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Facile Fabrication of Polyelectrolyte Complex Nanoparticles Based on Chitosan – Poly-2-Acrylamido-2-Methylpropane Sulfonic Acid as a Potential Drug Carrier Material

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Abstract. Polyelectrolyte complexes (PECs) are attractive materials for drug delivery application as they offer simple preparations and high drug-loading efficiency. In this study, a novel method for preparing polyelectrolyte complex nanoparticles using a simple mixing method of chitosan and poly-2-acrylamido-2-methylpropane sulfonic acid (PAMPS) solutions is presented. The effect of chitosan concentrations was examined by fixing the PAMPS concentration at 0.01 %w/v, while chitosan concentrations were varied from 0.01 to 0.05 %w/v. Based on dynamic light scattering (DLS) and zeta sizer results, increasing the chitosan concentration led to increased average PEC particle sizes with broader particle distributions from 249.1 (polydispersity index/PDI 0.13) to 318.2 nm (PDI 0.19) and changed the particle surface charges from -5.85±0.34 to 11.95±0.84 mV. The addition of glutaraldehyde (GA) followed by dialysis eliminated sodium chloride (NaCl) and produced spherical PEC nanoparticles, confirmed via scanning electron microscopy (SEM) results. Among those samples, PECs with a chitosan concentration of 0.01 %w/v are the most promising drug carrier materials due to their negative surface charges, which promote prolonged circulation time in the bloodstream.

Keywords: Chitosan; Drug carrier; Glutaraldehyde; PAMPS; Polyelectrolyte complex

1. Introduction

Recently, the utilization of nanoparticles as a drug carrier to minimize undesirable chemotherapeutic side effects has attracted researchers' attention because of their ability to selectively accumulate in tumor tissues through enhanced permeability and retention (EPR) effects (Alsehli, 2020). Many nanoparticles have been used as drug carriers for drug delivery systems (DDS), such as liposomes (Vahed et al., 2017), dendrimers (Madaan et al., 2014), hydroxyapatite-based nanoparticles (Prasanna and Venkatasubbu, 2018), porous polyion complexes (PICs) (Wibowo et al., 2014), and polyelectrolyte complexes (PECs) (Zhang et al., 2016). One promising drug nanocarrier material is polyelectrolyte complexes.

PECs are reportedly very useful for nano- or micro-encapsulation and controlled drug

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release because of their simple preparation, low toxicity, and prolonged circulation time in the bloodstream (Meka et al., 2017).

PECs are prepared from two opposite-charge polyelectrolytes, polycations (positively charged polymers) and polyanions (negatively charged polymers), which assemble complexes via electrostatic interactions in the form of a dense phase that is separated from the solvent (Jha et al., 2014; Meka et al., 2017). Numerous positively charged polymers can be used for PEC formation, such as chitosan, polyethyleneimine (PEI), poly-L-lysine (PLL), and poly(amidoamine) (PAMAM; (Kim et al., 2016). Chitosan is an excellent candidate for creating PECs for biomedical applications because of its good biocompatibility, very low toxicity, non-immunogenic nature, and numerous hydroxyl and amino functional groups. These properties enhance the conducting reactions, providing unique biological functions and polyelectrolyte formations (Usman et al., 2018; Anirudhan and Nair, 2019). Considering the above properties, chitosan could be orally applied as a micro- or nano-particle for bioactive compound delivery, enzyme immobilization, and as a drug carrier (Hamzah et al., 2019; Krisanti et al., 2019). Many reports also stated that chitosan could form complexes with various polyanions (Antunes et al., 2011; Arora et al., 2011; Luo and Wang, 2014). Several kinds of polyanion are frequently used to form PECs, such as poly-2-acrylamido-2-methylpropane sulfonic acid (PAMPS), alginate, and poly(methacrylic acid) (PMMA). PAMPS is appealing and negatively charges polysulfonated polymers for biomedical applications by acting as a heparin-like polymer with low toxic effects (García-Fernández et al., 2010). PAMPS has been reported as one of the most potent angiogenesis inhibitors (García-Fernández et al., 2010) and can be utilized as an effective cytokine growth factor (Platt et al., 2014). Hence, chitosan and PAMPS are presumably excellent candidates for PEC formation.

