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In vitro skin permeation of monoolein nanoparticles containing hydroxypropyl β-cyclodextrin/minoxidil complex

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ABSTRACT

Monoolein (MO) cubic phases entrapping hydroxypropyl β-cyclodextrin (HPβCD)/minoxidil (MXD) complex were prepared by hydrating molten MO with the complex solution, where the concentrations of HPβCD/MXD were 1.0%/0.32%–19.4%/1.98%. Without HPβCD, the maximum content of MXD loaded in the cubic phase was only 0.071%, but with aid of HPβCD, the content in the cubic phase increased up to 5.72%. The nanoparticles of the cubic phase were prepared by a bath type sonication using a Pluronic F127 as a dispersant. HPβCD/MXD complex had little effect on the size and the structure of cubic phase nanoparticles. *In vitro* skin permeation of MXD loaded in the cubic phase nanoparticles (2.44 mg/cm^2 for 18 h), were higher than that of MXD dissolved in propylene glycol/water/ethanol (20/30/50, v/v/v) (1.91 mg/cm^2 for 18 h), but the amount of MXD remained within skin was higher with the MXD solution (0.068 mg/cm^2 for 18 h) than with the nanoparticles (0.023 mg/cm^2 for 18 h).

1. Introduction

In order to deliver an active ingredient transdermally, the barrier function of skin is to be reduced and the permeation of the active ingredient through skin is to be enhanced. Extensive studies have been done to develop topical preparations which exhibit a high skin permeation of an active ingredient. Several approaches were proposed to enhance the skin permeation (Yu and Liao, 1996). Selection of adequate vehicles, chemical modification of an active gradient, complexation of an active gradient with other compounds, use of skin permeation enhancer and iontophoresis are representative examples of the approaches (Pillai and Panchagnula, 2003; Barry, 1991). Whatever the approaches are, the topical formulation is to be designed in a manner that the thermodynamic activity of an active ingredient increases in the vehicle but decreases in skin, the partition of the ingredient into skin increases, and the barrier function of skin decreases (Kogan and Garti, 2006). Recently, polymeric nanoparticles were reported to penetrate into lipidic space between corneocytes due to its small size (Shim et al., 2004). Representative particulate carriers adopted for transdermal delivery are lipidic nanoparticles such as liposomes, solid lipid nanopaticles (SLN) and cubic phase nanoparticles (so called "cubosomes"). Unlike polymeric nanoparticles, the lipid nanoparticles readily interact with skin lipids (Muller et al., 2000; Kirjavainen et al., 1999). On the other hand, Minoxidil (MXD) is being used topically in treating alopecia androgenetica. In order to enhance the percutaneous absorption of MXD into human skin, several researches have been done on vehicles for MXD. The mixture solvent of propylene glycol (PG)/water/ethanol (EtOH) (20/30/50, v/v/v) is the vehicle for commercially-available MXD solution (Rogaine, Pfizer). Liposomes were used to promote the transfer of the drug into hair follicles and ethosome (lipid vesicular system embodying ethanol) was studied to enhance the skin permeation of MXD (Kim et al., 2003; Tsai et al., 1993). Iontophoresis was employed to transport a cationic derivative of MXD effectively (Poulos et al., 1995). With topical preparations, one of the most important factors determining the percutaneous absorption of MXD is the concentration (Tsai et al., 1993). The vehicle of PG/DW/EtOH can solubilize enough amount of MXD for hair growth promotion, but the organic vehicle may cause an irritation to skin and eye. Therefore, water-based vehicles having a high skin permeation-enhancing potency need to be developed. The suspensions of lipid nanoparticles (liposomes, SLN, cubosome) would be one of the substitutes. However, MXD have been suffering from its low solubility in the lipid particle and in the water phase.

In order to overcome the poor solubility in the suspension of lipid nanoparticles while keeping the high percutaneous absorption, monoolein (MO) cubic phases containing hydroxypropyl β-cyclodextrin (HPβCD)/(MXD) complex were prepared by hydrating molten MO with the complex solution, and the nanoparticles were obtained from the cubic phases by a sonication method. HPβCD forms a complex with MXD, and it could act as a solubilizer

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without interrupting the structure of the cubic phase nanoparticles. The release profiles of MXD were observed with the cubic phases contacting with aqueous release medium. The structures of the nanoparticles were investigated on TEM. *In vitro* skin permeations and the skin retentions of MXD loaded in the nanoparticles and of MXD solution in PG/DW/EtOH (20/30/50, v/v/v), PG/water (5/95, v/v) and EtOH/water (5/95, v/v) were observed in diffusion cells.

