The 6th Asian Academic Society International Conference (AASIC)
A Transformative Community:
Asia in Dynamism, Innovation, and Globalization

QUATERNIZED CYCLODEXTRIN GRAFTED CHITOSAN ASSOCIATED WITH HIGH MOLECULAR WEIGHT HYALURONIC ACID NANOPARTICLES FOR COSMETICS

Sakhiran, Sakulwech[1], Nattaya, Lourith[1,2], Uracha, Ruktanonchai[3], Sarunya, Phunpee[3], Mayuree, Kanlayavattanakul[1,2]

[1] *School of Cosmetic Science, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[2] *Phytocosmetics and Cosmeceuticals Research Group, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[3] *National Nanotechnology Center, National Science and Technology Development Agency, Pathum Thani 12120, Thailand*
Corresponding author's email: mayuree@mfu.ac.th

ABSTRACT

An excellent moisturizer, hyaluronic acid (HA), provides biocompatibility and viscoelastic properties that is extensively utilized in cosmetics. High molecular weight HA (HMW-HA, 1.2 MDa) is a typical used form, however its benefits is limited due to its large molecule. In our previous study, the association with quaternized cyclodextrin grafted chitosan (QCD-g-CS) can improve skin penetration and provide positive charge promoting skin adhesion. Thus, the purpose of this study was to produce and characterize HMW-HA associated QCD-g-CS nanoparticles for cosmetic utilization. The HMW-HA nanoparticles (0.01 HA per 1 QCD-g-CS mole ratio) was the best formula with low particles size (348.20 ± 8.67 nm), narrow polydispersity index (0.083 ± 0.02) and positive zeta potential (16.00 ± 0.36 mV). The association efficiency and loading efficiency were analyzed by UPLC and shown to be 82.64 ± 0.39% and 20.09 ± 0.57%, respectively. The conjugation between carboxylic moieties of HMW-HA and amide of QCD-g-CS was confirmed by FTIR. The TEM imaging showed the spherical morphology of these nanoparticles. According to cytotoxicity test, these nanoparticles (0.01 to 0.10 mg/ml) were safe in human skin fibroblasts.

Keywords: High MW Hyaluronic acid, Nanoparticles, Ionotropic gelation, Moisturizer, Cosmetics

INTRODUCTION

The novel quaternized cyclodextrin grafted chitosan (QCD-g-CS) was synthesized with the combination of beneficial biopolymers composing β-cyclodextrin (β-CD) and chitosan (CS) (Sajomsang et al. 2011). The cup-shaped β-CD entraps hydrophobic active promotes solubility, dissolution rate, bioavailability, stability and controlled release. CS is widely used in nanoparticles preparation (Phunpee et al. 2016). However, the cyclodextrin grafted chitosan (CD-g-CS) is poor water-soluble nano-carrier. Addition of quaternization of cyclodextrin grafted chitosan (73 ± 2%) enhances its water solubility (Gonil et al. 2011). High molecular weight hyaluronic acid (HMW-HA, 1.2 MDa) is a commonly used hydrophilic negatively charged polymer in dermatological and cosmetics products for skin hydration (Farwick et al. 2008). However, its benefit on skin surface is limited due to its size (Chen et al. 2014).

QCD-g-CS is a versatile carrier for hydrophilic or hydrophobic molecules. Menthol and eugenol, the hydrophobic guest molecules, could form the inclusion complex within β-CD moieties (Phunpee et al. 2016), and the rest amides groups on chitosan backbone were able to associate with negatively charged polymer like hydrolyzed HA (20-50 kDa). The association between QCD-g-CS and hydrolyzed HA resulted positively charged nanoparticles with a smaller size (20-600 nm), which might promote skin penetration and skin adhesion (Sakulwech et al. 2018). However, this delivery system for HMW-HA that will promote skin adhesion exacerbating moisturizing efficacy has never been examined. Thus, the nanoparticles of QCD-g-CS associated with HMW-HA were prepared and characterized. Their association and loading efficacies, structure, morphology and safety in cell culture are presented herein.