Zhang et al. (Zhang et al., 2016) have been prepared chitosan and PAMPS based PECs nanoparticles for controlled delivery of doxorubicin (DOX). The result showed that the average diameter of obtained PECs is 255–390 nm, with an enhanced drug loading rate. However, they used multiple steps of the polymer-monomer pair reaction system to form their PECs. In our previous work, chitosan-PAMPS based PECs has successfully prepared by simple mixing of PAMPS solution and chitosan solution in sodium chloride (NaCl) (Wibowo et al., 2018; Wibowo et al., 2019). This method offers a simpler and faster strategy for obtaining chitosan-PAMPS-based PECs as they can be prepared directly from their polymer solution. However, the particle size of obtained PECs with a PAMPS concentration of 0.1 %w/v was in the micrometer range (Wibowo et al., 2018), which was too large. As such, it needed to be reduced for cancer-drug carrier application. Preparation of PECs at a lower concentration of the precursors might be an option to solve this problem because Kulkarni et al. (2016) were reported that the particle size of PECs could be significantly decreased by slightly reducing precursor concentrations. Herein, we report the successful fabrication of PEC nanoparticles in an aqueous medium with a physiologically relevant concentration of salt (150 mM NaCl), prepared using a simple polymer solution mixing method at a lower PAMPS concentration (0.01 %w/v). Optimization was carried out by varying chitosan concentrations—0.01; 0.025, and 0.05 %w/v—and investigating their effects on PEC properties—particle sizes, surface charge, and morphologies. This research finding provides an alternative strategy for the preparation of PEC nanoparticles with potency as drug carrier materials.

2. Methods

2.1. Materials

Chitosan (Mw = 232 kD g/mol) (Wibowo et al., 2018) was purchased from Biotech, Indonesia; poly-2-acrylamido-2-methylpropane sulfonic acid (PAMPS; Mw = 568 kD g/mol; (Wibowo et al., 2018) was procured from Brataco, Indonesia; sodium chloride, acetic acid glacial 98%, and glutaraldehyde were obtained from Merck, Darmstadt Germany; and double-distilled water was supplied by Sakura Medical Laboratory, Indonesia.

2.2. Preparation of Polyelectrolyte Complexes

Chitosan-PAMPS based PECs were prepared by a simple mixing method described in the previous report with slight modification in their precursor concentration (Wibowo et al., 2018). The PAMPS solution was prepared by mixing PAMPS powder in double-distilled water containing NaCl. The chitosan solution was prepared by mixing chitosan powder in two %v/v acetic acid solution. Both solutions were magnetically stirred at 500 rpm for 12 h to ensure that all of the polymers were entirely dissolved in their respective solutions. Subsequently, the PAMPS and chitosan solutions were mixed with a 1:1 volume ratio and magnetically stirred at 500 rpm for 3 h to obtain PEC solutions with a final concentration of 0.01 % w/v PAMPS, 0.15 M NaCl, and 0.01, 0.025, and 0.05 % w/v chitosan. These sample series were named 'PEC' followed by chitosan concentrations (i.e., PEC 0.01; PEC 0.025, and PEC 0.05, respectively).

Cross-linked PECs were prepared by adding an excess amount of glutaraldehyde (GA) to PEC solutions and magnetically stirred at 250 rpm for 15 min. After that, those solutions were inserted in dialysis tubing (Servapor, MWCO 12000–14000 with 21 mm diameter) for the dialysis process against double-distilled water for 3 d, which was replaced every 6 h to remove NaCl content in solution. These sample series would be named PEC-GA and followed by chitosan concentration (designated as PEC-GA 0.01; PEC-GA 0.025, and PEC-GA 0.05, respectively).

2.3. Characterization of PECs

The particle size of samples and zeta potential of the obtained PECs were investigated quantitatively by dynamic light scattering (DLS) and zeta sizer (Zetasizer Nano, Malvern Pananalytical Ltd, United Kingdom). DLS method determines the hydrodynamic size of particles in colloidal suspension, and particle sizes were reported as intensity particle sizes distribution (PSD). It was performed at room temperature (25°C), and the scattering angle was set at 165 degrees.