2. Materials and methods

2.1. Materials

Monoolein (1-monooleoyl glycerol, MO), Pluronic F127, phosphotungstic acid and 2-hydroxypropyl-β-cyclodextrin (HPβCD) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). Ethyl alcohol (EtOH) was provided by Dae Jung Co. (Siheung, Korea). Propylene glycol (PG) was purchased from Junsei Chemical Ltd. (Tokyo, Japan). Skins of female hairless mice (5 weeks old, type SKH1) were obtained from Orient Bio (seongnam, Korea). Water was distilled in a water purification system (Pure Power I$^+$, Human Corporation, Korea) until the resistivity was 18 MΩ/cm. All other reagents were in analytical grade.

2.2. Preparation of hydroxypropyl β-cyclodextrin/minoxidil inclusion complex

HPβCD/MXD inclusion complex was prepared following a method described in elsewhere (Kim et al., 2003). An excess amount of MXD was added to an aqueous solution of HPβCD contained in a 50 ml – conical tube. The concentration of HPβCD was adjusted to 0 M (0%), 0.0085 M (1%), 0.0169 M (2%), 0.0339 M (4%), 0.0678 M (8%), 0.1356 M (16%) and 0.2712 M (32%). The mixtures were gently revolved on a roller mixer (205RM, Hwashin Technology Co., Korea) overnight, and then they were filtered through a syringe filter (220 μm in pore diameter, Millex-GV, Millipore). The concentrations of the filtrates were determined by measuring the absorbances at 283 nm on a UV-spectrophotometer (Jenway 6505 UV/vis spectrophotometer, Bibby Scientific Ltd.).

2.3. Preparation of monoolein cubic phase and nanoparticles of cubic phase

MO cubic phases containing either HPβCD or HPβCD/MXD complex were prepared based on a method reported in elsewhere (Choi et al., 2007; Kim et al., 2004). Two gram of MO contained in 20 ml vial were molten in a water bath of 60 °C. Either HPβCD solutions (0%, 1.0%, 2.0%, 4.2%, 8.8%, 19.4%) or HPβCD/MXD complex solutions (1.0%/0.32%, 2.0%/0.44%, 4.2%/0.66%, 8.8%/1.12%, 19.4%/1.98%) in distilled water were laid carefully on the molten MO so that the contents of aqueous solutions water in the mixtures are 30%. After tightly sealed, the mixtures were kept at room temperature under a dark condition until the aqueous solutions were completely adsorbed and transparent gels were obtained. The cubic phases containing HPβCD/MXD complex solutions (1.0%/0.32%, 2.0%/0.44%, 4.2%/0.66%, 8.8%/1.12%, 19.4%/1.98%) are to be termed as cubic phase (1.0%/0.32%), cubic phase (2.0%/0.44%), cubic phase (4.2%/0.66%), cubic phase (8.8%/1.12%) and cubic phase (19.4%/1.98%). The nanoparticles of the cubic phases were prepared using Pluronic F127 as a dispersant. The cubic phases containing HPβCD/MXD complex solutions were put to 200 ml of Pluronic F127 solution (0.2%) in distilled water. And then, the gels were micronized in a bath type sonicator (VC 505, Sonic & Materials, USA, 30% energy intensity, 30 s pulse on, 30 s pulse off) at room temperature for 15 min. Nanoparticles prepared using cubic phase (1.0%/0.32%), cubic phase (2.0%/0.44%), cubic phase (4.2%/0.66%), cubic phase (8.8%/1.12%) and cubic phase (19.4%/1.98%) are to be termed as nanoparticle (1.0%/0.32%), nanoparticle (2.0%/0.44%), nanoparticle (4.2%/0.66%), nanoparticle (8.8%/1.12%) and nanoparticle (19.4%/1.98%).

2.4. Transmission electron microscopy

The suspensions of cubic phase nanoparticles were negatively stained with freshly prepared phosphotungstic acid solution (2%, pH 6.8) (Jo et al., 2009). The stained nanoparticle suspensions were transferred onto a formvar/carbon coated grid (200 mesh) and it was air-dried at room temperature. The electron microphotographs were taken on an electron microscopy (LEO-912AB OMEGA, LEO, Germany).

