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ZINC OXIDE NANOPARTICLES AS SKIN PERMEATION ENHANCER FOR SOLVENTS AND SURFACTANTS

Narges Shokri[1*], Hamid Akbari Javar[2]

[1]Department of Pharmaceutics, School of Pharmacy, Ardabil University of Medical Sciences, Ardabil, P.O. Box: 56189-53141, Iran. Tel : +98-04533522437 Fax : +98-04532522197

[2]Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Objective: Transdermal route of drug administration has absorbed largeinterests for its many advantages. Several materials, mainly different solvents and surfactants, have been used as excipients to enhance the skin permeation of drugs.Nanoparticles (NPs) also have been proved to affect the permeations of substances.ZnO-NPs, widely used in topical products, have been investigated in this study in terms of their effects on permeations of different substances (excipients) and therefore permeations of active ingredients.

Method: To determine the skin permeation of everysubstance, diffusion cell method and a cut of chicken skin were employed following by quantification of the substance concentration in the receiver medium after 1.5 hours.

Results: The substances showed different permeations. The ZnO-NPs increased the permeation of each substance. In the absence and also in the presence of the ZnO-NPs, the mean amounts permeated were respectively belonged to hydrophobic solvents, hydrophilic solvents, oily solvents and surfactants. The ZnO-NPs increased the permeation ofhydrophobic solvents, oily solvents, hydrophilic solvents and surfactants, with 31.33, 24, 20.33 and 5.34%, respectively. Such increases, were not dependent on the molecular weight (MW) of the oily and hydrophobic solvents but were dependent on the MW of the hydrophilic solvents and the surfactants.

Conclusion: The ZnO-NPs are suggested to be used for enhancing the skin permeations ofsolvents or surfactantsin topical productswhich potentially can improve absorption of active ingredients. Besides, such enhancing effect of the ZnO-NPs should be noticed in topical products where they may increase drug delivery dose and alsoincrease drug or excipient systemic toxicity.

Keywords: *ZnO nanoparticles; Skin permeation; solvent; surfactant.*

INTRODUCTION

Transdermal drug route of administration has lots of advantages including easy, safe, non-invasive, painless, decreased or loss of first-pass drug metabolism, no gastro-intestinal degradation, long time delivery (>24 hours) (Especially transdermal patches), controlled delivery, controlled termination, bypassing GI absorption steps, dramatic pH changes, enzyme effects and transit times,and ultimately easier preparation of the dosage forms than the parenterals[1]. The most important barrier for transdermal drug delivery is the skin's horny layer or stratum corneum (SC). This layer must be altered for penetration of drugs through the skin. This has been the subject of research for pharmaceutical scientists during the two latest decades[2]. Extensive research on chemical penetration enhancers (CPEs) has been performed during the latest 20 years which form the main strategy of formulation-design approaches for transdermal drug delivery[3]. It is now well known that formulation components can improve the quantity and rate of transdermal absorption of drugs[4]. Permeation of a drug through the skin in the presence of an enhancer is related to physico-chemical characteristics of the enhancer and the drug[5-7]. More than 200 chemicals have been shown to enhance skin permeation of drugs. Chemical penetration enhancers should construct a situation to make new skin microstructures[3].

Several enhancers have been used to enhance skin permeation of different drugs mainly including aliphatic acids, fatty acids, esters, alcohols, oils and terpens[1,8,9].

One group of penetration enhancers are hydrophobic nanoparticles (NPs) made from lipids, hydrophobic polymers, etc. Such polymers should be evaluated in terms of safety, biocompatibility and especially degradation kinetics. Therefore, they should be accurately designed to become suitable for use in medications[10-12].

Zinc is a relatively inexpensive, biocompatible and non-toxic essential element for human health[11]. Parat et al. proved that Zinc is safe and has antioxidant and cytoprotective effects on skin keratinocytes in cell (HaCaT) culture[13]. Zinc oxide (ZnO) (molecular weight (MW): 81.408 g/mol) has been applied topically to heal wounds and treat other skin disorders[14]. The zinc distribution peaked in the epidermal layer and decreased toward the SC, with the exception positioned in the SC[15-17].

