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PMMA-*N*,*N*,*N*-trimethyl chitosan nanoparticles for fabrication of antibacterial natural rubber latex gloves

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ABSTRACT

This paper presents one-pot synthesis of *N*,*N*,*N*-trimethyl chitosan (TMC) stabilized poly(methyl methacrylate) (PMMA) latex particles via the miniemulsion polymerization technique. From ^{1}H NMR, synthesized TMC contains 52% degree of quaternization. Compared to native biopolymer chitosan, TMC possesses permanently positive charges as well as provides greater antibacterial activity. Combining properties of PMMA and TMC, PMMA–TMC latex nanoparticles (hydrodynamic size ≈282 nm) could be used in place of inorganic lubricating powder in fabrication of latex gloves at pH ≥7. After immersing sulphur prevulcanized natural rubber (SPNR) film into 3 wt% of PMMA–TMC latex at pH 7, significant amount of nanoparticles uniformly deposited onto SPNR film was observed under SEM. A number of nanoparticles present on film surface would increase surface roughness of the rubber film and potentially inhibit the bacterial (*Escherichia coli* and *Staphylococcus aureus*) growth, which would be useful for fabrication of special gloves with antibacterial property.

1. Introduction

In order to reduce the surface friction of sulphur prevulcanized natural rubber (SPNR) latex gloves and to avoid the direct contact between rubber and human skin, talc or other fine powdered materials are generally sprinkled onto the glove surface. However aforementioned surface treatments could cause the contamination and/or allergic problem, alternative methods are needed. Attempts to replace the lubricating powders with poly(methyl methacrylate) (PMMA) nanoparticles firmly attached onto SPNR film surface have been systematically investigated. Negatively charged PMMA latex particles were deposited on the polyacrylamide-grafted SPNR film via the Layer-*by*-Layer technique (Anancharungsuk, Taweepreda, Wirasate, Thonggoom, & Tangboriboonrat, 2010; Sruanganurak, Sanguansap, & Tangboriboonrat, 2006). The surface hardness and roughness (*Ra*) of the coated SPNR film increased and, hence, its surface friction effectively decreased. The in vitro cytotoxicity of coated rubber films was tested using L-929 fibroblasts and the results showed that no toxicity potential detected at the extract concentration (using culture medium) of ≤ 12.5% of the coated film for 24 h at 37 °C (Anancharungsuk, Polpanich, Jangpatarapongsa, & Tangboriboonrat, 2010). These data suggested that this coating technique could potentially decrease the allergy issue caused by non-rubber components.

Although lowering allergic concern, coating PMMA particles on SPNR film is time-consuming because the film surface must be first grafted. To reduce the coating steps, PMMA nanoparticles stabilized by cationic polymers, e.g., polyethyleneimine and chitosan, were synthesized before being deposited onto the non-grafted SPNR film. Chitosan, the cationic (1 → 4)-2-amino-2-deoxy-β-D-glucan, is industrially produced from marine chitin, the second most abundant polysaccharide in nature (Muzzarelli, 2010, 2012; Muzzarelli et al., 2012). It is being studied extensively for applications owing to its biodegradability, biocompatibility, and absence of toxicity. The studies of SPNR film coated with PMMA–chitosan particles have been recently focused. Similar to chitosan-stabilized gold and poly(butyl cyanoacrylate) nanoparticles, PMMA–chitosan particles were prepared via the miniemulsion polymerization in acidic medium (Bhumkar, Joshi, Sastry, & Pokharkar, 2007; Kanjanathaworn, Kaewsaneha, Polpanich, Jangpatarapongsa, & Tangboriboonrat, 2012; Yang, Ge, Hu, Jiang, & Yang, 2000). At pH 2, the positively charged PMMA–chitosan particles could be directly

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deposited onto SPNR film via hydrogen bonding with the indigenous non-rubber substances, i.e., proteins and fatty acids. The *Ra* values increased from 46 to 64 nm after immersing SPNR film into 0.5 wt% PMMA–chitosan latex for 10 min. The coated SPNR film showed no cytotoxicity on L-929 cells. However, the antibacterial activity of the coated film against *Escherichia coli* and *Staphylococcus aureus* determined by the agar disk diffusion method could not be detected, possibly due to low quantity of chitosan adhered on the rubber surface (10^{-4} mg/mL) compared to the minimal inhibitory concentration (MIC) of chitosan, i.e., 1 mg/mL (Kanjanathaworn, Polpanich, Jangpatarapongsa, & Tangboriboonrat, 2013). Thus, it is challenging to improve antimicrobial activity by increasing the amount of chitosan coated on the SPNR film surface.