MATERIALS AND METHODS

Materials

Cosmetic grade sodium hyaluronate with molecular of 1.1-1.4 MDa was purchased from Ter Hell Haus (Hamburg, Germany). Sodium hydroxide (NaOH) and disodium hydrogen phosphate (Na_2HPO_4) were of analytical grade supplied from Merck (New Jersey, USA). QCD-g-CS was synthesized by the method of Gonil and coworker (2011) at the National Nanotechnology Center (NANOTEC) (Pathumthani, Thailand). The deionized (DI) water was produced from a Milli-Q water purification system (Millipore, Massachusetts, USA). Human skin fibroblasts (ATCC® CRL 2097, USA) at 14-20th passage were used for cytotoxicity assessment. Cell cultured medium were from Gibco (New Hampshire, USA). Trypsin and sulforhodamine B dye were obtained from Sigma-Aldrich (Missouri, USA).

Methods

Preparation of QCD-g-CS associated HA nanoparticles

QCD-g-CS and HMW-HA (1.2 MDa) were separately dissolved in DI water. The HMW-HA solution was ultra-sonicated (Sonics VCX-130 PB Vibra cell™, Connecticut, USA). The HMW-HA nanoparticles were prepared by a slow addition of HMW-HA solution into QCD-g-CS solution at different mole ratios (0.05 to 0.7 mole) with stirring (Sakulwech et al. 2018).

Determination of particle size, zeta potential and polydispersity index (PDI)

HMW-HA nanoparticles were analyzed with Zetasizer® (NanoZS4700 nanoseries, Malvern, UK) at 25^{o}C (Sakulwech et al. 2018).

Determination of loading efficiency (% Loading) and association efficiency (% AE)

Each prepared HMW-HA nanoparticles were freeze-dried, and dialysed with ultrasonication. The dialysates were filtered (0.4 μm) further to UPLC analysis (Waters Acquity H-Class system, Acquity UPLC PDA eλ detector, BEH C18 1.7 mm column (2.1 × 100 mm, Waters, Massachusetts, USA) by the validated condition. The results were calculated as followings (Sakulwech et al. 2018).

$$\% \text{ Loading} = \frac{\text{Detected amount of HA}}{\text{Weight of freeze dry pellets}} \times 100$$

$$\% \text{ AE} = \frac{\text{Detected amount of HA}}{\text{Initial HA added}} \times 100$$

FTIR structural confirmation of QCD-g-CS associated HA nanoparticles

QCD-g-CS, HMW-HA, and the selected HMW-HA nanoparticles were FTIR analyzed (Perkin Elmer Spectrum-GX FTIR spectrometer, Massachusetts, USA) from 4000 to 400 cm^{-1} (Sakulwech et al. 2018).

Morphology observation using TEM

The samples were stained with phosphotungstic acid (1%, w/v) or uranyl acetate (2%, w/v) on the carbon-coated copper grid, and observed by transmission electron microscopy (TEM, JEOL JEM-2010, Tokyo, Japan) observed at 120 kV (Sakulwech et al. 2018).

Assessment of the cytotoxicity on human skin fibroblasts

Fibroblasts were cultured in 75 cm^2 flask with DMEM medium contained 10% FBS and 1% penicillin/streptomycin. The cultured cells were incubated at 37 ^{o}C with 5% CO_2. The samples were dispersed in sterile deionized water (0.01-0.12 mg/ml), and examined by Sulforhodamine B (SRB) assay (Kanlayavattanakul et al. 2016) using a microplate reader (SPECTROstar Nano, BMG Labtech, Germany) at 540 nm.

Statistical analyses

Analysis of variance and least significant different test by using ANOVA test (SPSS version 21.0). The significant level was set at $p < 0.05$.

RESULTS AND DISCUSSION

Preparation and characterization of QCD-g-CS-associated HMW-HA nanoparticles

The nanoparticles were formed with ionotropic gelation technique by ionic linkages between CS and HA (Sakulwech et al. 2018). The ratios of HMW-HA were varied form 0.005 (H1), 0.01

(H2), 0.02 (H3), 0.03 (H4), 0.04 (H5), 0.05 (H6), 0.06 (H7) and 0.07 (H8) mole per 1 mole of QCD-g-CS as shown in **Figure 1**. The increasing HMW-HA ratios increased particle size and PDI, but decreased the zeta potential due to the addition of negative charge of HMW-HA, which altered the net zeta potential and dispersity of the nanoparticles. H1 and H2 were smallest in size (327.20 - 348.20 nm) with high positive charge and narrow PDI that would lead good stability (Lademann et al. 2007; Sakulwech et al. 2018). The positive charge of nanoparticles promotes skin adhesion prolonging the skin benefit of HMW-HA (Gillet et al. 2011). H3 to H5 formulae were the aggregated nanoparticles with larger size (>1000 nm) due to their low zeta potential (+10 to -10 mV). H6 to H8 were size increased as they were suspended nanoparticles in free HMW-HA medium with strong negative zeta potential. H2 was the smallest formula with highest HMW-HA molar ratio, which better adhere on skin (Lademann et al. 2007).

