Synthesis and characterization of PEG-P(MAA-SS-VCL) nanoparticles

L L Yu1,2, K Yang1, R H Mu2, N Zhang1 and L Su1

1Department of Pharmacy, Xi’an Medical University, Xi’an, shaanxi, 710021, China
2College of Environmental and Chemical Engineering, Xian Polytechnic University, Xian 710048, China

E-mail: yulili0218@163.com

Abstract. The PEG-P(MAA-SS-VCL) nanoparticles were obtained using disulfide containing dimethacrylate (SS) as cross-linking agent, using polyethylene glycol methyl acrylate (PEGMA), N-Vinyl-ε-caprolactam (VCL), and methacrylic acid (MAA) as monomers via homogeneous polymerization in aqueous. The PEG-P(MAA-SS-VCL) nanoparticles were characterized by FT-IR and TGA. The particle size and morphology variation in different environments were detected by dynamic light scattering (DLS) and scanning electron microscopy (SEM). It is the very method that PEG-P(MAA-SS-VCL) nanoparticles can be obtained in this study.

1. Introduction
Nanoparticles which can release drug at the targeted site have attracted much attention in recent years.[1] Nanoparticles which can respond to certain triggers including pH[2], redox[3], temperature[4], specific enzymes[5], and so on, have become a research hotspot. The literature reports show that disulfide bond is redox sensitivity, while poly (methacrylic acid) (PMAA) has good pH sensitivity. [2] Herein, we report on the design and fabrication of a redox and pH dual responsive nanoparticle by incorporating disulfide bond containing network into pH-responsive PMAA particle matrix. The preparation and the property of the nanoparticles were investigated by FT-IR, TGA, DLS, and SEM.

2. Materials and methods

2.1. Materials
Vinyl caprolactam (VCL), Methoxypolyethylene glycols 350 (PEG350OH) were purchased from Alfa Aesar. 2, 2'-dithiodiethanol was provided by Alarich. Methacryloyl chloride and methyl acrylic acid (MAA) were purchased from Energy Chemical. Glutathione (GSH) was obtained from Shanghai source biological technology co., LTD. Tetrahydrofuran (THF), triethylamine (TEA), and dichloromethane (DCM) were distilled over CaH2. Poly(ethylene glycol) methyl ether methacrylate (PEGMA) and the cross-linking agent (2, 2'-dithiodiethanol dimethacrylate, SS) were obtained according to the literatures [6, 7].
2.2. Preparation of PEG-P(MAA-SS-VCL) nanoparticles
0.97 g VCL, 0.03g MAA, 20 mg SDS, 25 mg NaHCO$_3$ and a certain amount SS (20 mg, 40 mg, 80 mg, 120 mg, and 160 mg respectively) were dissolved in 100 mL water in round bottom flask. The mixture was stirred for 30 mins at 70°C in N$_2$. Then, Potassium persulfate (KPS) solution (25 mg KPS in 2 mL distilled water) and 10 mg PEGMA were added to the mixture under N$_2$. Keeping the original temperature for another 6 hs, the solution was transferred into a dialysis bag, and dialyzed against 500 mL water for 24 hs, the dialysis medium was exchanged every 2 hs. The dried nanoparticles were obtained by freeze drying the dialyzed solution at -70 ºC.

2.3. Measurement
The compositions of PEG-P(MAA-SS-VCL) nanoparticles with different amount of cross-linking agent were determined by FT-IR spectra (Bruker, Tensor 27). TGA data were recorded on a Thermogravimetry analyzer (TA, Q500) under a flowing nitrogen atmosphere at a heating rate of 20 ºC/min from 100 to 650 ºC. Particle size and Zeta potential analysis were also determined by DLS (Malvern, ZEN3600). The surface morphology of PEG-P(MAA-SS-VCL) nanoparticles was characterized by SEM using Nanoscope IIIa.

3. Results and discussion

3.1. Synthesis of PEG-P(MAA-SS-VCL) nanoparticles
Homogeneous free radical polymerization is such an effective method for preparation of nanoparticles, so that this method has been employed to the preparation of PEG-P(MAA-SS-VCL) nanoparticles using PEGMA, VCL, MAA as monomers, and SS as cross-linking agent. The chemical formation of PEG-P(MAA-SS-VCL) nanoparticles with different cross-linking degree were confirmed by FT-IR spectra as illustrated in Fig. 1. The FT-IR data displayed that strong peaks for C=O stretching vibration at 1728 cm$^{-1}$ and 1631 cm$^{-1}$ were attributed to PMAA and PVCL. Specific peaks for C- N (1440 cm$^{-1}$) belonged to PVCL, and PEG (1144 cm$^{-1}$) was observed in all nanoparticles. Remarkably, the intensity peak ratio (1728 cm$^{-1}$ to 1631cm$^{-1}$) for nanoparticles with 20 and 40 mg cross-linking agent was much higher than samples with more cross-linking agent, suggesting that the reaction rate of MAA and VCL was relating to the dosage of the cross-linking agent. As in Fig. 1, a high cross-linking agent dosage was favorable for the reaction rate of MAA in the nanoparticles. Disulfide in both nanoparticles could not be confirmed by FT-IR spectra, for the signal for disulfide bond is rather weak. Thus, the content of disulfide for nanoparticles was determined according to Ellman’s titration, which was 51, 87, 115, 156, and 205μM/g, respectively, while for nanoparticles with different cross-linker concentration (20 mg, 40 mg, 80 mg, 120 mg, and 160 mg), things were different and that was why the existence of disulfide in nanoparticles was validated.