The functional group present in the PAMPS, chitosan, PEC representative, and PEC-GA samples were identified by Fourier transform infrared spectrometer (FTIR, Prestige 21 Shimadzu, Japan) using the KBr pellet method. Samples (10–15 mg) and potassium bromide (KBr) powder (150–250 mg) were mixed and pressed at 700 kN for 5 min to produce a pellet shape. The FTIR was recorded in the range of 4000–500 cm^{-1}. Before FTIR measurement, PEC representative and PEC-GA samples were freeze-dried to remove their water content.

Sample morphology identification was carried out by scanning electron microscopy (SEM, JEOL JEC-3000FC). SEM samples were prepared by dropping PEC samples onto cover glass and drying them in the air for 24 h. Then, SEM samples were coated with gold using sputtering equipment (JEOL JEC-3000FC). Average particle sizes are calculated from SEM images taken from several spots and analyzed using Image J software.

3. Results and Discussion

3.1. PECs without Glutaraldehyde (PEC Sample Series)

Determination of PEC particle sizes and the PEC sample series distribution were performed by the Dynamic Light Scattering (DLS) method. The average particle size and distribution of PECs particles were presented in Table 1. Particle distribution in Table 1 is expressed as a polydispersity index (PDI), and its distribution can be seen in Figure 1.a. In general, small PEC particles (average particle sizes: 249.1–318.2 nm; Table 1), with small PDI (0.13–0.19; Table 1) and narrow distribution (Figure 1.a), were observed in PEC sample series. The particle sizes of obtained PECs are smaller than the micron-sized PEC particles in a previous report (Wibowo et al., 2018), suggesting that PEC nanoparticles were successfully obtained by decreasing the precursor concentration from 0.1 to 0.01 %w/v. PEC particles obtained in these experiments can be considered homogeneous particles because their PDI values are less than 0.3 (Yoon et al., 2013).

Table 1 DLS results of PEC and PEC-GA sample series

[Chitosan] (%w/v)	PEC sample series		PEC-GA sample series	
	Average particle size (nm)	PDI	Average particle size (nm)	PDI
0.01	249.1	0.13	213.4	0.31
0.025	284.3	0.14	238.7	0.30
0.05	318.2	0.19	302.2	0.31

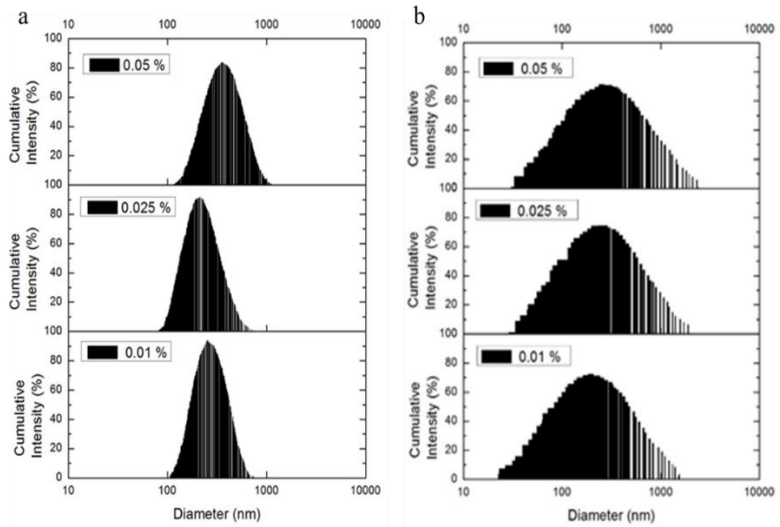

Figure 1 Intensity distribution of PEC particles in: (a) PEC; and (b) PEC-GA sample series (chitosan concentrations were varied from 0.01–0.05 %w/v)