To increase solubility in water at neutral pH as well as antimicrobial activity, the chitosan derivative, i.e., *N*,*N*,*N*-trimethyl chitosan (TMC), possessing permanently positive charges of quaternary amine groups is of great interest. The MIC and minimum bactericidal concentration (MBC) against *S. aureus* of TMC (250 μg/mL) is much lower than that of chitosan (Sadeghi et al., 2008). The water solubility of TMC synthesized from the methylation of chitosan with an excess of iodomethane in NaOH solution and *N*-methyl-2-pyrrolidone (NMP), depends on the degree of quaternization (DQ) (Curti, De Britto, & Campana-Filho, 2003; Verheul et al., 2008). TMC with 40% DQ was highly soluble in water at pH 1–12. However the water solubility of TMC with DQ of 80% decreased, it might stem from the high degree of *O*-methylation of 3- and 6-hydroxyl group (Jintapattanakit, Mao, Kissel, & Junyaprasert, 2008; Mourya & Inamdar, 2009). TMC can be used as a polycationic stabilizer by several means, such as forming complex structures with lysosomal enzyme α-GAL yielding TMC/α-GAL complex particles (ca. 200 nm) and with poorly water soluble drug leading to micron-sized particles (Sun et al., 2012). In addition, TMC-stabilized poly-(ε-caprolactone) nanoparticles, serving as DNA carriers, could be prepared via the emulsion–diffusion–evaporation method (Haas, Ravi Kumar, Borchard, Bakowsky, & Lehr, 2005). To the best of our knowledge, the preparation of PMMA latex via miniemulsion polymerization technique using TMC as stabilizers has not yet been reported. The advantage of this technique is that the final size of latex particles can be predicted from the size of monomer droplets acting as nanoreactors.

In this present study, TMC was synthesized prior being used as stabilizer for the synthesis of PMMA nanoparticles via miniemulsion polymerization. MBC values of TMC and chitosan against *E. coli* and *S. aureus* were determined. Particle size distribution, zeta potential and morphologies of synthesized PMMA–TMC as well as PMMA–chitosan nanoparticles were investigated using zeta sizer and transmission electron microscopy (TEM). The surface of SPNR film coated with PMMA–TMC or PMMA–chitosan particles was examined using scanning electron microscopy (SEM).

2. Materials and methods

2.1. Materials

SPNR latex (Lucky Four Co., Ltd., Nonthaburi, Thailand) was filtered through a 250 mesh aluminum. Methyl methacrylate (MMA) monomer (Merck, Purum) was purified by passing through a column packed with neutral and basic-aluminum oxide (Fluka, Purum). Chitosan (Taming-enterprises) having weight-average molecular weight of 2.7×10^4 g/mol (see Fig. S1 in supplementary data), iodomethane stabilized with copper (Merck, 99%), sodium iodide (Fluka, Purum), *N*-methyl-2-pyrrolidone (NMP) (Fluka, Purum), 2,2′-azobisisobutyronitrile (AIBN) (Fluka, Purum) and hexadecane (Fluka, Purum) were used as received.

2.2. Synthesis and characterization of N,N,N-trimethyl chitosan (TMC)

Chitosan (1.0 g), sodium iodide (2.4 g), NMP (40 mL) and NaOH solution (15%, w/v, 6 mL) were mixed and then heated to 60 °C. After stirring for 1 h, iodomethane (6 mL) was added and the mixture was refluxed at 60 °C for 2 h before adding NaOH solution (15%, w/v, 3 mL) and iodomethane (3 mL) under stirring for another 1 h. The reaction was terminated by precipitating the product in diethyl ether/ethanol (50/50, 100 mL). The sediment was dissolved in aqueous NaCl solution (10%, w/v, 50 mL) while shaking for 24 h (Verheul et al., 2008). The solution was dialyzed at room temperature against deionized (DI) water for 3 days and then freeze-dried (Thermo, Super module). The synthesized TMC was characterized by proton nuclear magnetic resonance (^{1}H NMR) spectrometer (Bruker, 300 MHz) using deuterium oxide as a solvent and the degree of quaternization (DQ) was calculated from the following equation:

$$\mathrm{DQ} = \left(\frac{[(\mathrm{CH_3})_3]}{[\mathrm{H}]} \times \frac{1}{9} \right) \times 100$$

where $[(CH_3)_3]$ is integral of protons of trimethyl amino groups at 3.3 ppm and [H] is integral of proton of C-1 of deacetylated unit between 4.7 and 5.7 ppm (Curti et al., 2003; Verheul et al., 2008).

The chemical composition of the synthesized TMC was examined by Fourier Transform Infrared spectrometer (FTIR; Perkin Elmer, Spectra GX) at 4 cm^{-1} resolution and 16 scans.

2.3. Evaluation of MBC of TMC or chitosan

The two-fold dilution of TMC (2 wt% in DI water) or chitosan (2 wt% in 0.1 M acetic acid solution) in tryptic soy broth was inoculated with *S. aureus* (ATCC 6538, 10^8 CFU/mL) or *E. coli* (ATCC 25,922, 10^8 CFU/mL) at 37 °C. After 24 h, the suspension (100 μL) was spread onto a mueller-hinton agar plate and then incubated at 37 °C for 24 h. The MBC defined as the lowest concentration of the compound to kill the microorganism was determined by counting surviving bacterial colony (Wiarachai, Thongchul, Kiatkamjornwong, & Hoven, 2012). After correction for the dilution factor, the results were expressed as the average number of colony forming unit per milliliter (CFU/mL) (Avadi et al., 2004).

2.4. Preparations and characterizations of PMMA–TMC or PMMA–chitosan latex particles

PMMA latex particles stabilized by TMC (PMMA–TMC) or by chitosan (PMMA–chitosan) were prepared via the miniemulsion polymerization technique (Kanjanathaworn et al., 2012, 2013). MMA monomer (2 g), mixed with AIBN (0.08 g) and hexadecane (0.25 g) were poured into 1 wt% TMC or chitosan in aqueous solution (24 g). After 1 h of stirring for pre-emulsion, the mixture was ultrasonicated (Branson, W 450 Digital) for 2 min at 90% amplitude in an ice-cooling bath. The polymerization was proceeded at 80 °C for 2 h. The synthesized PMMA–TMC and PMMA–chitosan latexes were centrifuged at 5000 rpm for 30 min (Hettich Zentrifugen, Universal 320) thrice prior to characterization. Effects of polymerization time on % conversion and of TMC concentrations on hydrodynamic diameter (D_h) of PMMA–TMC or PMMA–chitosan were investigated.

The D_h and zeta potential of latex particles were measured using the zeta sizer (Malvern, Nano ZS). Morphological study of dried latex particles onto a 300-mesh carbon-coated copper grid was characterized using TEM (FEI, TECNAI G^2) at an accelerator voltage of 200 kV.

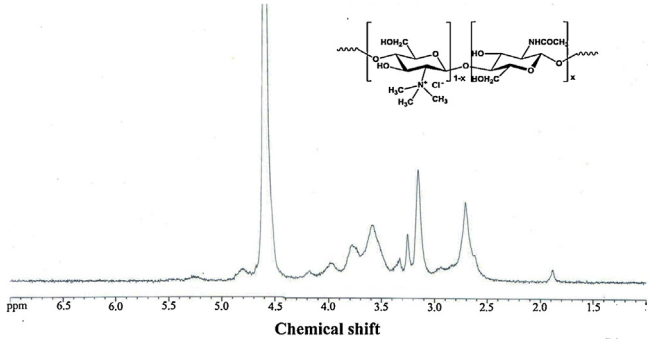

Fig. 1. ^{1}H NMR spectrum of the synthesized TMC.

