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Cationic poly(VCL–AETA) hydrogels and ovalbumin (OVA) release in vitro

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Abstract The objective of this research is to explore the synthesis of a new family of water soluble polycationic copolymeric precursors that could be photo-crosslinked into hydrogels. The in vitro control release of ovalbumin protein (OVA) from this family of hydrogels was also studied to assess the biomedical potential of this new family polycationic hydrogels. A series of novel poly(VCL–AETA) copolymer hydrogels was fabricated in an aqueous medium via photo-induced polymerization and crosslinking of hydrophobic *N*-vinylcaprolactam (VCL) and hydrophilic [2-(acryloxy)ethyl]trimethylammonium chloride (AETA) monomers over a wide range of VCL to AETA feed molar ratios of 2:1, 1:1, 1:2, 1:5. *N,N′*-methylene bisacrylamide (MBA) was used as a crosslinker. Ovalbumin (OVA), a model antigen, was preloaded into poly(VCL–AETA) hydrogel precursors and its release profiles in pH 7.4 PBS at 37°C were investigated as a function of VCL to AETA monomer feed ratios over a period of 4 weeks. The in vitro results showed that OVA initial burst and subsequent sustained releases could be controlled by 3 material parameters: the hydrophobic VCL to hydrophilic AETA monomer feed ratios, crosslinking density and hydrogel degradation rate. Thus, the hydrophobic-hydrophilic VCL–AETA hydrogel network for controlled OVA release could offer advantages over organic solvent-based single component polymer system. However, these in vitro OVA release profiles may change in an in vivo environment.

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1 Introduction

Ovalbumin (OVA), a non-toxic protein derived from chicken eggs, has been successfully used as an antigen, cell-mediated immune (CMI) responses as well as for oral vaccine delivery [1, 2]. As a result, OVA carrier and its delivery have been extensively investigated [3–13]. For example, Kissel's group has developed ABA triblock copolymers consisting of poly(lactide-*co*-glycolide) (PLGA) A-blocks attached to central hydrophilic poly (ethylene oxide) (PEO) B-blocks for OVA (43 kDa) carrier and studied the effects of ABA block copolymer composition and molecular mass on the release, degradation and compatibility of OVA [4, 5]. They found that microspheres prepared from these absorbable aliphatic polyesters show a continuous and molecular mass-dependent release, reminiscent of cross-linked hydrogels, which attributed to a combined mechanism of swelling and erosion, leading to a hydrogel-like structure. Murthy et al. [6] reported an acid-sensitive microgel with a 50% OVA encapsulation efficiency and released 80% of their encapsulated protein after 5 h at pH 5.0. Nakaoka et al. [8] prepared OVA incorporated poly(DL-lactic acid) (PDLLA) granules to enhance OVA loading level to 20% and a sustained OVA release for 50 days. These reported approaches, however, involved OVA pre-loading in organic solvents, which may lead to a

loss in protein biological activity and toxicity from residual solvents.

In this paper, we report a new family of cationic hydrogels as OVA carrier. These cationic hydrogels were fabricated in an aqueous medium via photo-polymerization and crosslinking of two monomers: hydrophobic *N*-vinylcaprolactam (VCL) and hydrophilic [2-(acryloxy)ethyl]trimethylammonium chloride (AETA) in the presence of a crosslinker. The aqueous system could maintain OVA biological activity. Moreover, OVA release behaviors could be controlled by the feed ratio of hydrophobic VCL and hydrophilic AETA precursors, crosslinking density and hydrogel degradation rate.

2 Experimental section

2.1 Materials

Ovalbumin (OVA, chicken egg albumin, Grade V, 98% pure, molecular mass 45 kDa), QuantiPro® bicinchoninic acid (BCA) Assay Kit and crosslinker *N,N′*-methylene bisacrylamide (MBA) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Photo-initiator (Irgacure® 2959), 4-(2-hydroxyethoxy) phenyl-(2-hydroxy-2-propyl) ketone, was donated by Ciba Specialty Chemicals Inc. [2-(Acryloxy)ethyl]trimethylammonium chloride (AETA, 80 wt.% solution in water) and *N*-vinylcaprolactam (VCL) were phurchased from Aldrich (Milwaukee, WI, USA). All other reagents were of analytical grade and were used as received.

2.1.1 Synthesis of poly(VCL–AETA) hydrogel networks

To synthesize poly(VCL–AETA) hydrogel networks, hydrophobic VCL and hydrophilic AETA monomers at predetermined feed molar ratios, crosslinker MBA and photo-initiator Irgacure® 2959 were dissolved in distilled water. The VCL to AETA feed molar ratios were pure VCL (100%), 2/1, 1/1, 1/2, 1/5 and pure AETA (100%). Crosslinking agent MBA (0.2–0.8% mol/mol of monomers) and a photo-initiator [Irgacure® 2959 of 3% w/w of the monomers] were added to the clear monomer solution (3.75–4.50 ml). Although VCL has a hydrophobic caprolactam ring, VCL has 4% water solubility. The mixture solution was then poured into an aluminum mold (each of 5.30-cm diameter) and kept under a long wavelength UV lamp (365 nm, 8 W) for a period of 6 h. After the polymerization and crosslinking were complete, the resulting hydrogels were removed from the mold, repeatedly washed by distilled water for 24 h, and then dried under vacuum for several days at room temperature until constant weights.

2.1.2 Characterization

The crosslinked poly(VCL–AETA) hydrogel networks were characterized by standard chemical and physical methods like FTIR, DSC, and SEM.

Fourier transforms infrared (FTIR) spectra of the crosslinked networks were obtained from a Nicolet Magna-IR 560 spectrophotometer (Madison, WI). The sample was ground with KBr (90%) and pressed into pellets for FTIR scanning in the range from 400 to 4,000 cm^{-1} with a resolution of 8 cm^{-1}.

