Pectinate Micro-/Nano-particles for Stabilization of Retinyl Palmitate

Hyeongmin Kim, Jieun Ro, Kyunghhee Park, Prakash Khadka, and Jaehwi Lee

College of Pharmacy, Chung-Ang University, Seoul 156-756, South Korea
hm.kim8905@gmail.com

ABSTRACT SUMMARY
Pectinate micro/nanoparticles for stabilizing retinyl palmitate (RP) were developed. The stability of RP was considerably improved when it was loaded in the pectinate particles because of the anti-oxidative effect of pectin and protective action of the particles from the oxidative environment. Therefore, pectinate particles could be a promising vehicle for stabilization of RP and used as carriers in the cosmeceutical formulations.

INTRODUCTION
Retinyl palmitate (RP) is an ester form of retinol (vitamin A) and palmitic acid, widely used as an active ingredient in numerous cosmetic products due to its anti-aging activity such as the prevention of wrinkles [1]. However, the stability of RP is problematic although RP is thermally more stable than the parent compound, retinol. Several formulation strategies for the stabilization of RP have therefore been developed including micro/nano-capsules, solid lipid nanoparticles, and liposomes. These systems, however, have exhibited disadvantages such as high manufacturing cost and use of toxic solvents.

Pectin, a polysaccharide constituting cell walls of plants primarily made up of a D-galacturonic acid, has appropriate physical properties for preparing micro-/nano-particles when of which gel is cross-linked with calcium ions [2]. Furthermore, in our previous study pectin has demonstrated outstanding stabilizing effect among polysaccharides tested due to abundant hydroxyl groups in its chemical structure [3]. In this study, we therefore developed RP-loaded pectin micro-/nano-particles (RP-loaded PMP/PNP) to further stabilize RP and thereby to be used as cosmeceutical carriers.

EXPERIMENTAL METHODS
To prepare RP-loaded PMP and PNP, 0.1 mL of RP ethanol solution (10 mg/mL) was premixed with 0.9 mL of pectin aqueous solution (30 mg/mL). For the preparation of PMP, the premixed solution was taken with a 1 mL syringe (25 gauge) and added dropwise to 20 mL of CaCl₂ aqueous solution (5 mg/mL) using a syringe pump and a stream of nitrogen gas. The particle size of PMP was controlled by changing the pressure of nitrogen gas. PMP formed were maintained in the CaCl₂ solution for 10 min, and then separated from the solution. PNP was prepared by introducing the premixed solution to a high pressure homogenizer (Nano Debee, BEE International Inc., MA, USA) along with the CaCl₂ solution. The homogenization was performed at 5000 psi for 3 cycles. PNP prepared was then separated by centrifugation (8000 g, 10 min, 20°C).

The anti-oxidative effect of two PMPs, PMP 1 (mean particle size = 843.37 ± 56.18 μm), 2 (mean particle size = 593.45 ±23.39 μm) and PNP was evaluated using a DPPH assay as reported previously, with some modification [4]. PMP 1, 2 and PNP (1 g) were suspended and separated from the solution. The suspensions were then filtered through a nylon membrane with pore size of 0.45 μm and the absorbance of the filtrate was measured at 517 nm with a microplate absorbance spectrophotometer (xMark, Bio-Rad Laboratories Inc., CA, USA). The DPPH radical scavenging effect was calculated by the equation below.

\[ \text{DPPH radical scavenging activity (\%) = } \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \right) \times 100 \]

The stability of RP in PMP 2, PNP suspension, pectin gel (0.5 mg pectin/mL), and
ethanolic solution was evaluated. The formulations were incubated at 40°C for 8 h. Every 2 h, samples were taken and dissolved in phosphate buffer (5 mL, pH 7.4) containing 5 mM EDTA. RP was then extracted from the solution using ethanol (100 mL), followed by the measurement of RP levels in the ethanol with HPLC. RP stability in pectin gel and ethanolic solution was monitored without above mentioned extraction procedures.

RESULTS AND DISCUSSION

PNP exhibited the greatest anti-oxidative activity among formulations tested as shown in Fig. 1. Of two types of PMP with different average particle sizes, PMP 2 showed slightly greater DPPH scavenging effect than PMP 1. Thus, the DPPH scavenging effect was increased with decreasing particle size. The reason for this may be because the large surface area of smaller particles allowed DPPH free radicals to freely interact with hydroxyl groups of pectin.

Fig. 1. DPPH radical scavenging effect measured with PMP 1, 2 and PNP. Asterisks (*) indicate a significant difference (p < 0.05). Data are presented as mean±SD (n=3).

Of samples tested, the stability of RP incorporated in PMP 2 and PNP was greater than pectin gel containing RP and RP ethanolic solution as shown in Fig. 2. In case of RP ethanolic solution devoid of pectin, the stability of RP was considerably lower than other samples formulated with pectin. The reason for this may be attributed to the stabilizing effect caused by the anti-oxidative activity of pectin, and the protection activity of PMP and PNP on RP in contact with oxidative environment.

Fig. 2. Changes in concentrations of RP in formulations tested. Data are presented as mean±SD (n=3).

CONCLUSION

The stability of RP was considerably improved when it was loaded in pectinate micro-/nano-particles may largely be due to the anti-oxidative effect of pectin and protection action of the particles from the oxidative environment. From the results obtained in this study, we suggest that pectinate particles have potential to be used as a promising vehicle for the stabilization of RP.

REFERENCES


ACKNOWLEDGMENTS

This study was supported by a grant of the Korean Health Technology R&D project, Ministry of Health & Welfare, Republic of Korea (Grant No.: 1465014399).