2.5. Deposition of PMMA–TMC particles onto SPNR film

SPNR film (0.1 cm × 2 cm × 5 cm) was first adhered on a poly(ethylene terephthalate) film without using adhesive before immersed into a beaker containing various concentrations of PMMA–TMC latex (0–3 wt%) for 60 min. The sample was subsequently washed with DI water via a series of three rinsing baths and allowed to dry under vacuum at room temperature. The morphologies of the coated SPNR film (0.5 cm × 0.5 cm) stuck on the stub before metallization with a specific affinity of gold by a sputter coater (Hitachi, E-102) for 10 min were investigated using SEM (Hitachi, S-2500).

3. Results and discussion

3.1. Analysis of TMC

Chemical structure of the synthesized TMC was examined using ^{1}H NMR and the spectrum is shown in Fig. 1.

The ^{1}H NMR spectrum in Fig. 1 shows the characteristic signals at 3.1 and 2.7 ppm corresponding to methyl protons of *N*,*N*,*N*-trimethylamine (—N(CH$_3$)$_3^+$) and *N*,*N*-dimethyl amine (—N(CH$_3$)$_2$) originated from substitution of primary amines of chitosan with methyl group (Sajomsang, Ruktanonchai, Gonil, & Warin, 2010). The *O*-methylation of hydroxyl groups at C-3 and C-6 of glucosamine units was also noticed at 3.3 and 3.2 ppm, respectively (Sajomsang, Tantayanon, Tangpasuthadol, & Daly, 2008). The %DQ of TMC, calculated from the relative ratio of trisubstitution of methyl group at 3.1 ppm to C-1 glucosamine unit at 4.7 ppm, was 52% (Wiarachai et al., 2012). Due to the hydrophilicity of quaternary amine group, TMC was greatly soluble in water over a wide pH range. The electrostatic repulsion among positive charges of quaternary amines along the polymer backbone decreased both intra- and intermolecular interactions and, hence, unfolded TMC chains (Kotzé et al., 1999). Moreover, the presence of partial *O*-methylation at 3- and 6-hydroxyl groups further reduced the intra- and/or intermolecular interactions leading to the increase of water solubility of TMC (Verheul et al., 2008).

FTIR spectrum of TMC shows the characteristic broad band at 3434 cm^{-1} (—OH and N—H stretching) and two sharp peaks at 1652 and 1594 cm^{-1} (C=O stretching and N—H bending) of glucosamine unit of chitosan (see Fig. S2 in Supplementary Data) (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). The appearance of new sharp peak at 1476 cm^{-1} in spectrum S2 (i) corresponded to C—H deformation of methyl group of TMC. The depletion of N—H bending peak at 1594 cm^{-1} resulted from direct methylation reaction on chitosan was clearly noticed (de Britto, de Moura, Aouada, Mattoso, & Assis, 2012).

MBC of TMC and chitosan against *E. coli* (gram negative bacteria) and *S. aureus* (gram positive bacteria) at pH 7 were then determined. The MBC values of TMC against both types of bacteria were 0.3 mg/mL, while those of chitosan against *E. coli* and against *S. aureus* were 2.5 and 0.6 mg/mL, respectively. The lower MBC values of TMC compared to chitosan supported that the quaternization of primary amine could improve the antibacterial activity of chitosan at neutral pH (Xu, Xin, Li, Huang, & Zhou, 2010). Permanently positive charges generated on TMC molecules were responsible for the effective attachment to the negatively charged cell membrane of bacteria, which caused the leakage of proteinaceous and intracellular components leading to cell rupture and death (Rabea, Badawy, Stevens, Smagghe, & Steurbaut, 2003; Vallapa et al., 2011)

The bacterial colonies formed on an agar plate added with TMC or chitosan were clearly observed under the optical photographs (see Fig. S3 in Supplementary Data). The yellow spots of both *E. coli* and *S. aureus* colonies appeared on agar surface of control samples as shown in Fig. S3(a) and (b), respectively. However the formation of bacteria colonies was not detected when incubating *E. coli* suspension with TMC (0.3 mg/mL) or chitosan (2.5 mg/mL), as presented in Fig. S3(c) and (e), respectively. Similarly, no *S. aureus* colony was formed on TMC (0.3 mg/mL) or chitosan (0.6 mg/mL) tests as presented in Fig. S3(d) and (f). Due to the higher antibacterial activity of TMC against both types of bacteria, TMC would be suitable in the preparation of the antibacterial SPNR glove.