Differential scanning calorimetric data (DSC) were obtained by a differential scanning calorimeter (TA Instrument DSC model 2920). A piece of dried sample (about 3–5 mg) was placed in a sealed aluminum pan and the temperature was cooled to −50°C and then heated from −50°C to 250°C at a heating rate of 10°C under nitrogen purging.

Scanning electron microscope (SEM) was employed to analyze the surface morphological change of the networks as a function of 2 precursors' feed ratio and immersion time. The dried sample was used as the controlled specimens. Individual sample was incubated in 15 ml pH 7.4 PBS at 37°C for over 2 weeks, and the PBS was replaced every 2 days to ensure a constant pH of the media. At a predetermined time, the sample was gently removed and immediately frozen in liquid nitrogen to retain its swollen structure. The sample was subsequently dried for 3 days in a Virtis Freeze Drier (Gardiner, NY) under vacuum at −45°C. The freeze drier was returned to room temperature and the sample was removed. The samples were then fixed on aluminum stubs and gold coated for SEM observation by Stereo Scan 440 (JEOL LTD. Tokyo, Japan).

2.1.3 Swelling test

A known weight of dried poly(VCL–AETA) network gel (~25 mg) was immersed in a vial containing a 15-ml PBS buffer solution of pH 7.4 at 37°C. The swollen hydrogel was removed from the buffer solution at pre-determined intervals and excess surface water was blotted. The hydrogel was then weighed until equilibrium. A swelling ratio was calculated by the formula shown below and expressed in terms of the percentage of initial weight of dried sample. Swelling ratios of a sample were determined in triplicate:

$$\text{Swelling ratio } (\%) = [(W_t - W_o)/W_o] \times 100 \quad (1)$$

where W_t is the weight of the sample at time t and W_o is the initial dry weight of the sample.

The effect of ionic strength of the immersion media on swelling ratios (%) of poly(VCL–AETA) hydrogel (at VCL/AETA feed molar ratio 1/1) was conducted in different ionic

strength media (0.1, 0.5 and 1.0 M NaCl) PBS pH 7.4 at 37°C.

2.1.4 *In vitro degradation*

Disc-shaped specimens (7 mm in diameter, approximately 1–1.5 mm thickness) were placed in a vial containing 10 ml 0.1 M PBS (pH 7.4) at 37°C for up to 30 days. At predetermined intervals, sample was removed from its vial, washed with water and dried under vacuum. Mass loss was calculated by comparing the initial mass (M_0) with the mass measured at a given time point (M_t), as shown in Eq. 2. An average from three replica experiments was used and the results were presented as means ± standard deviation ($n = 3$).

$$\text{Mass loss } (\%) = (M_0 - M_t)/ \times 100 \quad (2)$$

2.1.5 *OVA loading and in vitro release study*

In this study, the ovalbumin (OVA) was pre-loaded into hydrogel precursor solutions before gelation. A fixed amount of OVA (10% w/w of the monomers) was added to the 4% (w/v) of distilled water solution of VCL, AETA, and 0.2–0.8% (mol/mol) MBA crosslinker with a photo-initiator (Irgacure® 2959) (0.1% w/v) in an aluminum mold of 5.3-cm diameter. The solution mixture was then photocrosslinked under a long wavelength UV lamp (8 W, 365 nm) for a period of 6 h. The resulting OVA loaded hydrogels were removed from the mold, and dried under vacuum at room temperature until constant weights.

OVA-loaded VCL–AETA hydrogel (1.0×2.0 cm^2, ~30 mg) was immersed in 5 ml of PBS (37°C, pH 7.4) containing 0.02% sodium azide as a bacteriostatic agent. The supernant was collected at a predetermined time over 2 weeks, and fresh PBS was added back to maintain the original volume. The amount of OVA released (into the supernant) was analyzed in triplicates using the BCA assay (Sigma) [14]. In briefly, aliquots (150 µl) were transferred to 2.0 ml test tube and 250 µl of BCA reagent was added. Test tubes were maintained at 60°C for 60 min, and their absorbance at 562 nm was measured by a UV/Vis spectroscopy. The cumulative amounts of OVA released from the hydrogels were determined from established OVA calibration curves. The data from three replicas with mean ± s.d. were reported. The results were plotted in terms of cumulative release as a function of time, according to Eq. 3:

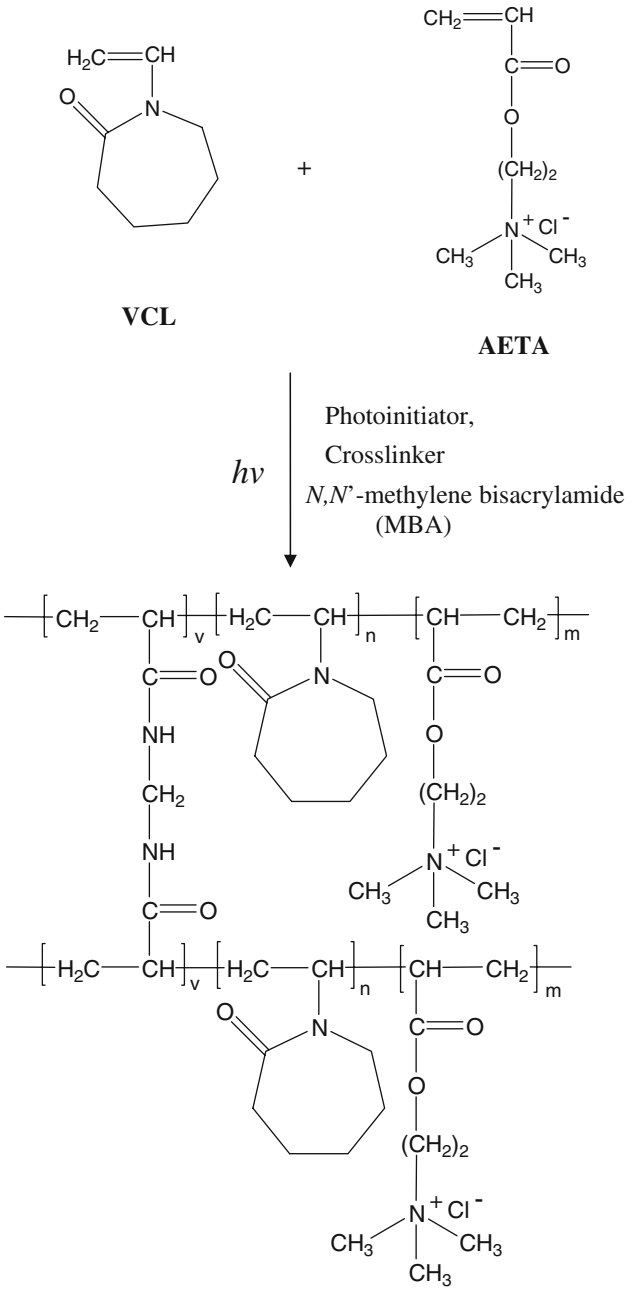
$$\text{Cumulative amount OVA released } (\%) = (O_t/O_o) \times 100 \quad (3)$$

