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Phosphonium hydrogels for controlled release of ionic cargo

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We describe the synthesis of three different phosphonium salts and their reaction with poly(ethylene glycol) dimethacrylate to create cationic hydrogels. The hydrogels were loaded with an anionic dye and an anionic anti-inflammatory drug through ionic interactions and compared with an analogous ammonium gel. The release rates of these anions depended on their structure and pK_a values, as well as the pH and ionic strength of the release medium.

Many traditional therapeutics suffer from limitations including side effects due to poor targeting, difficulty in controlling dosage and low patient compliance.¹ These challenges emphasize the need for superior controlled drug delivery systems. There are various approaches to delivering therapeutics that can release molecules in a controlled manner, ensuring sustained delivery.^{2,3} The use of hydrogels, cross-linked networks containing a high fraction of water, is one of the commonly employed strategies. Hydrogels have been used in many applications such as wound dressings,⁴ ocular aids,⁵ and drug delivery.⁶ Their high water content, and soft, rubbery properties can minimize inflammatory reactions of surrounding cells and tissues, imparting high biocompatibility.⁷ In addition, they can be shaped and molded into many different structures that can be designed to match anatomical requirements.⁸

Many early examples of hydrogels involved relatively inert and non-functional polymers. For example, poly(ethylene glycol) (PEG) has been widely used in hydrogels because it has weak immunogenicity and is FDA approved in certain applications.⁹ Recently, newer classes of hydrogels including those based on peptides¹⁰ and those capable of responding to changes in conditions such as pH¹¹ or temperature,¹² to enable triggered drug release have been reported. The release of drugs

can be controlled through network degradation,¹³ mechanical deformation¹⁴ or swelling/shrinking.¹⁵ Alternatively specific interactions between the hydrogel and the drug can be incorporated, including covalent conjugation,¹⁶ hydrophobic associations¹⁷ or electrostatic interactions.¹⁸

Quaternary ammonium salts carry a positive charge at nitrogen and have been extensively studied as antimicrobial agents and electrostatic drug delivery vehicles.^{19,20} Quaternary phosphonium cations also carry positive charges at phosphorus but exhibit different charge distributions and have been shown to have enhanced performance over their ammonium counterparts in areas including antimicrobial activity²¹ and nucleic acid binding.²² While a limited number of phosphonium-containing hydrogels have been reported,^{23,24} to our knowledge they have not been investigated for drug delivery. In this context, we report phosphonium-containing hydrogels as controlled delivery vehicles. The phosphonium monomers can be cured using UV light to create stable cross-linked hydrogels through a simple process. The hydrogel properties and drug release rates can be tuned by varying the monomer structures.

First, tris(hydroxypropyl)phosphine, tri-*n*-butylphosphine and triphenylphosphine were reacted with 4-vinylbenzyl chloride to generate the corresponding polymerizable phosphonium salts **T(hp)-P**, **Bu-P**, and **Ph-P**, respectively (Scheme 1). These phosphonium derivatives were of specific interest because they have varying structures and hydrophobicities, potentially allowing their interactions with molecules in the hydrogels to be tuned. Tri-*n*-butyl(4-vinylbenzyl)ammonium chloride (**Bu-N**) was also investigated to enable comparison between phosphorus and nitrogen

Scheme 1. Synthesis of phosphonium monomers

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Fig. 1. Schematic illustration of the synthesis and drug loading of phosphonium hydrogels.

analogues. PEG dimethacrylate (PEGDMA) with an M_n of 11,900 g/mol was incorporated to provide cross-linking. The hydrogels were prepared from water-based formulations containing 89 wt% PEGDMA, 10 wt% onium salt (**T(hp)-P**, **Bu-P**, **Ph-P** and **Bu-N**) and 1 wt% photoinitiator (Irgacure 2959; Fig. 1). Formulations were transferred to round Teflon® molds, purged with N_2 then cured by irradiation with UV light for 30 min.