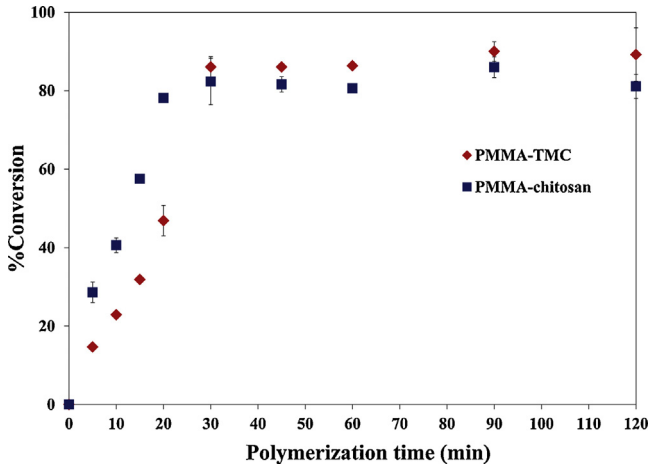

Fig. 2. Percent conversions of PMMA–TMC and PMMA–chitosan latexes versus polymerization times (1 wt% AIBN, 80 °C, 1 wt% TMC or chitosan).

3.2. Characterization of latex particles

The percent conversions of PMMA latexes prepared at 80 °C using 1 wt% AIBN, 1 wt% TMC (or chitosan) at various polymerization times were gravimetrically determined and the data are presented in Fig. 2.

In the initial period, the percent conversion of both latexes rapidly increased with increasing polymerization times and then attained the constant value of 86% and 82% for PMMA–TMC and PMMA–chitosan, respectively, within 30 min. More hydrophilic TMC could better stabilize the latex particles in aqueous medium leading to slightly higher MMA conversion (Zhang et al., 2010). Nevertheless, the conversion could not reach 100%, it might come from the increase in viscosity of polymer chains in PMMA particle, which then induced the diffusion controlled radical termination (Landfester, 2003). As previously reported, the miniemulsion polymerization was started in MMA monomer droplet consisting of AIBN and hexadecane as hydrophobe. The polymerization proceeded in the monomer droplet, which its stability was maintained by adsorbing TMC or chitosan chains at the surface of droplet. The high stability of monomer droplet was also attributed to the

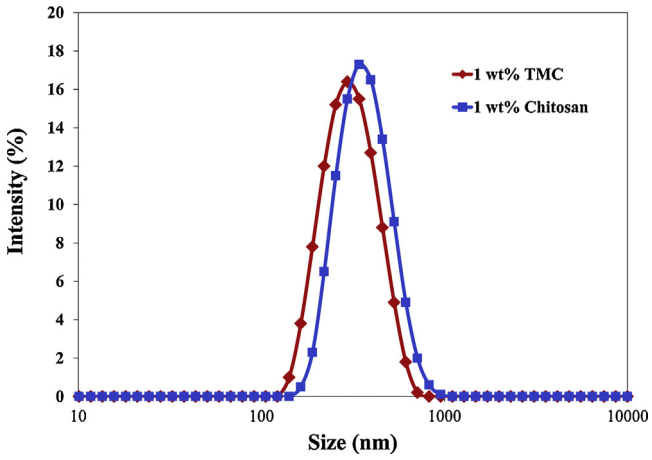

Fig. 3. Size distribution curves of PMMA–TMC and PMMA–chitosan latex particles.

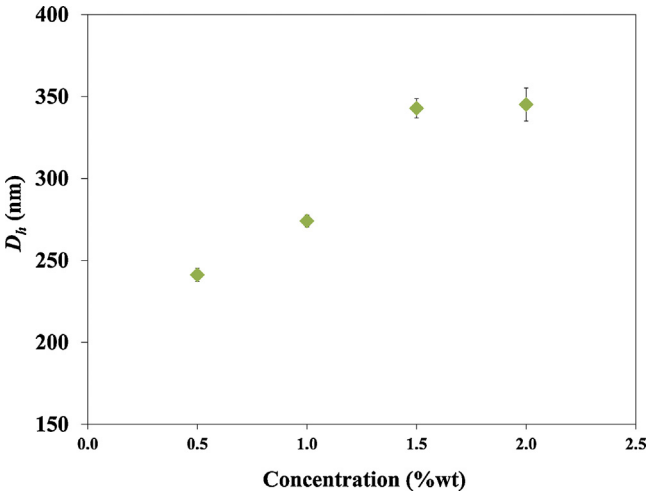

Fig. 5. Effect of TMC concentration on D_h of PMMA–TMC latex.

existence of hexadecane, which efficiently suppressed the Ostwald ripening as well as prevented the coalescence of MMA droplets.