2.5. Release of MXD from cubic phases

The degrees of MXD release from cubic phases were determined, following a method reported in a previous work (Choi et al., 2007; Kim et al., 2004). Distilled water of 5 ml, release medium, was laid over the cubic phases prepared using the saturation solution of MXD, and cubic phase (1.0%/0.32%), cubic phase (2.0%/0.44%), cubic phase (4.2%/0.66%), cubic phase (8.8%/1.12%) and cubic phase (19.4%/1.98%). The 20 ml-vials containing the cubic phases and the release medium was stood at room temperature and they are occasionally upside-downed. The supernatant, 0.1 ml, was taken for the assay of MXD released at predetermined time intervals, and the same amount of fresh distilled water was added to the release medium to compensate for the reduction in the volume of release medium. The absorbance of supernatant was measured at 283 nm on a UV-spectrophotometer. The percent releases of MXD was defined as the percent of the released amount of MXD at a given time based on the initial amount of MXD loaded in the cubic phases.

2.6. In vitro skin permeation of MXD

In vitro skin permeations of MXD loaded in nanoparticle (8.8%/1.12%) and nanoparticle (19.4%/1.98%) were observed following a method reported in a previous work (Shim et al., 2004). In brief, the dorsal skins of female hairless mice (type SKH) aged 5 week were used and the skin permeation was investigated on a Franz diffusion cells (Lab Fine Instruments, Korea). The receptor cells were filled with phosphate buffered saline (PBS, pH 7.4) and it was held at 37 °C under stirring. The suspensions of MXD-containing nanoparticles were diluted with distilled water so that the concentrations of MXD are 0.24 mg/ml. As controls, MXD solutions (0.24 mg/ml) in PG/DW/EtOH (20/30/50, v/v/v), PG/DW (5/95, v/v) and EtOH/DW (5/95, v/v) were used. The nanoparticle suspensions and the control solutions, 200 μl, were applied onto the skins (0.636 cm^2) and the receptor solutions, 300 μl, were taken for the assay of MXD at a predetermined time. After 18 h-skin permeation experiment, the skins exposed to MXD-containing samples were washed using PBS buffer solution, and they were cut out using a biopsy blade (Surgical Blade No. 10, Feather Safety Razor Co., Ltd.). In order to dissolve MXD out of the skin, the skins were put to 5 ml of ethanol contained in 20 ml-vials, tightly sealed and kept at room temperature for 24 h. And then the concentration of MXD in ethanol was determined using HPLC. The MXD assay was performed in a liquid chromatograph (M600E, M7725i/Waters, 996PDA) equipped with a UV detector (0.05 AFUS). A reversed phase column (4.6 mm × 150 mm, Agilient Eclipse XDB-C18 5 μm) was eluted with methanol/water/glacial acetic acid (750/250/10, v/v/v) at a flow rate of 1.0 ml/min and a sample of 20 μl was injected. The detection wave length was 281 nm.

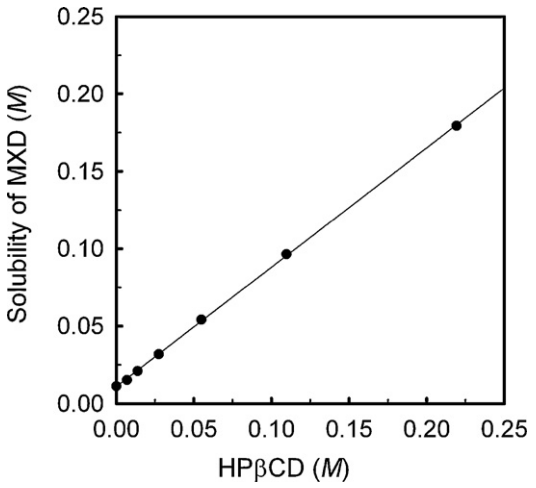

Fig. 1. Solubility of MXD with increasing concentration of HPβCD.