The gel content was similar for the different phosphonium networks, ranging from 71–74%, while the ammonium network had a higher gel content of 92 % (Table 1). Dried hydrogels were then placed into either deionized (DI) H_2O or into citric acid/sodium phosphate buffer with pH of 5, 6 or 7.4 and the hydrogels were swelled at 37 °C. Swelling occurred over 2–6 h for each system (Fig. S9). In each case, the swelling was highest in DI water, ranging from 1170 to 3700% of the dry weight. The swelling capacity was highest in pure H_2O due to the osmotic pressure. Conversely, when the hydrogels were placed into salt solutions, the osmotic pressure was much lower, resulting in less swelling.²⁵ Swelling varied slightly with pH due to the different buffer compositions (Table S1).²⁶ Swelling was highest for the **T(hp)-P** hydrogel, followed by **Bu-P**, and was lowest for **Ph-P** and **Bu-N**, correlating with the decreasing hydrophilicities of the phosphonium salts and suggesting differences between the P and N-based gels perhaps due to their different gel contents. SEM confirmed the microporous structures of all of the gels (Fig. S10). **T(hp)-P** and **Bu-P** gels appeared to have the largest pores, which correlated with their higher degrees of swelling. Based on thermogravimetric analysis, all of the phosphonium-based networks had decomposition temperatures of ~240 °C, whereas the **Bu-N** network decomposed at 180 °C.

To explore the abilities of the hydrogels to load anionic molecules and release them in a controlled manner, each hydrogel was first loaded with fluorescein sodium salt, an anionic dye. Loading was performed by immersing the hydrogels in a 1 wt% fluorescein sodium salt solution for 24 h. The gels were then washed in DI water to release all non-

Table 1. Swelling percentages, gel content and loading capacities of hydrogels.

Hydrogel	Swelling (%)	Gel Content (%)	Loading Capacity (%) ^a
T(hp)-P	DI Water: 3700 ± 1100 pH 5: 1560 ± 12 pH 6: 1550 ± 33 pH 7.4: 1360 ± 75	73 ± 0.2	Fluorescein: 0.77 ± 0.03 Diclofenac: 3.9 ± 0.06
Bu-P	DI Water: 2290 ± 240 pH 5: 1510 ± 170 pH 6: 1460 ± 98 pH 7.4: 1260 ± 130	71 ± 2	Fluorescein: 0.60 ± 0.03 Diclofenac: 3.8 ± 0.08
Ph-P	DI Water: 1750 ± 340 pH 5: 870 ± 26 pH 6: 880 ± 33 pH 7.4: 800 ± 33	74 ± 1	Fluorescein: 0.71 ± 0.08 Diclofenac: 4.1 ± 0.34
Bu-N	DI Water: 1170 ± 170 pH 5: 760 ± 65 pH 6: 770 ± 17 pH 7.4: 750 ± 60	92 ± 1	Fluorescein: 3.2 ± 0.27 Diclofenac: 15 ± 2

^a% relative to one gram of dry hydrogel

ionically bound fluorescein until no further dye was detected in the washings (Fig. S11A). A control hydrogel based only on PEGDMA was also evaluated. It was found that the onium-containing gels retained a deep red color after the washing procedure, while the PEGDMA gel did not retain any detectable dye (Fig. S12). This indicated that anionic molecules could be retained in the onium-based networks. The ionically bound dye could be released from the hydrogels by immersion in a 1 wt% solution of cetyltrimethylammonium chloride (CTAC), allowing the amount of bound dye to be calculated as loading capacity (Table 1). The **Bu-N** gel released much more unbound dye during the washing compared with the phosphonium gels. However, it also retained a higher loading after the washing.

To measure the release rates under different conditions, each fluorescein-loaded hydrogel was placed into either DI water or buffered solutions at pH 5, 6 or 7.4 at 37 °C. The release rate of fluorescein was fastest from the **T(hp)-P** hydrogels in all buffers, with complete release over 24 h, likely due to their high hydrophilicity and high degree of swelling (Fig. 2A). In contrast, **Ph-P** hydrogels released the fluorescein much more slowly, over at least 100 h (Fig. 2B and S13C). This can be attributed to the lower degree of swelling of this gel, which would slow diffusion of molecules out of the network. Furthermore, the phenyl groups on the phosphonium cation may interact with fluorescein through π stacking and van der Waals forces in addition to ionic interactions. Despite their different degrees of swelling, the **Bu-P** and **Bu-N** hydrogels exhibited similar fluorescein release rates in buffers and they were intermediate between those of the other phosphonium hydrogels (Fig. S13B,D).