The hydrodynamic diameter (D_h) of PMMA particles stabilized by 1 wt% of TMC or chitosan was characterized using the dynamic light scattering (DLS) technique and data are displayed in Fig. 3.

The size distributions of PMMA–TMC and PMMA–chitosan latex particles were narrow with low polydispersity index of 0.20 and 0.07, respectively. The average D_h of PMMA–TMC (282 ± 4 nm) was slightly lower than that of PMMA–chitosan (318 ± 4 nm) reasonably due to the electrostatic repulsion between permanently positive charges of quaternary amine groups of TMC. Not only acting as electrostatic and steric stabilizer, TMC and chitosan also prevented the aggregation of PMMA particles by reduction of interfacial tension between oil and water (Marie, Landfester, & Antonietti, 2002; Sun et al., 2012). Nevertheless, the partial amine groups of chitosan could form bridging with other molecules forming multi-layer shell around PMMA particle, which might increase D_h of PMMA–chitosan (Kaewsaneha, Opaprakasit, Polpanich, Smanmoo, & Tangboriboonrat, 2012).

TEM images in Fig. 4(a) and (b) revealed that PMMA–TMC and PMMA–chitosan particles are spherical with the average sizes of 98 ± 8 and 228 ± 12 nm, respectively.

As expected, particle size of dry PMMA–TMC and PMMA–chitosan particles obtained from TEM analysis was smaller than D_h because of the shrinkage of TMC or chitosan layer upon drying in sample preparation. (Bravo-Osuna, Ponchel, & Vauthier, 2007). In addition, a content of quaternized moieties of TMC left a long hydrophilic chain extended toward the aqueous phase yielding a larger different of particle size in wet and dried states of PMMA–TMC compared to that of PMMA–chitosan.

Due to a well-defined structure with smaller particle size which provided higher specific surface area, PMMA–TMC particles were then deposited onto SPNR film. Effect of TMC concentrations on D_h of PMMA–TMC latex particles was studied and the data are presented in Fig. 5.

It was observed that D_h values of PMMA–TMC latex were directly proportional to the TMC content. The high density of TMC around PMMA particles at high concentration would lower mobility or flexibility of TMC chains stabilizing MMA droplets. Consequently, efficiency of particle size reduction would decrease (Sun et al., 2012). In addition, the increase in particle size might be caused from the multilayer adsorption of TMC chains onto PMMA particle surface leading to the high water-swollen of TMC chains which extend into aqueous phase as loops and tails. As reported in the previous work, the particle diameter of poorly soluble drug droplet stabilized by TMC increased when increasing TMC concentration from 0.4 to 0.5 wt% (Sun et al., 2012). It was mentioned that the high concentration of TMC led to high viscosity of TMC solution in water phase which decreased shear stress of insoluble drug during ultrasonication. Since the agglomeration of particles or the gelation formed during polymerization easily occurred when using 1.5–2 wt% TMC