where O_t is the amount of OVA released from the VCL–AETA hydrogels at time t and O_o is the initial amount of OVA pre-loaded into the VCL–AETA hydrogels.

3 Results

3.1 Preparation and characterization of VCL–AETA hydrogels

Scheme 1 illustrates the chemical structures of the precursors (VCL and AETA) as well as the formation of VCL–AETA cross-linked hydrogel networks using a water-soluble crosslinker, *N,N′*-methylene bisacrylamide (MBA) *via* photo-induced free-radical polymerization/crosslinking. The



Scheme 1 Scheme of poly(VCL–AETA) hydrogel formation *via* photo-polymerization and crosslinking

photo-initiator chosen, Irgacure® 2959, is water-soluble with 0.1% (w/w) of the precursors in this study. VCL/AETA feed molar ratio ranged from 2/1, 1/1, 1/2 to 1/5, and the MBA crosslinker changed from 0.2% to 1.8% (mol/mol) of the precursors. About 100% AETA network did not retain its 3D network structure in PBS, i.e., deformed immediately like a viscous fluid gel. While 100% VCL did not form a network under this study condition that was similar to literature results [15]. The hydrogel networks obtained in this study, however, were mechanically strong to handle and insoluble after wash and dried. Table 1 summarizes the VCL–AETA hydrogel fabrication conditions like precursors' feed molar ratios and crosslinker concentration. The DSC results showed that as the concentration of MBA crosslinker increased, the formed hydrogel network exhibited a higher melting temperature, T_m. The T_m of the hydrogel network also increased with a decrease of the VCL/AETA precursors' feed molar ratio from 2/1, 1/1, 1/2 to 1/5. It demonstrated that the caprolactam ring of VCL component may introduce steric hindrance which could prevent a denser macromolecular packing and hence influenced VCL/AETA network curing and formulation.

Figure 1 shows the FTIR spectra of poly(VCL–AETA) with VCL/AETA feed molar ratios 2/1 (Spectrum A) and 1/5 (Spectrum B) along with the FTIR spectra of precursors VCL (Spectrum C) and AETA (Spectrum D). There are four characteristic absorption peaks in Fig. 1. The big absorption band at 1,732 cm^{-1} is ascribed to ester carbonyl stretching from AETA component. This band intensity increases as the AETA component in the network increases (Spectra A–B). The 1,635–1,625 cm^{-1} band is amide absorption including PVCL cyclic amide I band (ν_{CO}) and acyclic amide I band (ν_{CO}) of crosslinker MBA. This band intensity increases with increasing VCL content in the network (Spectra B–A). The bands at 1,483 and 1446 cm^{-1} are C–N stretching (ν_{C-N}) and C–H deformation bands (δ_{C-H}), respectively. A comparison of these network FTIR spectra with precursors VCL and AETA (Spectra C and D) shows that the ester carbonyl stretch vibration of AETA (ν_{CO}) was shifted to a higher value when AETA is incorporated into poly(VCL–AETA) network (from 1,728 to 1,732 cm^{-1}). While the carbonyl stretch vibration of VCL (ν_{CO}) was shifted to a lower value when VCL is incorporated in poly(VCL–AETA) network (from 1,658 to 1,635 cm^{-1}). The C=C double bond stretches at 1,650 and 822 cm^{-1} are completely disappeared after photoinitiation for 6 h [16]. Based on the FTIR data, it is concluded that AETA and VCL precursors were successfully integrated into a network with the MBA crosslinker. In other words, the formed network structure had both pendant

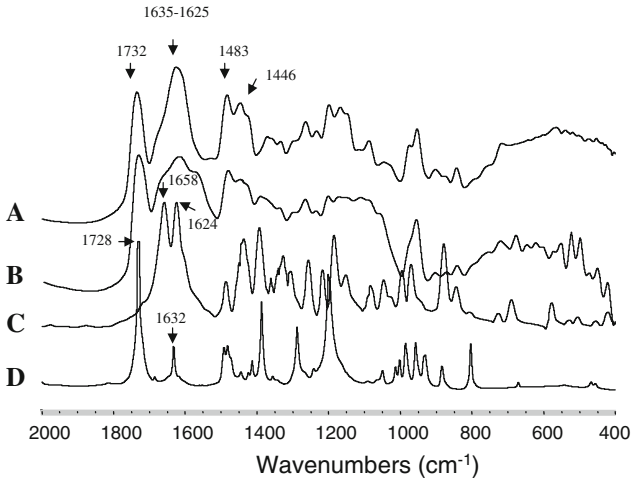


Fig. 1 FTIR spectra of poly(VCL–AETA) hydrogel networks. (**a**) VCL/AETA monomer feed molar ratio 2/1; (**b**) VCL/AETA monomer feed molar ratio 1/5; (**c**) VCL monomer; (**d**) AETA monomer