3. Results and discussion

3.1. Preparation of hydroxypropyl β-cyclodextrin/minoxidil inclusion complex

The calibration curve of MXD in distilled water was $A_{283} = 559.3X + 0.050$ with $R^2 = 0.9999$, where A_{283} is absorbance at 283 and X is concentration of MXD in % (w/w). Fig. 1 showed the solubility of MXD with increasing concentration of HPβCD. The solubility was proportional to the concentration of HPβCD. It was reported that an inclusion complex is formed by a hydrophobic interaction between the pyridine residue of MXD and the hydrophobic cavity of HPβCD (Kim et al., 2003). Accordingly, the increased solubility could be ascribed to the formation of inclusion complex. The equation for the solubility of MXD, obtained by a least square method, was expressed as $S_M = 0.7709C + 0.011$, where S_M is the solubility of MXD in M and C is the concentration of HPβCD in M. In a previous report where the solubility was determined in the HPβCD concentration range of 0–0.0678 M, the equation for the MXD solubility was $S_M = 0.7877C + 0.0106$. The slight difference from the result obtained in this work is possibly due to a difference in the purity of MXD used in each work.

3.2. Preparation of monoolein cubic phase and nanoparticles of cubic phase

Fig. 2 shows the MO gels prepared using HPβCD aqueous solutions (0% (a), 1.0% (b), 2.0% (c), 4.2% (d), 8.8% (e), 19.4% (f)). All the gels were transparent, whatever the concentrations of HPβCD in the aqueous solutions were (the numerical labels were posted behind vials containing gels.). Since cubic phases are known to be isotropic (Efrat et al., 2007; Spicer et al., 2001), the transparent gels shown in Fig. 2 are believed to be cubic phases. The structures of self-assemblies in an aqueous phase are determined by the packing parameter of an amphiphilic molecule (a building block) (Sagalowicz et al., 2006). If hydrophobic or surface-active additives are loaded in the self-assemblies, they could interposition among the amphiphiles. For example, MO cubic phase could accommodate triclosan (hydrophobic anti-bacterial agent) up to 32% (based on the mass of cubic phase) without structural disruption, however it transformed to a hexagonal phase when the content of triclosan was more than 16%. HPβCD has a hydrophobic cavity, but it is water-soluble and surface-inactive since the hydrophobic cavity is surrounded by hydroxyl groups. As a consequence, HPβCD could be included in the cubic phases as much as 5.7% (based on the mass of cubic phase) without interrupting the structure (this is the case of (f) in Fig. 2). Fig. 3 shows the MO gels prepared using the saturated solution of MXD (a) and HPβCD/MXD complex solutions (1.0%/0.32% (b), 2.0%/0.44% (c), 4.2%/0.66% (d), 8.8%/1.12% (e), 19.4%/1.98% (f)). All the gels were transparent and yellowish, whatever the concentrations of HPβCD/MXD complex in solutions, used for the preparation of MO gels, were (the alphabetical labels were behind vials containing gels). Accordingly, the transparent gels are believed to be cubic phases. MXD is poorly water-soluble (about 0.24% in Fig. 1) and it is also hardly soluble in the lipid matrix of the cubic phase. With aid of HPβCD, however, the amount of MXD loaded in the cubic phase increased. For example, when the cubic phase was prepared using the saturated solution of MXD (about 0.24%), the content of MXD in the cubic phase was only 0.07% (based on the mass of cubic phase). In case the cubic phases were prepared using the solutions of HPβCD/MXD complexes (1.0%/0.32%, 2.0%/0.44%, 4.2%/0.66%, 8.8%/1.12% and 19.4%/1.98%), the content of MXD in the cubic phases were 0.30%, 0.61%, 1.25%, 2.61% and 5.7% (based on the mass of cubic phase). Hence, the amount of MXD loaded in the cubic phase could be controlled by the amount of HPβCD loaded. Fig. 4 shows the nanoparticles of cubic phase prepared using the saturated solution of MXD (a) and HPβCD/MXD complex solutions (19.4%/1.98% (b)). Whether the complex was loaded in the cubic phase or not, the sizes of nanoparticles were 10–100 nm on the TEM photos, and the stripes and dots were observed on the nanoparticles. Lots of water channels pass through the lipid matrix of cubic phase. Phosphotungstic acid will stain the water channels but not the lipid matrix. The stained water channels would responsible for the stripes and dots. Fig. 5 shows the size distribution of the nanoparticles of cubic phase prepared using the saturated solution of MXD. The two populations were observed. The sizes of smaller population were less than 100 nm and that of larger population were about 150–400 nm. Mild energy generated from a bath type sonication led to the bimodal distributions of cubic phase nanoparticles (Yang et al., 1996). Extensive and intensive energy could give a homogeneous nanoparticles but it could also chemically deteriorate MO, HPβCD and MXD. Similar size distributions were obtained with the nanoparticles of cubic phases prepared using the solutions of HPβCD/MXD complexes. The inset shows the mean diameters of the nanoparticles. The mean size of nanoparticle (0%/0.24%), nanoparticle (1.0%/0.32%), nanoparticle (2.0%/0.44%), nanoparticle (4.2%/0.66%), nanoparticle (8.8%/1.12%) and nanoparticle (19.4%/1.98%) were 189.7 nm, 229.1 nm, 224.3 nm, 241.4 nm, 242.2 nm and 234.1 nm, respectively. Following TEM photos and mean sizes, the loading of HPβCD/MXD complex in the cubic phase has little effect on the structure and the size of the nanoparticles. HPβCD/MXD complex is hydrophilic and surface-inactive and it will hardly intercalate in the lipid matrix of MO cubic phase. This may explain the reason why HPβCD/MXD complex could hardly affect the structure of cubic phases.