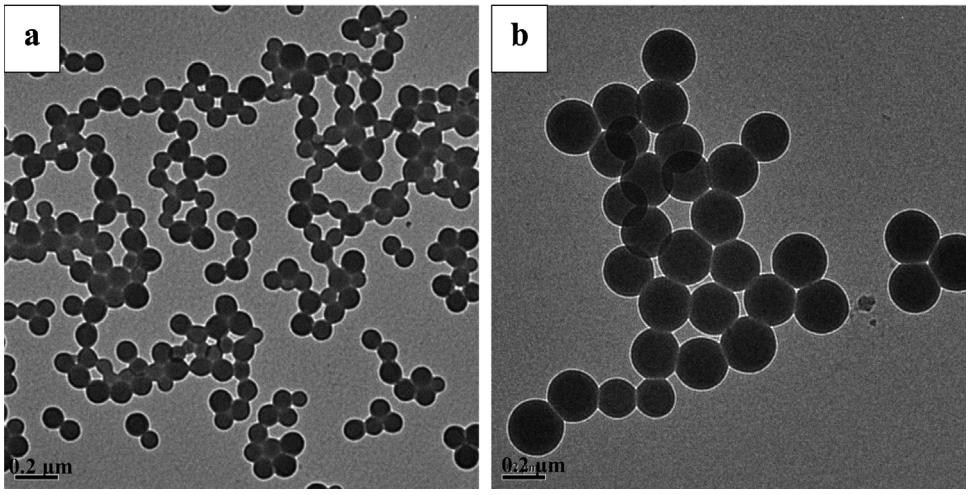

Fig. 4. TEM micrographs of (a) PMMA–TMC and (b) PMMA–chitosan particles.

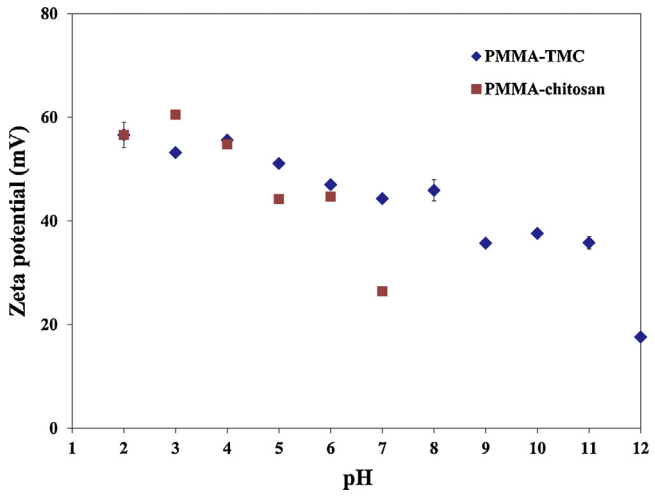

Fig. 6. Zeta potentials of PMMA–TMC and PMMA–chitosan particles as a function of pH.

possibly due to the hydrogen bonding between the partial amino and hydroxyl groups of TMC chains (Yang et al., 2000), 1 wt% TMC solution was chosen for further synthesis of PMMA–TMC particles.

The zeta potential values of PMMA–TMC compared to PMMA–chitosan particles as a function of pH (2–12) were determined and the data are presented in Fig. 6.

The positive zeta potentials in both cases confirmed the presence of TMC or chitosan onto PMMA surfaces. The zeta potential of PMMA–chitosan at pH 8–12 could not be determined due to the deprotonation of $-NH_3^+$ group of chitosan which then induced the aggregation of particles (Kanjanathaworn et al., 2012). Since the absolute zeta potential of PMMA–TMC was >30 mV, it was assumed that the particles were stable at wide pH range (Thanou, Florea, Geldof, Junginger, & Borchard, 2002). The large amount of charges on the particle surface promoted the electrostatic repulsion among the particles and hence, increased energy barrier against coalescence (Yang et al., 2000).

3.3. Deposition of PMMA–TMC latex particles onto SPNR film

The PMMA–TMC latex particles were deposited onto SPNR film surface at pH 7. Particles attachment occurred through the electrostatic interaction between the negative charges on SPNR film derived from non-rubber components, i.e., proteins and phospholipids (isoelectric point (pI) of SPNR latex = 3.8), and the positive charges on particle surface (Ho, 1989; Kanjanathaworn et al., 2012). SEM micrographs of the SPNR films coated with 0–3 wt% PMMA–TMC latexes are presented in Fig. 7(a)–(d), respectively.