Table 1 Poly(VCL–AETA) hydrogel preparation feed and thermal properties

Code	VCL (mmol)	AETA (mmol)	VCL/AETA (mmol)	MBA (mmol)	MBA (mmol%)	Irg (mg)	H_2O (ml)	T_m (°C)
VCLAETA1	1.0	0.5	2/1	0.009	0.596	10	3.75	82.5
VCLAETA2	1.0	1.0	1/1	0.012	0.596	10	3.75	87.4
VCLAETA3	1.0	2.0	1/2	0.018	0.596	10	3.75	92.0
VCLAETA4	1.0	5.0	1/5	0.036	0.596	10	3.75	95.0
AETA	–	2.0	–	0.012	0.596	10	3.75	147
VCL	1.0	–	–	0.006	0.596	10	3.75	35
VCLAETA2-1	1.0	1.0	1/1	0.004	0.199	10	3.75	77.5
VCLAETA2-2	1.0	1.0	1/1	0.008	0.398	10	3.75	82.0
VCLAETA2-3	1.0	1.0	1/1	0.016	0.793	10	3.75	86
VCLAETA2-4	1.0	1.0	1/1	0.020	0.990	10	3.75	92
VCLAETA2	1.0	1.0	1/1	0.012	0.596	10	3.75	87.4
VCLAETA2'	1.0	1.0	1/1	0.012	0.596	10	4.50	87
VCLAETA2''	1.0	1.0	1/1	0.012	0.596	10	5.50	86

hydrophobic caprolactam ring and hydrophilic quaternary ammonium cationic group (N^+) along the backbone.

3.2 Swelling behavior

Figure 2a exhibits swelling behaviors of poly(VCL–AETA) hydrogels as a function of different VCL/AETA precursors' feed molar ratios (2/1, 1/1, 1/2 and 1/5) in 0.1 M PBS at pH 7.4 at 37°C over a period of 1 week. Generally, an initial burst phase followed by a gradual phase of swelling was found. The rate and the extent of swelling depended on the hydrophobic (VCL) to hydrophilic (AETA) feed ratio. An increase in hydrophilic component led to a higher swelling ratio, and such an effect became more pronounced at a longer period. For example, initial swelling ratios (4 h) of poly(VCL–AETA) networks at 2/1, 1/1, 1/2, and 1/5 VCL/AETA feed molar ratio were 768, 1,012, 1,312 and 1,512%, respectively. At 8 h, the swelling ratios increased to 1,500, 1,618, 2,088 and 2,488%, respectively. At the end of 7 days, the maximum swelling ratios were 4,978, 5,088, 6,971 and 11,350% for 2/1, 1/1, 1/2, and 1/5 feed molar ratios of VCL/AETA, respectively, i.e., near triple the swelling ratio as the VCL to AETA feed ratio changed from 2/1 to 1/5. The poly(VCL–AETA) hydrogels did not show any sign of degradation until after 18 days.

Figure 2b shows the effect of different MBA crosslinker amounts (as % of the two precursors) on the swelling ratios of poly(VCL–AETA) hydrogels (at VCL/AETA feed molar ratio 1/1) in 0.1 M PBS pH 7.4 at 37°C. A higher crosslinker amount led to a lower swelling ratio of the hydrogels. For example, the hydrogel with 0.2% MBA crosslinker (VCLAETA2-1) swelled to 1,512% at 4 h, while the hydrogel with 1.0% MBA (VCLAETA2-4) swelled to only 641% at the same internal. At the end of 5 days, the swelling ratios of the hydrogels with 0.2, 0.4, 0.8 and 1.0% MBA were 4,900, 5,100, 4,196 and 3,558%, respectively. The data in Fig. 3 indicate that the crosslinking level of poly(VCL–AETA) hydrogels (controlled by the amounts of the MBA crosslinker used) could control both initial and equilibrium swelling ratios.

Figure 2c shows the effect of different ionic strength media (0.1, 0.5 and 1.0 M NaCl) on swelling ratios (%) of poly(VCL–AETA) hydrogel (at VCL/AETA feed molar ratio 1/1) in PBS pH 7.4 at 37°C. In general, a higher ionic strength medium reduced swelling ratio. For example, at the 12th day, the swelling ratios were 5,058, 4,898 and 4,618% for 0.1, 0.5 and 1.0 M, respectively. This observed ionic strength effect on swelling property of poly(VCL–AETA) hydrogels was consistent with Kim et al. [17] study of dextran-maleic acid-based hydrogels as well as Pratt et al. study of water uptake of polyglycolide (PGA) disc [18]. Kim et al.