Determination of % Loading and % AE

The loading (%loading) and association efficiencies (%AE) were evaluated on the selected formula. The %loading represents the amount of HMW-HA delivered per unit weight of nanoparticles, while the %AE represents the percentage of successfully associated HMW-HA with nanoparticles (Phunpee et al. 2016; Sakulwech et al. 2018). Loading efficacy (%) was shown to be increased at the greater amount of HMW-HA, although %AE was decreased. The diminution of %AE was due to the elimination of the excess amount of HMW-HA during centrifugation (**Figure 2**). H1, H2, H3 and H4 were significantly higher in %AE than the others (81.88 – 84.05%, $p < 0.001$). An addition of HMW-HA increased %loading as shown in H2 to H8 (17.26 – 21.85%), but excess HMW-HA ratio was unable to enhance loading because the free positive charges on QCD-g-CS were insufficient for HMW-HA ionic linkages (Sakulwech et al. 2018). Thus, H2 was the best formula with high %AE and %loading as well as size and zeta potential.

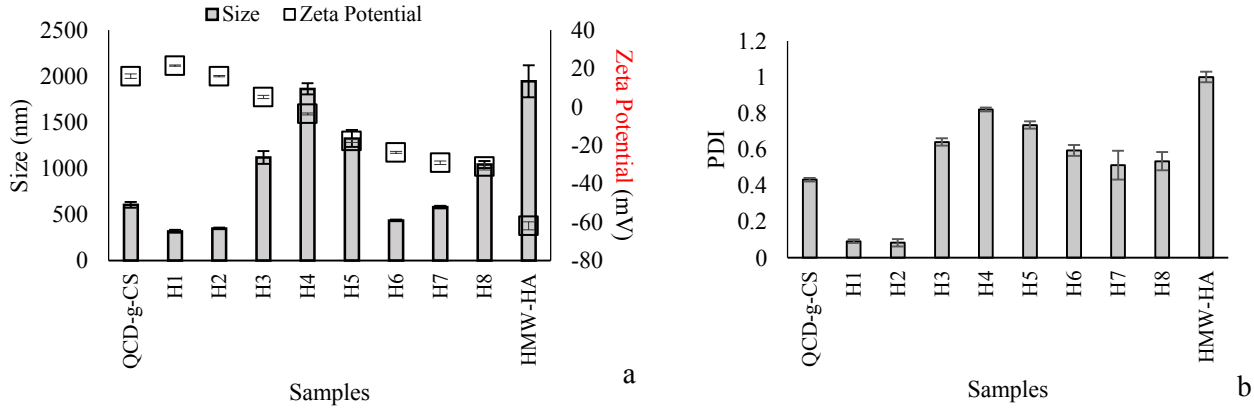

Figure 1: The particle size and zeta potential (a) and the polydispersity index (b) of each sample.

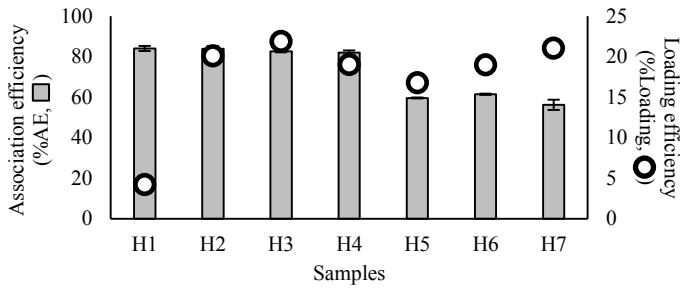

Figure 2: Association efficiency and loading efficiency of each sample.

FTIR structural confirmation of QCD-g-CS associated HMW-HA nanoparticles

HMW-HA and QCD-g-CS bonding was confirmed with FTIR (**Figure 3**). The spectrum of QCD-g-CS had amide dominant peaks at 1650 and 1560 cm^{-1}, indicating N-H bending within the primary and secondary amides. N-H stretching at 2860 cm^{-1} indicated the presence of these amides

(Phunpee et al. 2016). The spectrum of HMW-HA was similar to that of enzymatically hydrolyzed HA from the previous study, which provided sharp asymmetric stretching of C-O of carbonyl group at 1063 cm^{-1} and the overlapping peak 1475 to 1690 cm^{-1} representing amide group (Sakulwech et al. 2018). HA associates with QCD-g-CS via the electrostatic interaction and evidently supported by the H2 spectra. There were disappearing or weakening CS amides (1560 and 2860 cm^{-1}) and HA carboxylics (1063 cm^{-1}), and broadening peak of O-H and N-H (2340 - 3800) due to the ionic absorption (Sakulwech et al. 2018).