***Correspondence :** email: n.shokri@arums.ac.ir

In this study, ZnO-NPs(nominal average particle size of 100 nm) have been used to investigate their effect on the skin permeations of different substances including (oily, hydrophobic and hydrophilic) solvents and surfactants[18]. We divided these substances into four groups: Group 1: Oily solvents including sunflower oil, olive oil and coconut oil, Group 2: Hydrophobic solvents including Liquid paraffin (LP), dimethyl sulfoxide (DMSO), tetrahydrofuran (THF) and ethyl acetate (EA), Group 3: Hydrophilic solvents including propylene glycol (PG), ethylene glycol (EG), glycerol, ethanol, isopropyl alcohol (IPA) and acetic acid solution (1M), and Group 4: Surfactants including tween 80, Sodium lauryl sulfate (SLS), triethanolamine (TEA), cholesterol and glycine. All these substances have been widely used in different pharmaceutical or cosmetic products for their various advantages[19].Their MWs are: sunflower oil: 882.9 (average),olive oil: 882 (average), coconut oil: 645.6 (average), LP: 348 (average), DMSO: 78.13, THF: 72.11, EA: 88.11, PG: 76.09, EG: 62.07, glycerol: 92.09, ethanol: 46.07, IPA: 60.10, acetic acid: 60.05, tween80: 1300, SLS:288.38, TEA:149.2, cholesterol:386.65 and glycine:75.07 g/mol[20-25].

Skin permeations were determined using an ex-vivo method employing a diffusion cell and a piece of chicken skin. Using this model is also supported by the fact that SC, the site of enhancer action, presents similar behavior in vivo and in vitro because it is not a live layer[25].

MATERIALS AND METHODS

ZnO-NPs, sunflower oil, olive oil, coconut oil, LP, DMSO, THF, EA, 8PG, EG, glycerol, ethanol, IPA, acetic acid, tween80, SLS, TEA, cholesterol and glycine, were purchased from Sigma-Aldrich company, USA.

Preparation of formulations

Data related to the formulations are presented in the Table 1 (formulations 1-18). Every formulation of 1 to 14 containedonly 2ml of every liquid solvent or surfactant.Every formulation of 15 to 18 contained 100mg of every powder surfactant mixed by 2ml of deionized water (DW) to make a paste (by stirring for 10 minutes). The addition of DW was to make the powder skin permeation possible.

Table 1 : *Constituents of formulations 1-18.*

Formulation No.	Solvent or Surfactant (amount)	DW (amount)
1	Sunflower oil(2 ml)	-----
2	Olive oil(2 ml)	-----
3	Coconut oil(2 ml)	-----
4	LP (2 ml)-----5DMSO (2 ml)	-----
6	THF (2 ml)	-----
7	EA (2 ml)	-----
8	PG (2 ml)	-----
9	EG (2 ml)	-----
10	Glycerol (2 ml)	-----
11	Ethanol (2 ml)	-----
12	IPA (2 ml)	-----
13	Acetic acid (1 M) (2 ml)	-----
14	Tween80 (2 ml)	-----
15	SLS (100 mg)	✔ (2 ml)
16	TEA (100 mg)	✔ (2 ml)
17	Cholesterol (100 mg)	✔ (2 ml)
18	Glycine (100 mg)	✔ (2 ml)

Permeation test

The transdermal penetration of every substance was determined by a diffusion cell with an effective diffusion area of 10 cm^2 with a glass cap. Its 30 ml volume receiver chamber was filled with 30 ml of phosphate buffer solution, pH 7.4 as medium. Whenthe three oily solvents, LP, DMSO and THF were used, 30 ml of n-hexane was used instead of DW. An isolated piece of skin of a 3 months old chicken was fixed between the two chambers as the diffusion membrane, making an almost stretched condition of the skin. Isolated skins were carefully selected in order to have low underlying fat tissue. They were accurately selected in order to be completely similar in terms of thickness of the fat tissue and the number of feather follicles. For every permeation test experiment, each formulation was placed and spread on the skin. Experiments were repeated with addition of 200 mg ofZnO-NPs to every formulation on the skin. Then the cap (donor chamber) was placed and fixed at the top to avoid evaporation. The cell was placed in a shaker-incubator (Heidolph incubator 1000, Heidolph co., Germany) at 32°C for an exposure time of 1.5 hours[3,24]. Every 15 minutes the cap was taken away and the formulation was rubbed evenly by a swab to help the penetration. After 1.5 hours, the medium was analyzed for the concentration of the related solvent or surfactant using a UV-Vs spectrophotometer (Perkin-Elmer-Lambda25, USA). The maximum absorption wavelengths for the substances were considered as: sunflower oil, olive oil and coconut oil: 232 nm, LP: 278 nm, DMSO: 289 nm, THF: 290 nm, EA: 207 nm, PG: 272 nm, EG: 276 nm, glycerol: 285 nm, ethanol: 205 nm, IPA: 210 nm, acetic acid: 204 nm, tween80: 505 nm, SLS: 460 nm, TEA: 520 nm, cholesterol: 500 nm and glycine: 220 nm.

Statistical analysis

The permeation test experiment for every substance (solvent or surfactant), was repeated three times (also three more times in the presence of the ZnO-NPs for every substance). The concentration of the permeated substances was determined every time.The reported data aremean±SD (n=3). One-way analysis of variance (ANOVA) was used for comparing the mean differences. SPSS for Windows (release 11.5.0) was employed for statistical analysis and p-value<0.05 was considered to be significant.