Figure 1. FT-IR spectra of PEG-P (MAA-SS-VCL) with different crosslinking degree.

Figure 2. TGA thermograms of PEG-P(MAA-SS-VCL)-80 mg and PEG-P(MAA-SS-VCL)-60 mg.
Furthermore, the structural differences for PEG-P(MAA-SS-VCL) nanoparticles with different cross-linker concentration (80 mg and 160 mg) were also confirmed by TGA data twice. As shown in Fig. 2, pyrolysis temperature of PEG-P(MAA-SS-VCL) nanoparticles was 302 °C, and the thermal decomposition processes of nanoparticles were composed of two stages. Meanwhile, pyrolysis temperature of PEG-P (MAA-SS-VCL) with 80 mg crosslink agent was lower than PEG-P(MAA-SS-VCL) with 160 mg (Fig. 2), which indicated that the cross-linked structure was conducive to the thermal stability of nanoparticles.

3.2. **GSH and pH triggered variation of PEG-P(MAA-SS-VCL) nanoparticles**

As shown in Table 1, particle size variation is not pH dependent, while GSH concentration has a certain effect on the particle size. At low GSH level, the effect is not significant, but the particle size has a trend to be larger at the high GSH level. Because of disulfide bond breakage at the high GSH level, the contact between the polymer chain segments can be weakened with the nanoparticles' structures swelling. In addition, with the increase of the amount of cross-linking agent the change trend can be weakened too.

| Table 1. Particle size variation in PEG-P(MAA-SS-VCL) nanoparticles in different condition. |
|-----------------------------------------------|
| **SS**=20 mg | **SS**=40 mg | **SS**=80 mg | **SS**=120 mg | **SS**=160 mg |
| Size (nm) | PDI | Size (nm) | PDI | Size (nm) | PDI | Size (nm) | PDI | Size (nm) | PDI |
| pH=2.2 | 165.0 | 0.10 | 189.9 | 0.04 | 166.3 | 0.16 | 127.8 | 0.04 | 169.3 | 0.08 |
| pH=5.0 | 233.9 | 0.25 | 233.3 | 0.20 | 161.7 | 0.13 | 130.5 | 0.08 | 176.0 | 0.12 |
| pH=7.4 | 178.4 | 0.09 | 490.7 | 0.02 | 160.7 | 0.15 | 155.6 | 0.26 | 181.2 | 0.07 |
| GSH 0.002 mmol/L | 169.1 | 0.10 | 199.8 | 0.05 | 167.8 | 0.18 | 140.3 | 0.11 | 183.5 | 0.06 |
| GSH 2 mmol/L | 173.4 | 0.14 | 294 | 0.17 | 254.7 | 0.24 | 138 | 0.11 | 207.3 | 0.18 |
| GSH 10 mmol/L | 459.5 | 0.35 | 933.4 | 0.53 | 242.4 | 0.31 | 144.4 | 0.04 | 192.5 | 0.07 |

| Table 2. Zeta potential variation in PEG-P(MAA-SS-VCL) nanoparticles in different condition. |
|-----------------------------------------------|
| **SS**=20 mg | **SS**=40 mg | **SS**=80 mg | **SS**=120 mg | **SS**=160 mg |
| pH=2.2 | pH=5.0 | pH=7.4 | GSH 0.002 (mmol/L) | GSH 2 (mmol/L) | GSH 10 (mmol/L) |
| SS=20 mg | -20.8 | -24.4 | -28.5 | -35.2 | -11.90 | -8.20 |
| SS=40 mg | -13.4 | -45.0 | -33.3 | -36.2 | -10.10 | -7.16 |
| SS=80 mg | -16.2 | -28.9 | -37.7 | -23.7 | -6.88 | -5.85 |
| SS=120 mg | -15.5 | -15.3 | -36.2 | -32.8 | -14.20 | -10.40 |
| SS=160 mg | -23.2 | -36.8 | -40.8 | -34.3 | -10.30 | -13.50 |

As shown in Table 2, when the pH value decreased, the Zeta potential of nanoparticles decreased obviously, this was attributed to the dissociation degree for carboxylic acid groups in nanoparticles declining in the acidic environment, and negative charge on the surface of nanoparticles declining. In addition, when GSH concentration increased, Zeta potential of nanoparticles increased, this showed that the fracture of crosslinking agent was not conducive to the stability of system.

In Fig.3a, PEG-P(MAA-SS-VCL) nanoparticles maintained good spherical morphology, and the particle size distribution was relatively uniform. The particle diameter was around 200 nm in diameter as shown in SEM image. After 24 hs treatment by 10 mmol/L GSH solution, the spherical shapes crashed. In addition, nanoparticles were bonding in the medium with pH 5.4.
Figure 3. SEM images of nanoparticles (a), nanoparticles in 10 mmol/L GSH (b), and nanoparticles in pH 5.4.

4. Conclusion
In summary, the novel redox responsive biodegradable PVCL based on nanoparticles (PEG-P(MAA-SS-VCL)) were developed successfully. The structures and properties of nanoparticles with redox sensitive network were characterized by FT-IR, DLS, SEM, and TGA. The redox sensibility of nanoparticles was confirmed by SEM and DLS.

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