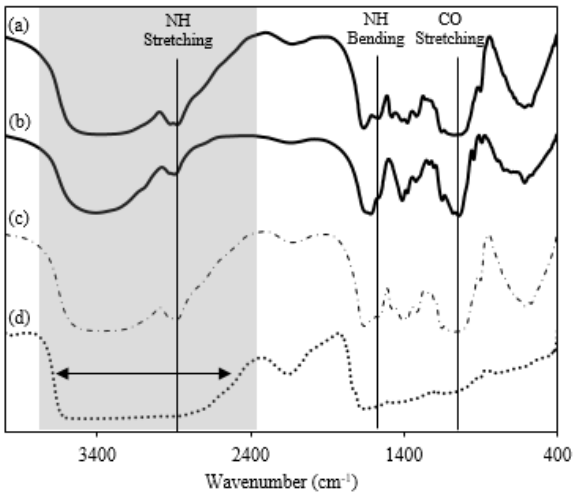

Figure 3: FTIR spectra of (a) QCD-g-CS, (b) HMW-HA, (c) a physical mixture of QCD-g-CS and HA and (d) H2.

Morphological observations using TEM

TEM was widely used for morphological observation of the nanoparticles evaluating the particle size in dry state. QCD-g-CS is a spherical shape nanoparticle as Sajomsang et al. (2011) had demonstrated that QCD-g-CS formed self-aggregates micelle with hydrophobic β-CD core (a dark center of the sphere) and surrounding hydrophilic CS backbone as shown in **Figure 4a**. HMW-HA is a linear polysaccharide chain, which can be contracted into globular form via its inter- or intramolecular interactions (Cowman and Matsuoka 2005) as shown in **Figure 4b**. HMW-HA spreads into linear chain at low concentration and then contacts its negatively charged carboxylics with positive charge of amides on the QCD-g-CS micelle during an addition of HMW-HA solution into QCD-g-CS micelle solution (**Figure 4c**), resulting condensed spherical micelle (Sakulwech et al. 2018).

Assessment of the cytotoxicity on human skin fibroblasts

The toxicity test in mammalian cells was used for screening the safety of nanoparticles for healthcare applications (Kanlayavattanakul et al. 2015). At 0.01-0.1 mg/ml, the QCD-g-CS, HMW-HA and H2 were non-cytotoxic (**Figure 5**). The QCD-g-CS and H2 were slightly cytotoxic at 0.12 mg/ml as their positive charges might adhere on cell membrane leading disruption (Sakulwech et al. 2018).

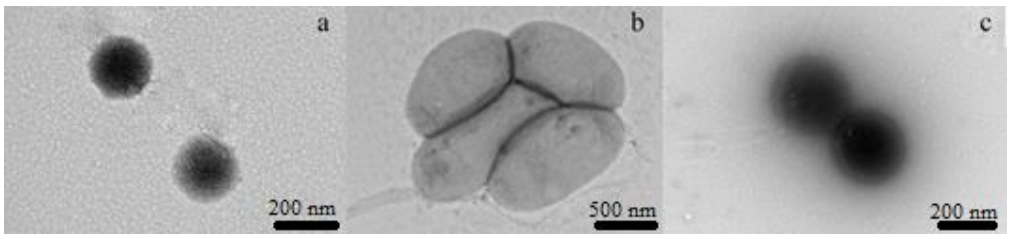

Figure 4: TEM image of (a) QCD-g-CS, (b) HMW-HA and (c) H2.

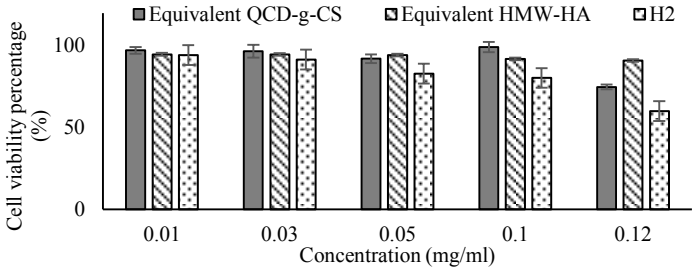

Figure 5: Cytotoxicity of QCD-g-CS, HMW-HA and H2.