Besides particle size, the surface charge of particles also plays an essential role in their circulation time in the bloodstream and interaction with phagocytic cells in the spleen, lymph nodes, and liver. These properties are widely known as the mononuclear phagocytes system (MPS) (Blanco et al., 2015). In this study, zeta sizers were performed to measure the zeta potential of PECs at various chitosan concentrations. The measurement results could help in understanding the electronic interaction between chitosan and PAMPS in the PECs. As shown in Table 2, increasing the chitosan amount led to a more positive PEC net surface charge. Based on the previous estimation of the molecular weight of chitosan (M_v 232 kD) and PAMPS

(M_v 568 kD) (Wibowo et al., 2018), it requires roughly 2.5 times chitosan to neutralize PAMPS. Therefore, it is reasonable that the zeta potential result of PEC 0.025 (with chitosan concentration of 0.025 %w/v) is 0.29±0.33 because the chitosan/PAMPS ratio is 2.5. Meanwhile, PEC 0.01 can be considered PAMPS rich PECs with negatively charged PECs (-5.85±0.34) because the chitosan/PAMPS ratio is less than 2.5. While PEC 0.05 can be considered chitosan-rich PECs with positively charged PECs (11.95±0.84) because chitosan/PAMPS ratio is higher than 2.5.

Regarding their surface charge, PEC 0.01 is more promising for drug carriers than other PEC samples because its negative surface charge could prolong its circulation time in the bloodstream and less interaction with MPS macrophages (Blanco et al., 2015). Due to the rigidity of the chitosan backbone (Terbojevich et al., 1991; Delair, 2011), chitosan cannot be folded easily to form compact and small particles. Consequently, the particle sizes of the chitosan-rich PEC (PEC 0.025) sample were larger than that of other PEC samples (Table 1). Additionally, their particle distributions were broader and shifted to bigger particles (Figure 1).

Table 2 Zeta potential of PEC and PEC-GA sample series

Samples	[PAMPS] (%w/v)	[Chitosan] (%w/v)	Chitosan/PAMPS ratio	Zeta potential (mV)
PEC	0.01	0.01	1	-5.85±0.34
	0.01	0.025	2.5	0.29±0.33
	0.01	0.05	5	11.95±0.84
PEC-GA	0.01	0.025	2.5	-0.58±0.62

FTIR spectrums of chitosan, PAMPS, PEC 0.025 as a PEC sample series representative, and PEC-GA 0.025 as a PEC-GA sample series representative are presented in Figure 2.

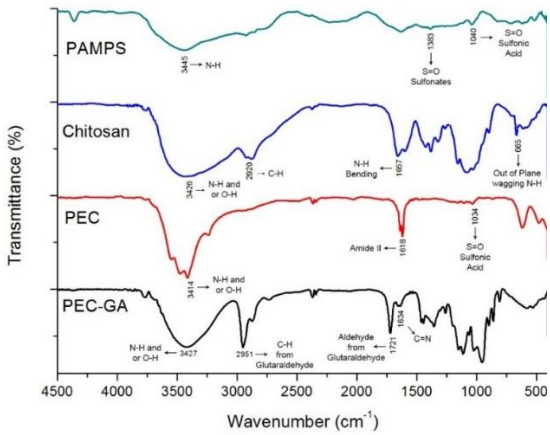

Figure 2 FTIR spectrum of Chitosan, PAMPS, as well as PEC 0.025, and PEC-GA 0.025 as representatives of PEC and PEC-GA samples, respectively

FTIR spectra of PAMPS showed their typical peaks: broad peak at 3445 cm^{-1} from N-H stretching vibrations, peak at 1632 cm^{-1} from amide II band, peak at 1383 cm^{-1} from sulfonate, and 1040 cm^{-1} from sulfonic acid (Silverstein and Bassler, 1962; Durmaz and Okay, 2000). FTIR spectra of chitosan notably showed their amine characteristic peaks: peak at 3426 cm^{-1} is correlated with N-H and/or O-H stretching vibrations, peak at 1657 cm^{-1} is associated with N-H bending vibrations, and peak 665 cm^{-1} is belonged to out of the

plane (OOP) of N-H wagging vibrations (Silverstein and Bassler, 1962; Wibowo et al., 2018). FTIR spectrum of the PEC 0.025 sample revealed that N-H bending vibration (1657 cm^{-1}) and OOP of N-H wagging vibrations (665 cm^{-1}) from chitosan and the sulfonate characteristics of PAMPS (1383 cm^{-1}) no longer emerged. This FTIR result implies that amine from chitosan and sulfonate from PAMPS already bound each other through electrostatic interaction.