3.3. Release of MXD from cubic phases

Fig. 6 shows the degree of MXD released from cubic phase prepared using the saturated solution of MXD, and cubic phase (1.0%/0.32%), cubic phase (2.0%/0.44%), cubic phase (4.2%/0.66%), cubic phase (8.8%/1.12%) and cubic phase (19.4%/1.98%). The degree of release increased in a saturation manner and the rate of release seemed to be a first-order release. It was reported that the release rate of a water-soluble ingredient loaded in MO cubic phase followed a first-order release (Lara et al., 2005). MXD available for the diffusion through cubic phase is depleted with time elapse from the surface of cubic phase. Accordingly, the region where MXD is exhausted will be extended with time so the length of diffusion will increase. That is, the boundary where MXD is about to diffuse (so called “moving boundary”) will recede with time from the surface

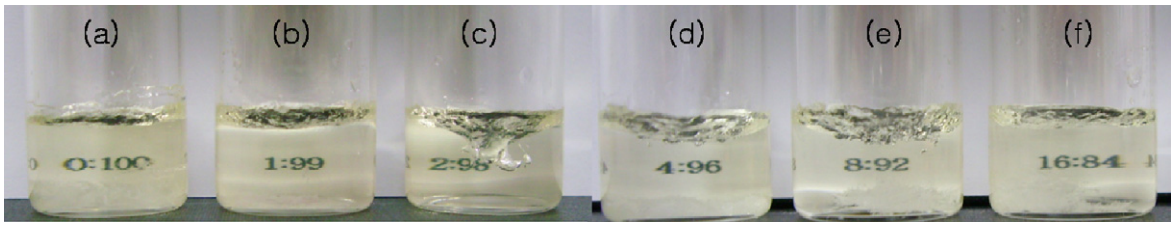

Fig. 2. MO gels prepared using HPβCD aqueous solutions (0% (a), 1.0% (b), 2.0% (c), 4.2% (d), 8.8% (e), 19.4% (f)).

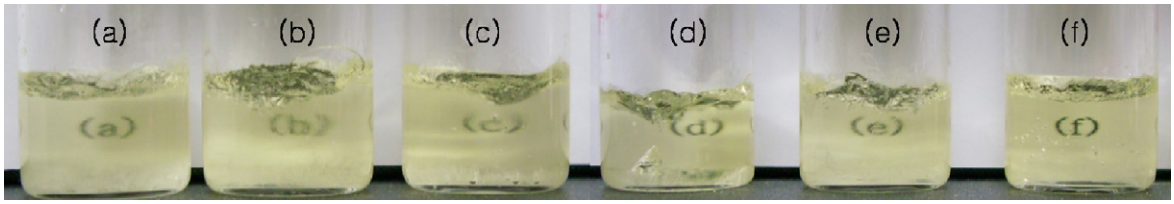

Fig. 3. MO gels prepared using the saturated solution of MXD (a) and HPβCD/MXD complex solutions (1.0%/0.32% (b), 2.0%/0.44% (c), 4.2%/0.66% (d), 8.8%/1.12% (e), 19.4%/1.98% (f)).