CONCLUSION

These results proved that the versatile QCD-g-CS nanoparticle can be used for carrying HMW-HA in addition to a small molecule. The nanoparticle delivery system of HMW-HA was prepared and characterized. The positively charge spherical nanoparticles differed from HMW-HA natural negative linear chain form that skin adhesion obstructed by the lipid barrier of the skin are resolved. Lademann et al. (2007) suggested that small nanoparticles (less than 600 nm) might promote skin penetration and skin adhesion. High positive charge and the small size (348.20 ± 8.67 nm) of the prepared nanoparticles would therefore promote skin adhesion exacerbating moisturizing efficacy of HMW-HA (Gillet et al. 2011; Lademann et al. 2007). Moreover, the available CD cavity can entrap other biologically active hydrophobic guest molecules, while the nanoparticles could provide additional benefits of HMW-HA. Accordingly, these nanoparticles are challenged for cosmetics such as oral care products and others.

ACKNOWLEDGMENTS

Mae Fah Luang University and National Nanotechnology Center (NANOTEC) are acknowledged for facilities and financially. This study was funded by Thailand Graduate Institute of Science and Technology (TGIST), National Science and Technology Development Agency (NSTDA), Thailand (Project No. SCA-CO-2558-1026-TH). Dr. Puxvadee Chaikul is acknowledged for her kind suggestion on cell culture study.

REFERENCES

Chen, M., Gupta, V., Anselmo, A. C., Muraski, J. A., & Mitragotri, S. (2014). "Topical delivery of hyaluronic acid into skin using SPACE-peptide carriers." J. Control. Release, 173(1), 67-74.

Cowman, M. K., & Matsuoka, S. (2005). "Experimental approaches to hyaluronan structure." Carbohydr. Res., 340(5), 791-809.

Farwick, M., Lersch, P., & Strutz, G. (2008). "Low molecular weight hyaluronic acid: its effects on epidermal gene expression & skin ageing." SÖFW J., 134(11), 2–6.

Gillet, A., Compère, P., Lecomte, F., Hubert, P., Ducat, E., Evrard, B., & Piel, G. (2011). "Liposome surface charge influence on skin penetration behaviour." Int. J. Pharm., 411(1-2), 223-231.

Gonil, P., Sajomsang, W., Ruktanonchai, U. R., Pimpha, N., Sramala, I., Nuchuchua, O., et al. (2011). "Novel quaternized chitosan containing β-cyclodextrin moiety: Synthesis, characterization and antimicrobial activity." Carbohydr. Polym., 83(2), 905-913.

Kanlayavattanakul, M., Lourith, N., Tadtong, S., & Jongrungruangchok, S. (2015). "Rice panicles: New promising unconventional cereal product for health benefits." J. Cereal Sci., 66(1), 10-17.

Kanlayavattanakul, M., Lourith, N., & Chaikul, P. (2016). "Jasmine rice panicle: A safe and efficient natural ingredient for skin aging treatments." J. Ethnopharmacol., 193(1), 607-616.

Lademann, J., Richter, H., Teichmann, A., Otberg, N., Blume-Peytavi, U., Luengo, J., et al. (2007). "Nanoparticles–an efficient carrier for drug delivery into the hair follicles." Eur. J. Pharm. Biopharm., 66(2), 159-164.

Phunpee, S., Saesoo, S., Sramala, I., Jarussophon, S., Sajomsang, W., Puttipipatkhajorn, S., et al. (2016). "A comparison of eugenol and menthol on encapsulation characteristics with water-soluble quaternized β-cyclodextrin grafted chitosan." Int. J. Biol. Macromol., 84(1), 472-480.

Sajomsang, W., Gonil, P., Ruktanonchai, U. R., Pimpha, N., Sramala, I., Nuchuchua, O., et al. (2011). "Self-aggregates formation and mucoadhesive property of water-soluble β-cyclodextrin grafted with chitosan." Int. J. Biol. Macromol., 48(4), 589-595.

Sakulwech, S., Lourith, N., Ruktanonchai, U., & Kanlayavattanakul, M. (2018). "Preparation and characterization of nanoparticles from quaternized cyclodextrin-grafted chitosan associated with hyaluronic acid for cosmetics." Asian J. Pharm. Sci., 13(1), 498-504.