Further observation to unveil PEC morphologies in PEC 0.025 (as a PEC sample series representative) was performed via SEM. As presented in Figure 3, cubic morphologies were detected in all areas of the sample. Unfortunately, those kinds of structures are not common in PECs particles (Zhang et al., 2016). Considering that PECs solution containing 150 mM NaCl, those cubic particles most probably were related to NaCl particles due to the precipitation of NaCl as impurities during the evaporation of the SEM sample. This result is consistent with previous research by Taziwa and Mayer that found NaCl particles with cubic structures as impurities in their effort to obtain TiO_2 nanoparticles (Taziwa and Meyer, 2017).

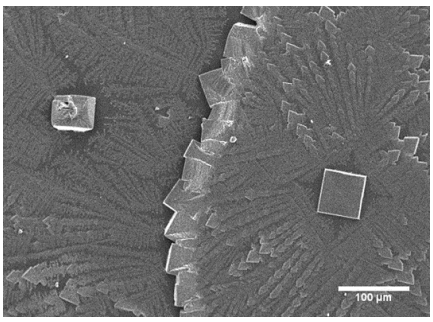

Figure 3 SEM photogram of PEC 0.025 as a PEC sample series representative

Consequently, PEC particles in the PEC sample series could not be seen because NaCl particles with cubic structures dominate the SEM image. Hence, an additional process to remove NaCl impurities would be needed to reveal PEC particle morphology. One of the purification processes that can be used to remove NaCl is the dialysis process. PEC particles can be separated from Na^+ and Cl^- ion during this process and remained in the dialysis bag. However, the structure of PEC particles after the dialysis process might not be the same as their original structures because electrostatic interaction is enormously dependent on salt concentration in the solution. Therefore, the addition of cross-linking agents might be required to enhance their stability before the dialysis process. Previously, Wibowo et al. used an excessive amount of GA to conserve the original structure of porous PICs from PIC solutions containing 150 mM NaCl before the removal of NaCl (Wibowo et al., 2014). Thus, a similar strategy was used in this study by adding an excess amount of GA to the PEC sample before dialysis in order to preserve the original PEC's structures during the NaCl dialysis removal process. By utilizing this strategy, we expected the observation of PEC structures (via SEM) to be performed without NaCl crystal impurity disturbance.

3.2. PECs with Glutaraldehyde (PEC-GA sample series)

Particle size characterization using the DLS method (Table 1) revealed that the average particle size of the PEC-GA sample series (213.4–302.2 nm) is relatively similar to the average particle size of the PEC sample series (249.1–318.2 nm). However, the PDI of the PEC-GA sample series is larger than that of the PEC sample series, implying that particle distributions of the PEC-GA sample series are broader than the PEC sample series. Compared to the PEC sample series (Figure 1a), micron-size particles were exhibited in the

PEC-GA sample series (Figure 1b). This phenomenon might be occurred due to an excess amount of GA that led to the formation of large particles.

The effect of GA addition on the zeta potential of PECs was studied by examining PEC-GA 0.025 sample as a PEC-GA sample series representative. As shown in Table 2, the zeta potential of the PEC-GA 0.025 sample was -0.58±0.62 mV, which is slightly lower than the PEC 0.025 sample (0.29±0.33 mV). This result is consistent with the previous result by He et al. (1999), which showed that the zeta potential of chitosan microspheres decreased after the addition of GA due to interaction between the aldehyde part of GA and the amine part of chitosan. Even though the surface charge of PEC-GA is more negative than its original PEC sample, which is more favorable as a drug carrier material, the addition of excess GA will increase the rigidity of PEC particles. Geng et al. (2007) found that flexible worm-like filomicelles were potential drug carrier materials that could remain in the bloodstream for 7 d. However, cross-linked filomicelles will be removed immediately from the bloodstream due to their higher rigidity (Geng et al., 2007). Regarding their flexibility, original PEC samples are superior drug carrier materials than their cross-linked counterparts. The primary purpose of adding GA to the PEC samples in this study is to preserve the original structure of PEC particles during the removal of NaCl before SEM observation.