Fig. 7(a) revealed the smooth surface of the unmodified SPNR film. SEM images of SPNR latex films after immersed into 1–3 wt% PMMA–TMC latexes in Fig. 7(b)–(d) confirmed the presence of PMMA–TMC particles on the rubber film surfaces. At low concentration of latex (1–2 wt%), the small amount of PMMA–TMC particles were adsorbed on SPNR film surface. When increasing PMMA–TMC concentration to 3 wt%, the number of PMMA–TMC particles onto SPNR film was significantly increased because of the repulsion among similar charged particles providing the collision between PMMA and TMC particles and SPNR film. The formation of two-dimensional aggregates of the adsorbed PMMA–TMC particles in Fig. 7(d) might be due to the bridging effect caused by TMC chains dangled into the solution or the evaporation of solvent in the sample preparation. This result correlated well with that reported in the positively charged polyethyleneimine-functionalized PMMA nanoparticles deposited on long chain fatty acids and polypeptides of SPNR film (Sunintaboon, Duangphet, & Tangboriboonrat, 2009). However, the PMMA–TMC particles did not cover the whole SPNR surface which might be due to the insufficient amount of TMC on PMMA particles. An attempt to increase the TMC content on PMMA particles and, hence, the antibacterial activity of the SPNR coated with PMMA–TMC would be further investigated.

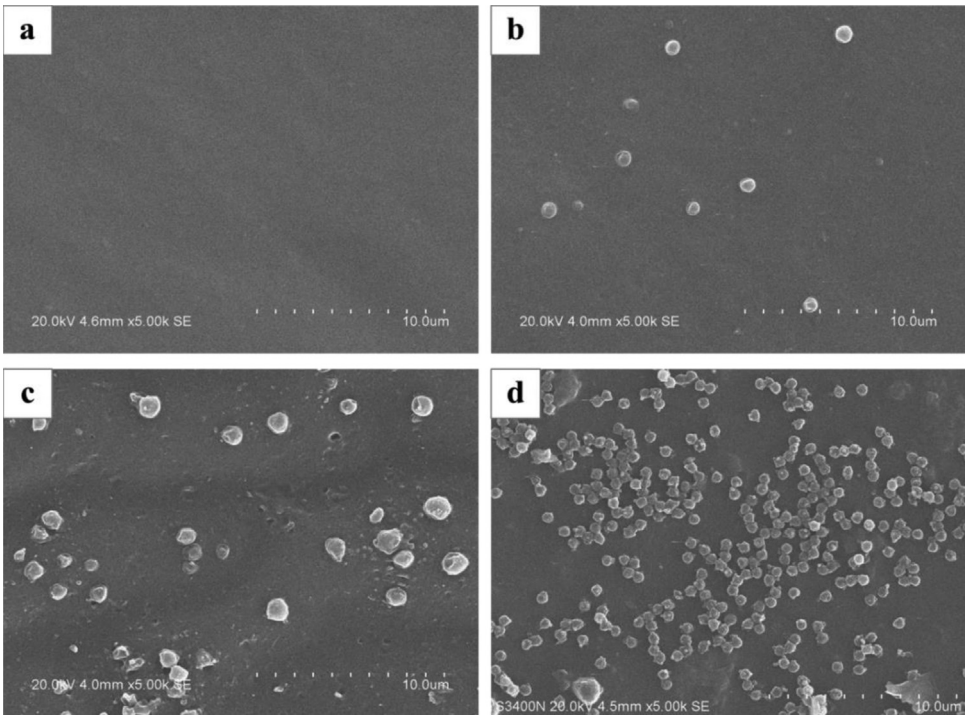

Fig. 7. SEM micrographs of SPNR films after immersing in (a) 0, (b) 1, (c) 2 and (d) 3 wt% PMMA–TMC latex (pH 7, dipping time 60 min).

4. Conclusions

We report the synthesis of TMC-stabilized PMMA latex via the miniemulsion polymerization technique. Derivative of chitosan, TMC with 52%DQ was first prepared. MBC of TMC against *E. coli* and *S. aureus* was 0.3 mg/mL. The D_h of PMMA–TMC nanoparticles increased from 241 to 345 nm with increasing TMC from 0.5 to 2 wt%. The cationic PMMA–TMC particles (282 nm) were stable at pH ranging from 2 to 11, enabling them to be deposited directly onto negative SPNR film at pH 7 via the electrostatic interaction. However, at 3 wt% of PMMA–TMC latex solution, significant amount of PMMA–TMC particles were adsorbed onto SPNR film, the non-coated area could still be seen. Some two-dimensional aggregates of the adsorbed particles were also found from SEM analysis. To improve surface coverage of nanoparticles on SPNR films, the increase in TMC content on PMMA particles would be necessary. The study will be reported in the future work along with the antibacterial activity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.03.030.

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