The release rates also depended on the pH, with fastest release at pH 7.4 and slowest release at pH 5 (Fig. 2A,B and S13). Although the swelling was slightly lower at pH 7.4 than at the other pH values, which would favour slower release, the

Fig. 2. Release rates of: A) fluorescein from **T(hp)-P** hydrogels; B) fluorescein from **Ph-P** hydrogels; C) diclofenac from **T(hp)-P** hydrogels; D) diclofenac from **Ph-P** hydrogels (Blue line – DI Water, Red – pH= 5, Orange – pH= 6, Black – pH= 7.4)

behaviour was dominated by the protonation state of fluorescein. Fluorescein has a pK_a value of 6.7,²⁷ and as the pH of the solution was decreased from pH 7.4 to pH 5, the molecule became less anionic, and consequently more hydrophobic. This favoured its retention in the hydrogel and slowed its release. Finally, although the hydrogels swelled much more in DI water than in the buffer solutions, the release in DI water was much slower for hydrogels **T(hp)-P**, **Bu-P**, **Ph-P**, as there were no anions to exchange with the fluorescein to enable its release. However, the release of fluorescein from the **Bu-N** hydrogel was much faster than from any of the phosphonium gels in DI water (Fig. S13D). Thus, the release rate depended on the ion structure as well as the pH and the presence of salt.

The loading and release of the non-steroidal anti-inflammatory drug diclofenac were then investigated. Notably, the loading capacities of the gels for diclofenac were ~5-fold higher than for fluorescein (Table 1), which may result from its higher hydrophobicity or the presence of a single ionizable site compared to fluorescein which can undergo multiple deprotonation events. The release of diclofenac from the hydrogels was then studied under the same conditions described above. As for fluorescein, **T(hp)-P** hydrogels released diclofenac most rapidly, with release complete in 24 h (Fig. 2C). **Bu-P**, **Ph-P** and **Bu-N** hydrogels released diclofenac more slowly, with complete release requiring ~120 h (Fig. 2D, S14). Like for fluorescein, the release of diclofenac from the **Bu-N** gel was much faster than from the other gels in DI water, suggesting that there may have been more non-specific binding of the drug in the **Bu-N** gel (Fig. S14D). However, unlike fluorescein, the release rates were not very dependent on the pH for any system. The pK_a value for diclofenac is reported to be 4.1,²⁸ so it should remain primarily in its anionic state from pH 5–7.4. Lowering of the pH to 4 resulted in visible precipitation of

diclofenac in the hydrogel. Thus, the pH-dependent release of drugs from the network depends on their specific chemical structures and pK_a values. Loading of an amino acid, tryptophan, was also attempted in deionized water, but due to its isoelectric point at 5.89, it existed predominately in its neutral form upon loading. When washed with water, all of the tryptophan was rapidly released (Fig. S11C).

The growth of C2C12 mouse myoblast cells on the hydrogels was also studied. The hydrogels were seeded with C2C12 cells, incubated for 2 h, then washed to remove unadhered cells. After 72 h, the cells were fixed and stained with 4',6-diamidino-2-phenylindole (DAPI, blue, cell nuclei) and Alexa Fluor 568 (cytoskeletons, red). The glass slide (control) was an excellent substrate for cell attachment and proliferation (Fig. 3A). The cells appeared healthy with normal cytoskeletons and were well spread over the entire surface. In contrast, for each of the **T(hp)-P**, **Bu-P**, and **Ph-P** hydrogels, no cells were detected on the

Fig. 3. Confocal microscopy images of C2C12 cells adhered to A) glass slide (control); B) **T(hp)-P** hydrogel (only weak autofluorescence of the gel was observed). The cell nuclei were stained blue and cytoskeletons were stained red. Each image represents an area of 0.45 x 0.45 mm.

surfaces (Fig. 3B, S15). To probe this further, the hydrogels were immersed in cell culture medium, then this medium was added to C2C12 cells. High cell viability was retained for each gel, as indicated by -(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assays (Fig. S16), suggesting that the gels did not leach toxic species that would prevent the growth of cells. We also investigated the toxicities of the onium monomers using MTT assays and found that it was dependent on the monomer structure. Greater than 80% viability was observed for **T(hp)-P** up to 1 mg/mL, whereas the other monomers exhibited toxicity at much lower concentrations (Fig. S17). The fact that resistance to cell adhesion occurred for all gels, regardless of monomer structure suggests that adhesion properties were dominated by the high PEG content, as PEG is known to resist protein adsorption and consequently cell adhesion.²⁹ However, it is also possible that direct contact between cells and monomers covalently incorporated into the gels played a role.

In conclusion, we have developed a new method for incorporating phosphonium cations into PEG based hydrogels to enable the ionic entrapment of anionic dyes and drug molecules. The hydrogel formation was completed rapidly and efficiently using UV light. The initial work to load anionic dyes and drug molecules has shown that the release rates can be tuned based on the chemical structures of the phosphoniums and anions, as well as the ionic strength and pH of the release medium. No growth of C2C12 cells was observed on the hydrogel surfaces, suggesting that they may resist fouling in biological systems.

Conflicts of Interest

There are no conflicts to declare.

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