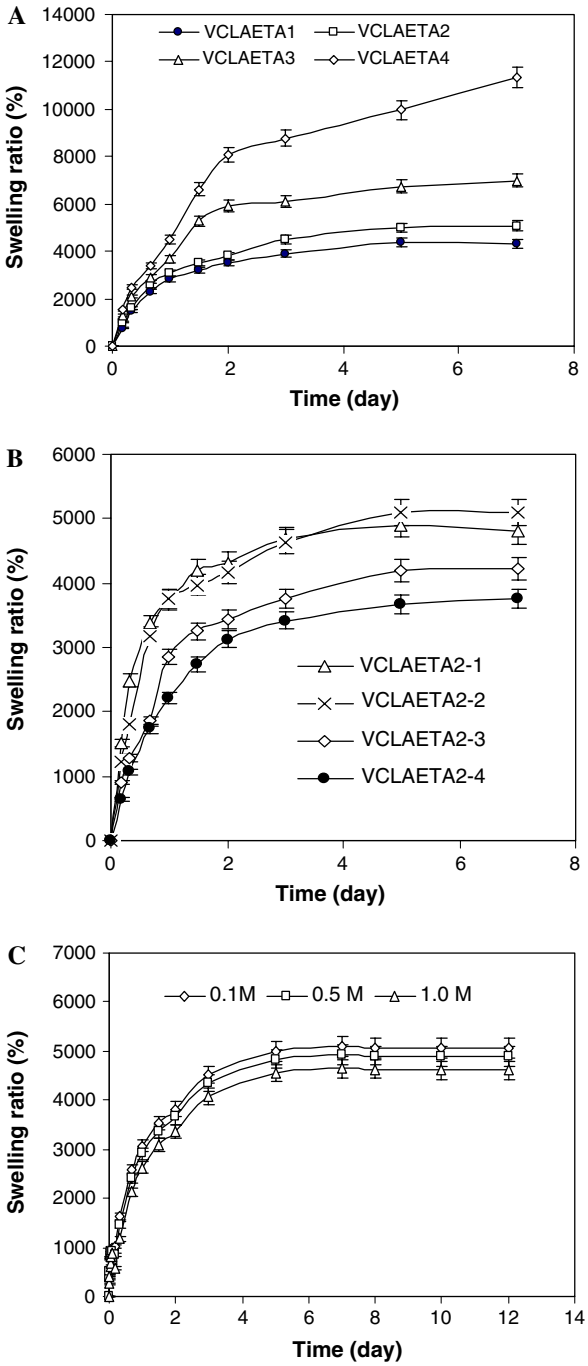


Fig. 2 (**a**) Swelling ratios (%) of poly(VCL–AETA) hydrogels having different VCL to AETA feed molar ratios. (•) VCLAETA1 at VCL/AETA 2/1, (□) VCLAETA2 at VCL/AETA 1/1, (Δ) VCLAETA3 at VCL/AETA 1/2, (◇) VCLAETA4 at VCL/AETA 1/5. 0.1 M PBS pH 7.4 at 37°C. (**b**) The effect of MBA crosslinker concentration on the swelling ratios (%) of poly(VCL–AETA) hydrogels at a fixed VCL/AETA feed molar ratio 1/1. MBA crosslinker concentration as a percentage of monomers: 0.2% for VCLAETA2-1; 0.4% for VCLAETA2-2; 0.8% for VCLAETA2-3; 1.0% for VCLAETA2-4. 0.1 M PBS pH 7.4 at 37°C. (**c**) The effect of ionic strength of media on swelling ratios (%) of poly(VCL–AETA) hydrogel (at VCL/AETA feed molar ratio 1/1) Ionic strength ranged from 0.1 M, 0.5 M and 1.0 M NaCl in PBS pH 7.4 at 37°C

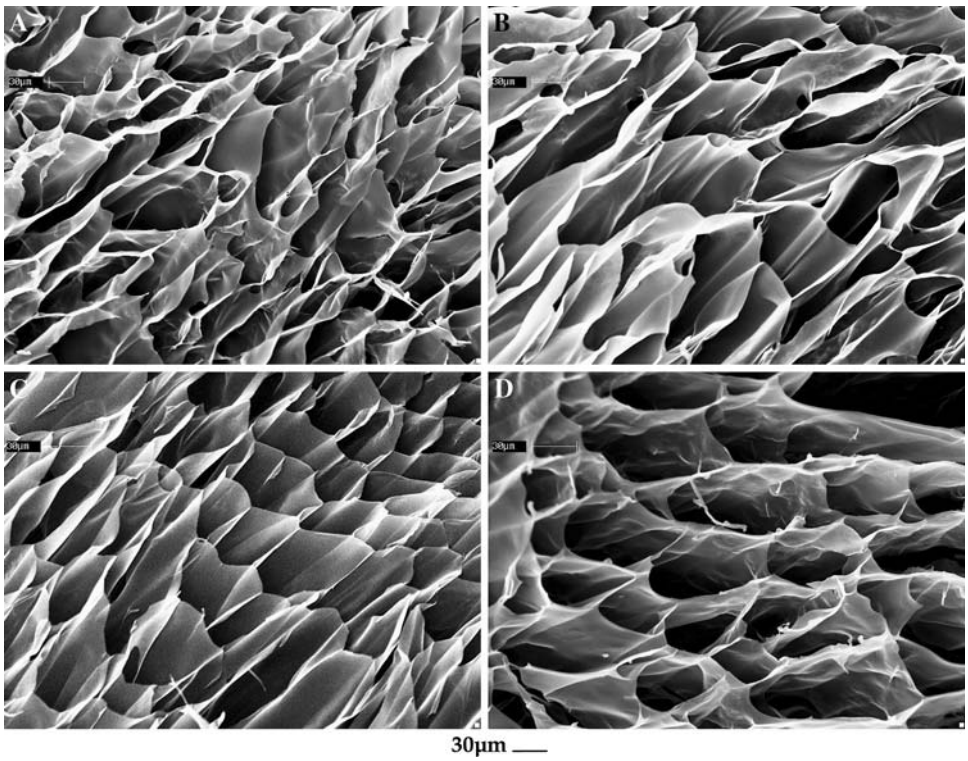


Fig. 3 Morphology of equilibrium swollen poly(VCL–AETA) hydrogels having different VCL/AETA monomer feed ratio. (**a**) VCL/AETA 2/1; (**b**) VCL/AETA 1/1; (**c**) VCL/AETA 1/2; (**d**) VCL/AETA 1/5

reported a near 65% reduction in swelling ratio of dextran-maleic acid hydrogel when the ionic strength of aqueous-based medium increased from 0 to 15. They attributed the ionic strength induced reduction in swelling ratio to the shielding of the ionized carboxylic acid group in pendant maleic acid segment by counter-ions from electrolytes. Such counter-ion shielding would reduce the electrostatic repulsion of carboxylic acid anions, i.e., reduction in swelling. Pratt et al. attributed the effect of electrolytes on the reduction in water uptake of PGA discs to the chemical potential differences of water between electrolyte solutions and pure water [18] and a lower water chemical potential in an electrolyte solution would slow the diffusion process and result in a slower and smaller water uptake.