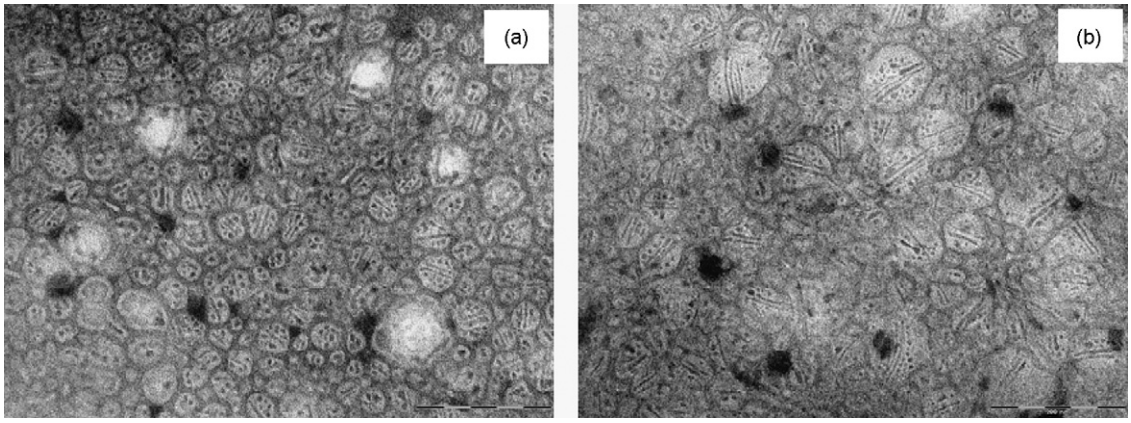

Fig. 4. Nanoparticles of cubic phase prepared using the saturated solution of MXD (a) and HPβCD/MXD complex solutions (19.4%/1.98%) (b). The nanoparticles were negatively stained using phosphotungstic acid solution (2%, pH 6.8). Bar in each photo represents 200 nm.

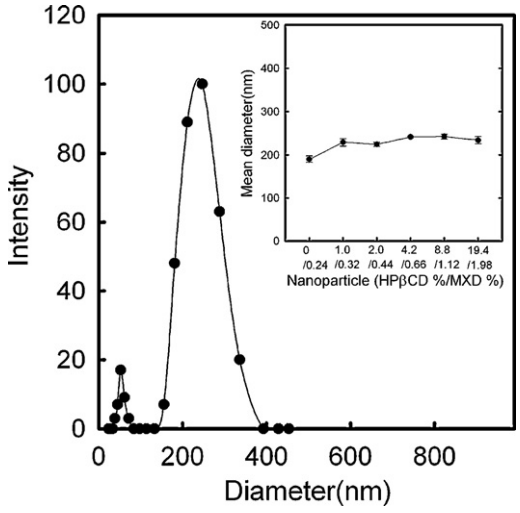

Fig. 5. Size distribution of the nanoparticles of cubic phase prepared using the saturated solution of MXD. Inset is the mean diameters of nanoparticle (0%/0.24%), nanoparticle (1.0%/0.32%), nanoparticle (2.0%/0.44%), nanoparticle (4.2%/0.66%), nanoparticle (8.8%/1.12%) and nanoparticle (19.4%/1.98%).

of cubic phase. Assuming there is no mass transfer resistance in water phase, the rate of release could be governed by Fick's law. Hence, major factors determining the rate of release would be the concentration difference (ΔC) between the surface of cubic phase and the moving boundary, and the diffusion distance (L, the length from the surface of cubic phase to the moving boundary), once the diffusivity (D) through the cubic phase is assumed to be constant. ΔC will decrease with time because the concentration of MXD at the surface will decrease, and L will increase with time. Accordingly, the rate of release will decrease as shown in Fig. 6.

