at 40°C overnight.

The **PAR** release profile of **MSHY/PECALD** hydrogel was evaluated in various solutions at different pH values, such as pH 1.2 (gastric fluid) and pH 7.4 (PBS saline). The dry **PAR** loaded **MSHY/PECALD** hydrogel was immersed in 4 mL of release solutions and removed from the solutions at different time intervals and exchanged with 4 mL of fresh solution. The aliquots were measured at 243 nm by UV-Vis spectrophotometer, and cumulative released percentages at the measured time point were calculated from the calibration curve.

3.4. In Vitro Cytocompatibility Assessment

The cytocompatibility of the synthesized hydrogels was evaluated using the MTT assay against BJ fibroblast cells. Cells were cultured in high-glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin at 37 °C in a humidified atmosphere containing 5% CO₂. BJ fibroblast cells were seeded into 96-well plates at a density of 3×10^3 cells per well and incubated for 24 h to allow cell attachment.

Hydrogel extracts were prepared according to the literature [70]. Briefly, sterilized hydrogel samples (4 mg) were incubated in 8 mL serum-free DMEM at 37 °C for 24 h. The obtained extracts were filtered through a sterile 0.22 µm membrane filter and used as stock solutions (500 µg/mL). Different concentrations (10, 20, 30, 40, 60, and 90 µg/mL) were prepared using complete culture medium and applied to the cells. Cell viability was evaluated after 1, 3, 5, and 7 days of incubation.

For the MTT assay, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well and incubated for 3 h at 37 °C in the dark. Subsequently, the medium was removed, and the resulting formazan crystals were dissolved in 100 µL dimethyl sulfoxide (DMSO). Absorbance values were measured at 570 nm using a FLUOstar Omega microplate reader (BMG LABTECH, Germany). All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation.

3.5. In Vitro Degradation Study

The biodegradation behavior of **MSHY/PECALD** hydrogels was evaluated under physiological and acidic conditions using phosphate-buffered saline (PBS, pH 7.4) and acidic buffer solution (pH 1.2). Briefly, pre-weighed lyophilized hydrogel samples (W_0) were immersed in 1 mL of the respective degradation media and incubated at 37 °C under continuous shaking at 100 rpm.

At predetermined time intervals up to 72 h, the hydrogel samples were removed from the medium, gently washed with distilled water to eliminate residual buffer salts, and dried at 70 °C until constant weight was achieved. The final dry weight of the samples (W_t) was then recorded.

The degradation behavior of the hydrogels was determined by calculating the remaining weight percentage according to the following equation:

$$\text{Remaining Weight (\%)} = \frac{W_t}{W_0} \times 100$$

where W_0 represents the initial dry weight of the hydrogel and W_t represents the dry weight after degradation at a specific time point. All experiments were performed in triplicate, and the results were expressed as mean $\pm$ standard deviation.

3.6. Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean $\pm$ standard deviation (SD). Statistical analyses were performed using GraphPad Prism 9 software (GraphPad Software, San Diego, CA, USA). A p-value lower than 0.05 was considered statistically significant.

4. Conclusions

MSPCT, **MSHY**, and hydrogel (**MSHY/PECALD**), which are new paracetamol derivatives of cyclotriphosphazene, were successfully synthesized. Standard spectroscopic methods have been used to describe all of the compounds, and single crystal X-ray crystallography demonstrated that the monomer's (**MSHY**) structure is solid. The results show that the Schiff-base formation reaction of **PECALD** with **MSHY** resulted in the effective synthesis of **MSHY/PECALD** hydrogel. The hydrogel's morphology is examined using a scanning electron microscope. The SEM images reveal that the hydrogel exhibits a porous structure with uniformly distributed pores throughout, which is also in a good agreement with swelling studies. The **PAR** release profile of **MSHY/PECALD** hydrogels was investigated in a range of solutions with varying pH values, including pH 1.2 (gastric fluid) and pH 7.4 (PBS saline). The pH controlled **PAR** release was mainly observed in a strong acidic medium, which is provided as a medium that mimics gastric fluid. Additionally, pH controlled **PAR** release was 7 times higher at acidic medium than that of at pH 7.4. The synthesized hydrogel made from amine-terminated phosphazene and pectin aldehyde is the first example of Schiff reaction between -NH_2 group of phosphazene derivatives and aldehyde group of **PECALD**, whereas

reported studies are on aldehyde groups of the phosphazene reacting with $-NH_2$ groups of a biopolymer. In vitro studies further demonstrated that the **MSHY/PECALD** hydrogel made from amine-terminated phosphazene and pectin aldehyde, which are thought to be biocompatible degradation products, was well tolerated by BJ fibroblast cells and underwent gradual degradation under different pH conditions. These findings complement the swelling and drug release results and further strengthen the potential of the developed hydrogel system for controlled drug delivery applications. The synthesized hydrogel is thought to be biocompatible degradation products, have the potential to be useful and inventive controlled drug delivery devices in the pharmaceutical and therapeutic domains. We believe future iterations with other drugs will be capable of doing so.

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