3.3 Morphology of poly(VCL–AETA) hydrogels

Figure 3 exhibits morphology of swollen poly(VCL–AETA) hydrogels of different VCL to AETA feed molar ratios from 2:1 (a) to 1:5 (d) at 37°C. The data illustrate composition-depended network morphology in which an increase in hydrophilic AETA component in the poly(VCL–AETA) network (from a to d) resulted in a more open and less compact three-dimensional porous network structure. The cell wall appeared to be very thin and wavy. Figure 4b exhibits interior open channel structure of swollen poly(VCL–AETA) hydrogel (at VCL/AETA feed molar ratio 1/1). These channels appeared relatively straightforward and went through the whole hydrogel and became the conduits for swelling and OVA release.

Figure 5 shows the effect of different MBA crosslinker amounts (from 0.2% to 0.8%) on mass loss kinetics of poly(VCL–AETA) hydrogels (at VCL/AETA feed molar ratio 1/1) in 0.1 M PBS pH 7.4 at 37°C over a period of 30 days. The data suggest that poly(VCL–AETA) hydrogels having a lower amount of MBA crosslinker would degrade more and faster than those hydrogels having a higher MBA amount, e.g., VCLAETA2-1 (0.2% MBA) versus VCLAETA2-3 (0.8% MBA). The weight loss data also indicated that, in the first 4 days of immersion, no apparent degradation occurred for all three hydrogels having 0.2–0.8% MBA cross-linker; thereafter, a lower MBA crosslinker gel (like 0.2%) degraded earlier, faster and reached 14% weight loss at the end of 20 days, while a higher amount of MBA crosslinker gel (VCLAETA2-3 with 0.8% MBA) did not shown any apparent weight loss until about 12 days, a whole 8 days longer than VCLAETA2-1 gel (0.2% MBA).

3.4 OVA release from the poly(VCL–AETA) hydrogels

The in vitro cumulative release profiles of OVA (%) from poly(VCL–AETA) hydrogels in PBS at 37°C are shown in Fig. 6a. The data show that, regardless of hydrogels formulations, OVA release from VCL/AETA hydrogels all

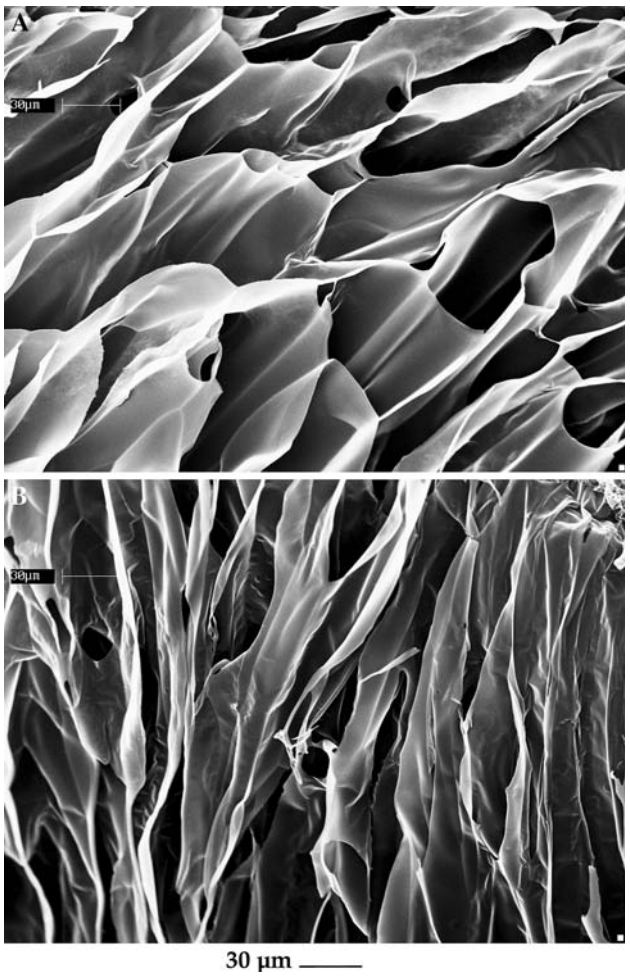


Fig. 4 Morphology of equilibrium swollen poly(VCL–AETA) hydrogel (at VCL/AETA monomer feed ratio 1/1). (**a**) pore structure; (**b**) open water channel structure inside the hydrogel

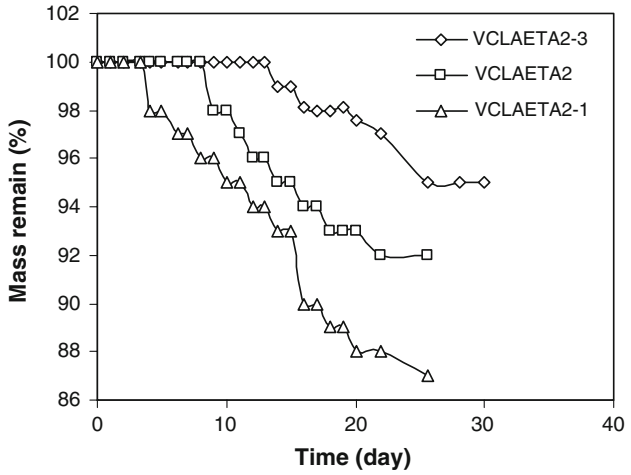


Fig. 5 The effect of MBA crosslinker concentration (as a percentage of monomers) on mass retention (%) of poly(VCL–AETA) hydrogels (at a VCL/AETA monomer feed ratio 1/1). MBA crosslinker concentration: 0.2% for VCLAETA2-1; 0.6% for VCLAETA2; 0.8% for VCLAETA2-3; Immersion medium: 0.1 M PBS pH 7.4 at 37°C