3.4. In vitro skin permeation of MXD

Fig. 7 shows the fluxes of MXD, loaded in the nanoparticle (8.8%/1.12%) and the nanoparticle (19.4%/1.98%), and the fluxes of MXD dissolved in liquid vehicles. The fluxes increased with time whatever the vehicles for MXD were. It means that the transfer rate from the surface of skin to receptor solution increased with time. Components (MO, HPβCD, PG, EtOH) of vehicles for MXD would accumulate within the skins so that the transfer rate may be accelerated with time. In fact, the components are known to be skin permeation enhancers (Hyde et al., 1984; Masson et al., 1999; Oh et al., 2001). The effectiveness of the skin permeation is in the order of nanoparticle (8.8%/1.12%) >nanoparticle (19.4%/1.98%) >MXD solution in PG/DW/EtOH (20/30/50, v/v/v)

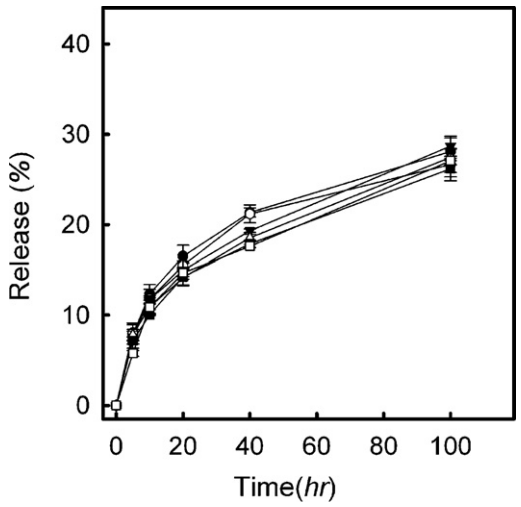

Fig. 6. Degree of MXD released from cubic phase prepared using the saturated solution of MXD (●), and cubic phase (1.0%/0.32%) (○), cubic phase (2.0%/0.44%) (▼), cubic phase (4.2%/0.66%) (△), cubic phase (8.8%/1.12%) (■) and cubic phase (19.4%/1.98%) (□).

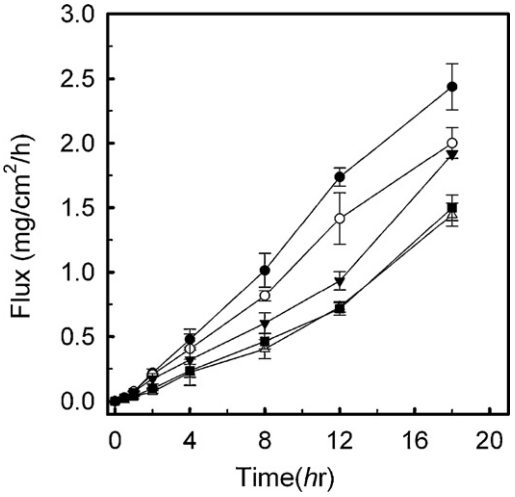

Fig. 7. Fluxes of MXD, loaded in nanoparticle (8.8%/1.12%) (●) and nanoparticle (19.4%/1.98%) (○), and fluxes of MXD dissolved in PG/DW/EtOH (20/30/50, v/v/v) (▼), PG/DW(5/95, v/v) (△) and EtOH/DW (5/95, v/v) (■).

>MXD solution in EtOH/DW (5/95, v/v) =MXD solution in PG/DW (5/95, v/v). For example, the fluxes at 18 h of MXD contained in each vehicle were 2.44 mg/cm^2/h, 2.00 mg/cm^2/h, 1.91 mg/cm^2/h, 1.49 mg/cm^2/h and 1.45 mg/cm^2/h, respectively. The skin permeability is defined as KD/L, where K is the partition coefficient of solute (MXD) between skin and vehicle, D is the diffusion coefficient of MXD through skin and L is the thickness of skin (Pirot et al., 1997). In case of cubic phase nanoparticles, MO could intercalate into the extra-cellular lipid matrix of skin, and disrupt the well-organized compact structure. Furthermore, although the concentrations of HPβCD in the nanoparticle suspensions (0.187% for nanoparticle (8.8%/1.12%) suspension and 0.23% for nanoparticle (19.4%/1.98%) suspension) were much less than those of MO (5.0% for nanoparticle (8.8%/1.12%) suspension, and 2.82% for nanoparticle (19.4%/1.98%) suspension), HPβCD could also reduce the skin barrier function, since it was reported to interact with skin lipids (Masson et al., 1999). As a result, MO and HPβCD will increase the diffusion coefficient (D). In addition, polymeric nanoparticles were reported to readily penetrate through extra-corneocyte route owing to their small size (Alvarez-Roman et al., 2004), and MO