FTIR spectrum of the PEC-GA 0.025 sample as a PEC-GA sample series representative can be seen in Figure 2. Compared to PEC 0.025, the FTIR result of PEC-GA 0.025 displays an indication of excess GA in PEC-GA samples: a strong peak at 2951 cm^{-1} that belong to methylene (-CH_2-) stretching vibration and a strong peak at 1721 cm^{-1} that associated with C = O aldehyde stretching vibration (Silverstein and Bassler, 1962). Also, a weak peak at 1634 cm^{-1} from C = N stretching vibration indicated an amine functional group's presence due to the reaction between chitosan and GA (Silverstein and Bassler, 1962). This information suggests that GA successfully cross-linked PECs.

Morphological identification for the PEC-GA sample series can be seen in Figure 4. In general, spherical morphologies and well-distributed particles were observed in all PEC-GA sample series, consistent with chitosan-PAMPS-based PECs prepared by the polymer-monomer pair reaction system (Zhang et al., 2016). Moreover, the absence of cube-shaped morphology in the SEM results of the PEC-GA sample series concluded that the addition of GA followed by dialysis successfully conserved PEC particles and eliminated NaCl from the samples. Determination of particle sizes from SEM images using ImageJ software showed that the average particle sizes of PEC-GA 0.01, PEC-GA 0.025, and PEC-GA 0.05 were 153.7±104.9 nm, 169.9±102.5 nm, and 171.0±103.3 nm, respectively; these are relatively smaller than PEC sizes measured by DLS. Notably, DLS measures the hydrodynamic diameters of particles (Tomaszewska et al., 2013), while SEM images calculate the actual particle sizes. Thus, it is reasonable that the average particle size results from SEM images are smaller than the DLS results of the PEC-GA series (Table 3). Based on DLS and SEM results, PEC nanoparticles were successfully prepared using a simple mixing method; their average particle sizes (249.1–318.2 nm) were comparable to those of PECs prepared via the polymer-monomer pair reaction system (255–390 nm; (Zhang et al., 2016).

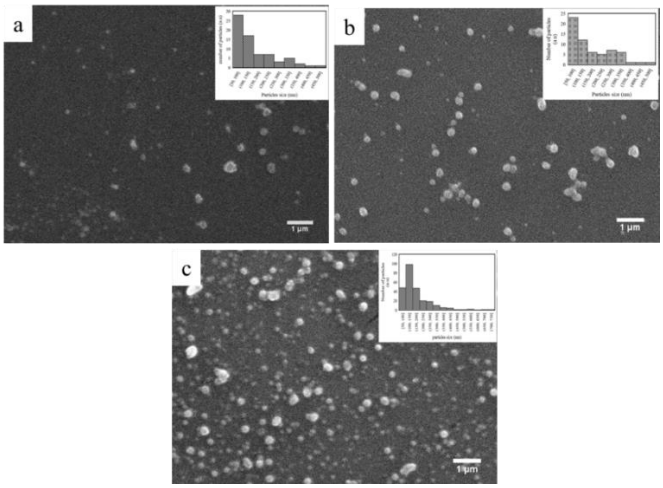

Figure 4 SEM photogram of PEC-GA sample series: (a) PEC-GA 0.01; (b) PEC-GA 0.025; (c) PEC-GA

4. Conclusions

Polyelectrolyte complex nanoparticles based on chitosan and PAMPS were successfully prepared using a simple polymer solution mixing method with a PAMPS concentration of 0.01 %w/v and chitosan concentration variations of 0.01–0.05 %w/v. However, increasing the chitosan concentration led to larger particles with broader particle distributions due to chitosan's rigidity. PECs with a chitosan concentration of 0.01 %w/v were better options for cancer drug carriers than other PEC samples due to their negative surface charges. The addition of GA, followed by dialysis, preserved PEC morphologies and removed NaCl as impurities in the PEC solutions.

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