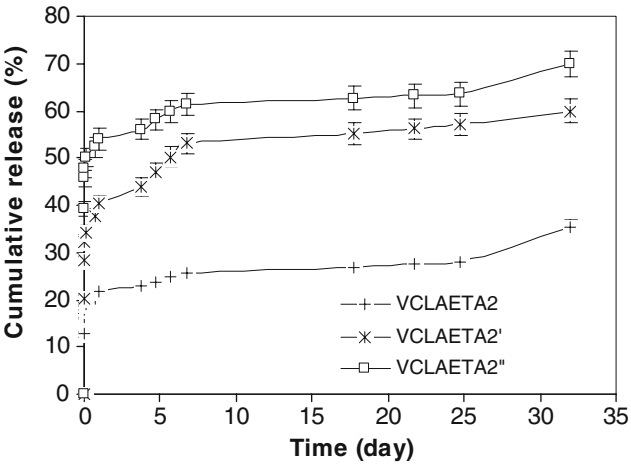


Fig. 6 (**a**) The effect VCL to AETA monomer feed molar ratio during gelation process on the OVA cumulative release profiles from poly(VCL–AETA) hydrogels in pH 7.4 PBS at 37°C (OVA loading level 10% of monomer (w/w)). VCLAETA1 at VCL/AETA feed ratio 2/1; VCLAETA2 at VCL/AETA feed ratio 1/1; VCLAETA3 at VCL/AETA 1/2; VCLAETA4 at VCL/AETA feed ratio 1/5. (**b**) The effect of MBA crosslinker level on the OVA release profiles from poly(VCL–AETA) hydrogels (OVA loading level 10% of monomer (w/w)). VCLAETA2-1: 0.2% MBA; VCLAETA2-2: 0.4% MBA; VCLAETA2: 0.6% MBA; VCLAETA2-3: 0.8% MBA, and VCLAETA2-4: 1.0% MBA. Immersion medium: 0.1 M PBS pH 7.4 at 37°C. (**c**) The effect of VCL and AETA monomer concentrations on OVA cumulative release profiles (%)from poly(VCL–AETA) hydrogel (at VCL/AETA feed ratio 1/1, and OVA loading level 10% of monomer (w/w)). VCLAETA2: 8.8% monomer concentration; VCLAETA2′: 7.3% monomer concentration; VCLAETA2″: 6.0% monomer concentration w/v). Immersion medium: 0.1 M PBS pH 7.4 at 37°C

had a characteristic of an initial burst release followed by a gradual release. For example, the first 20 min initial cumulative release percentages were 9.4, 14, 24 and 26% for poly(VCL–AETA) hydrogels with VCL/AETA feed molar ratios of 2/1, 1/1, 1/2, and 1/5, respectively. Thus, poly(VCL–AETA) hydrogels having a lower VCL to AETA feed molar ratio showed a larger OVA burst release. After the initial burst release period (first 40 min), the sustained OVA release phase from poly(VCL–AETA) hydrogels having feed molar ratio 2/1 and 1/1 were very slow, while the release of OVA from poly(VCL–AETA) hydrogels having higher AETA content (i.e., feed molar ratio 1/2 and 1/5) were quite fast. At the end of 30 days, cumulative OVA release from poly(VCL–AETA) hydrogels having higher or equal VCL precursor in the feed ratio (2/1 and 1/1) reached to 27% and 34%, respectively; while hydrogels having higher AETA precursor in the feed ratio (1/2 and 1/5) showed a complete OVA release at 25 days and 18 days, respectively. Thus, an increase in a hydrophobic precursor like VCL in the precursor feed ratio would lead to a lower initial burst and more sustained release of OVA from the poly(VCL–AETA) hydrogels.

The effect of crosslinking density (in terms of the concentration of MBA crosslinker from 0.2% to 1.0%) on OVA release kinetics from poly(VCL–AETA) hydrogels in 0.1 M PBS pH 7.4 at 37°C is shown in Fig. 6b. As the concentration of MBA crosslinker increased in the poly(VCL–AETA) hydrogel formulation, the initial burst release of OVA at 1 h was reduced. For example, as MBA concentration increased from 0.2% to 1.0%, the first 1 h OVA burst release was reduced from 22.9% to 9.8%, a near 60% reduction. At the end of 30 days study period, 46–29% cumulative OVA releases were observed for poly(VCL–AETA) hydrogels formulated at the crosslinker concentrations 0.2–1.0%. Clearly, the crosslinking level of the resulting gel network structure would have a profound effect on the magnitudes of OVA initial burst as well as subsequent sustained releases.

The effect of VCL and AETA precursor concentrations (VCLAETA2: 8.8%; VCLAETA2′: 7.3%; VCLAETA2″: 6.0%) during the formulation of poly(VCL–AETA) hydrogels on the OVA cumulative release profiles in 0.1 M PBS pH 7.4 at 37°C is shown in Fig. 6. The data in Fig. 6 suggest that the precursors' concentration effect was similar to the effect of MBA crosslinker concentration shown in Fig. 6b, i.e., an increase in precursors' concentrations leads to a reduction in both initial burst and sustained releases of OVA. For example, at the first 20 min, the amounts of OVA cumulative releases decreased from 39.3, 20.1, to 13.6%, as the precursors' concentration increased from 6.0, 7.3, to 8.8%, respectively; and this relationship was held throughout the whole 32 days study period. In addition, a higher of precursor concentration during hydrogel formulation led to a slower release rate. For example, at 2 h, 50% OVA released from the poly(VCL–AETA) hydrogel formulated from 6.0% (w/v) precursor concentration, while it took 5.6 days for the poly(VCL-ETA) hydrogel formulated at 7.3% monomer concentration to reach the same level of OVA release. VCLAETA2 (8.0% w/v) showed the lowest initial burst release and the longest sustained release among the 3 samples (6.0, 7.3, and 8.0% w/v) over the entire 35 days.