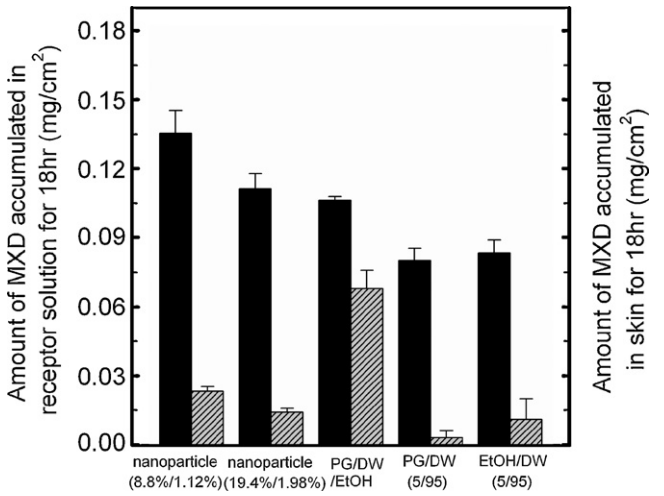

Fig. 8. Amount of MXD accumulated in receptor solution for 18 h (filled bars) and the amount MXD accumulated within skin for 18 h (shaded bars).

nanoparticles (so called "cubosomes") are known to permeate into skin (Caboi et al., 1997). As a consequence, K of MXD will apparently increase since MXD is loaded in the MO nanoparticles and the partition of MXD into skin would be enhanced by MO nanoparticles. It is concluded that the higher permeability (P) of MXD loaded in MO nanoparticles is ascribed to the increased D and K. Even though the concentrations of MXD in the MO nanoparticle suspensions were the same (0.24 mg/ml), the flux of MXD loaded in nanoparticle (8.8%/1.12%) was somewhat higher than that of MXD loaded in nanoparticle (19.4%/1.98%). This is because that the MO concentration in the former suspension, 50 mg/ml, was higher than the MO concentration in the latter one, 28.2 mg/ml. On the other hand, among the controls, the vehicle of PG/DW/EtOH exhibited the highest flux of MXD. The composition of the vehicle is the same as that of commercially-available MXD solution (Rogiane, Pfizer). PG and EtOH were included not only to solubilize MXD but also to enhance the skin permeation of MXD. It is thought that PG permeates into hairy skin and it helps the skin absorb MXD. That is, MXD permeation into the skin could be increased due to the co-solvent effect of PG. EtOH also would have a co-solvent effect and, in addition, it could increase the thermodynamic activity of MXD in the vehicle due to its volatile property. Nevertheless, the flux of MXD in the vehicle was less than that of MXD in the MO nanoparticles. Fig. 8 shows the amount of MXD accumulated in receptor solution for 18 h and the amount MXD accumulated within skin for 18 h. The amounts of MXD accumulated in the receptor solutions, when MO nanoparticles suspensions were applied, were higher than the amount, when MXD solutions were used. But the amount of MXD accumulated within skin (about 9.5 mg), when MXD solution in PG/DW/EtOH was applied, was three to four times higher than the amount, when MO nanoparticle suspensions were used. The nanoparticles contained HPβCD/MXD complex in their water channels and they released a significant amount of MXD to water phase (Fig. 6). Similarly, the skin-penetrated nanoparticles would readily release MXD to the receptor solution. This could account for the reason the amount of MXD retained in skin was lower even though the amount in the receptor solution was higher. The one of the reasons why the skin retention of MXD was the highest when the solution in PG/DW/EtOH was applied could be that PG and EtOH act as co-solvents for MXD in skin. In order to obtain an effective hair growth promotion, MXD is required to be localized around blood vessels surrounding hair bulbs so that it could dilate the blood vessels effectively for a long time. In this circumstance, the skin retention of MO nanoparticles needs to be increased.

4. Conclusion

With aid of the inclusion complexation with HPβCD, MXD could be loaded in MO cubic phase so that the content in the cubic phase is as much as 5.72% (without HPβCD, the maximum content of CD in the cubic phase was only 0.071%). The nanoparticles of the cubic phase could be obtained by micronizing the cubic phases using a bath type sonicator. HPβCD/MXD complex had little effect on the size and the structure of the cubic phase nanoparticles. MO nanoparticles exhibited a higher *in vitro* skin permeation than the vehicle of PG/DW/EtOH (20/30/50, v/v/v) did. However, the skin retention of MXD loaded in the nanoparticles were lower than that of MXD in PG/DW/EtOH (20/30/50, v/v/v).

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