4 Discussion

As shown in Scheme 1, the poly(VCL–AETA) network was formulated in an aqueous system at room temperature. This aqueous condition is considered to be more favorable for the pre-loading of water-soluble biologically active agents like OVA into the gel network. OVA can mix homogenously with both VCL and AETA precursors as well as crosslinkers before network formation. Moreover, OVA (OVA's pI 4.6) bears a negative charge at neutral pH medium. The negative charge characteristic of OVA could have the potential to be attracted into the positive quaternary ammonium salt of the AETA segment in the poly(VCL–AETA) hydrogel network. Thus, depending on the relative concentration of OVA to the AETA in poly(VCL–AETA), a complex between positive quanternary ammonium and negative charge OVA might form and be dispersed in the VCL–AETA matrice. In this study, a 10% OVA pre-loading level of monomer (w/w) in a VCL–AETA matrix (molar ratios of VCL/AETA = 2/1, 1/1, 1/2, 1/5) was achieved. A similar strategy was applied by Van Tomme et al. for protein encapsulation [19]. They entrapped model proteins (lysozyme, BSA and IgG) by hydration of mixtures of oppositely charged hydroxyethyl methacrylate-derivatized dextran microspheres with a protein solution. A negative charge BSA at neutral pH was adsorbed onto the surface of positively charged microspheres.

The present study has demonstated clearly that 10% OVA pre-loaded poly(VCL–AETA) hydrogels had an controllable OVA release profiles in pH 7.4 PBS at 37°C, depending on the feed ratio of VCL to AETA monomers (Fig. 6a). The controllable OVA release was attributed to the swelling-induced 3D porous network structures that provided open channels for OVA diffusion (Figs. 2a, 4 and 5). A higher AETA component of poly(VCL–AETA) hydrogel, such as VCLAETA3 (1:2 VCL to AETA feed ratio) and VCLAETA4 (1:5 feed ratio) promoted OVA released easily and quickly. This fact indicates that a complex formation between positive quanternary ammonium (from AETA) and negative charge OVA is not a domain factor during the OVA release. The charge attraction may be too weak to be effective because OVA may not be negative charge enough to displace the Cl counter anion in quanternary ammonium salt of AETA. On the other hand, a higher swelling ratio of those poly(VCL–AETA) hydrogels having higher AETA contents led to a severe OVA concentration gradient that could promote fast OVA release. However, the initial burst release of OVA can be controlled by changing feed radio of VCL–AETA. This study demonstrated that an initial burst release % during the first 20 min decreased from 26% to 9.4% as the VCL to AETA feed ratio changed from 1:5 to 2:1. This reduction in the initial burst release of OVA is significantly better than others OVA release studies. For example, Nakaoka et al. reported a 25% burst release of 10% OVA-loaded PDLLA granules in PBS at 37°C [8], and Kissel et al. reported 80% burst release of 5%OVA-loaded ABA block copolymer microspheres in PBS pH 7.2 at 37°C [4]. We attributed the significant reduction in our OVA burst release to a more hydrophobic domain of our poly(VCL–AETA) hydrogel network, and such hydrophobic domain could promote a stronger OVA–VCL hydrophobic interaction to retard both water uptake and OVA overall release. The observation is consistent with other published studies [20–23]. For example, Kishida's [22] reported that adding a

hydrophobic benzyl group into hydrophilic poly(γ-glutamic acid) matrices suppressed the initial penetration of water into polymer films, and hence was able to inhibit burst release of 5-fluorouracil.

This present study also demonstrated that crosslinker density could also affected OVA release. For example, VCLAETA3 sample exhibited a slightly higher release after 22 days. The reason behind this observation is that this particular poly(VCL–AETA) hydrogel had a lower amount of MBA crosslinker and would degrade faster at that late period (Fig. 5). Similar strategy was used by Kishida et al. in their study of poly(γ-glutamic acid)(γ-PGA) benzyl ester (γ-PBG) as a drug delivery [22]. Because γ-PBG degraded very slowly in a phosphate buffer solution (pH 7.4), the release of 5-fluorouracil (5-FU) was sustained for 130 days after an initial burst-release. Thus, present study of the hydrophobic–hydrophilic VCL–AETA network for controlled OVA release apparently offers an advantage over either AETA or a VCL based single-component gel system.

However, in an in vivo environment, the OVA release profiles from this protein carrier system could be changed because of the factors, such as the formation of a protein biofilm on the polymeric surface when in contact with body fluid and the possible formation of a fibrin clot around the carrier.

5 Conclusions

A series of novel copolymer hydrogels for OVA delivery have been fabricated from photo-induced polymerization and crosslinking of hydrophobic VCL and hydrophilic AETA monomers over a wide range of monomer feed ratios in an aqueous medium. The hydrophobic and charge characteristics of these gel networks could be controlled by a simple adjustment of the VCL to AETA monomer feed ratios as well as the concentration of MBA crosslinker for achieving a wide range of OVA release profiles. An increase in hydrophobic VCL monomer in the feed ratio during gelation led to a significant reduction in initial OVA burst release and provided a sustain release over a period of 30 days. Because the gelation process was done in an aqueous environment, an organic solvent-induced adverse effect on OVA bioactivity could be eliminated. The controlled release mechanism of this poly(VCL–AETA) copolymer gels was based on the combination of three material factors: the rate and degree of formation of swelling-induced 3D porous structure in the hydrogel, the hydrolytic degradation of networks and the interaction between components and OVA. The ease of gel fabrication in an aqueous environment and control of final gel properties may be the advantages for a range of biotechnological applications including drug delivery.

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