RESULTS

The formulation components are shown in Table 1. Dermal permeations of the formulations are shown in Figure 1, as well as their related percentages.In the absence of the ZnO-NPs, the permeated amounts of the substances ranged from 0.22 ml (for acetic acid solution) to 1.11 ml (for LP), and about powders, from 5.8 mg (for glycine) to 11.7 mg (for cholesterol), resembling 11, 55, 5 and 11%, respectively.The mean permeated amount for oily solvents (sunflower oil, olive oil and coconut oil) (group 1) was 20.33±1.52%, for hydrophobic solvents (LP, DMSO, THF and EA)(group 2) was32.33±5.50%, for hydrophilic solvents (PG, EG, glycerol, ethanol, IPA and acetic acid solution) (group 3)

was 29.33±15.94%and for surfactants (tween80, SLS, TEA, cholesterol and glycine) (group 4) was 7.66±3.05%.

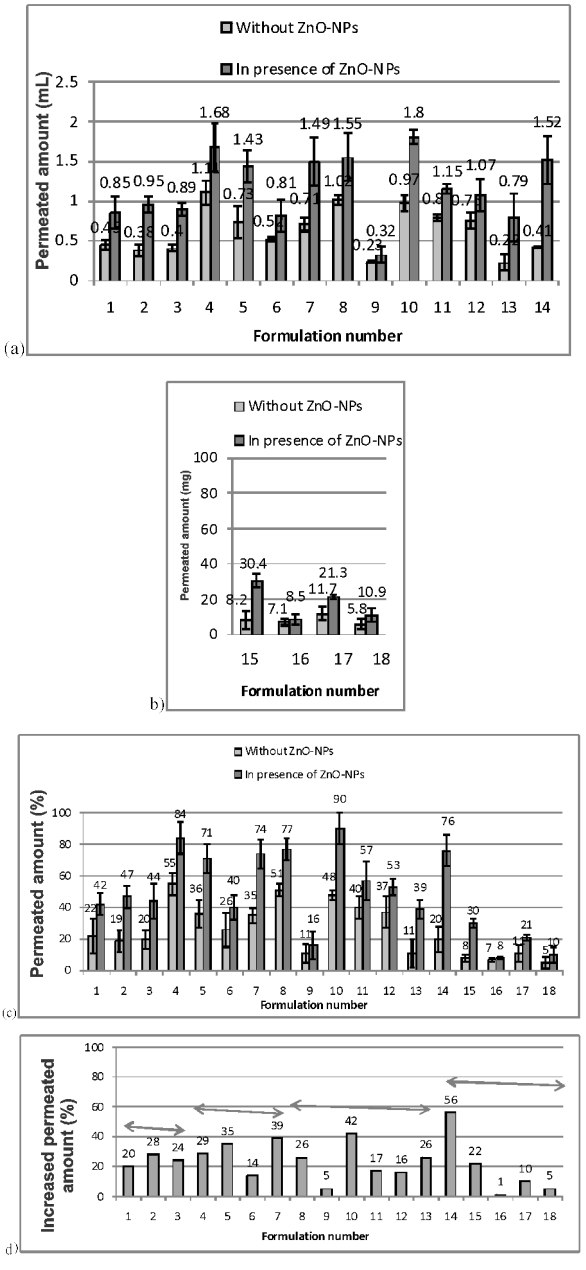

Fig. 1. *Permeated amountof every substance;(a): ml, (b): mg, (c): %, without the ZnO-NPs (□) and in the presence of the ZnO-NPs (□),(d): Increased permeated amounts (%)in the presence of the ZnO-NPs. The mean related to eachgroup is brought at the top(n=3), (1: sunflower oil, 2: olive oil, 3: coconut oil, 4: LP, 5: DMSO, 6: THF, 7: EA, 8: PG, 9: EG, 10: glycerol, 11: ethanol, 12: IPA, 13: acetic acid solution, 14: tween80, 15: SLS, 16: TEA, 17: cholesterol and 18: glycine),(Group 1: Oily solvents including sunflower oil, olive oil and coconut oil, Group 2: Hydrophobic solvents including LP, DMSO, THF and EA, Group 3: Hydrophilic solvents including PG, EG, glycerol, ethanol, IPA and acetic acid solution, Group 4: Surfactants including tween80, SLS, TEA, cholesterol and glycine).*

Simultaneous use of the substances and ZnO-NPs, lead to permeated amounts ranging from 0.32 ml (for EG) to 1.80 ml (for glycerol), and from8.5 mg (for TEA) to 30.4 mg (for SLS), resembling 16, 90, 8 and 30%, respectively.Using the ZnO-NPs, the mean permeated amount for oily solvents was 44.33±2.51%, for hydrophobic solvents was63.66±20.55%, for hydrophilic solvents was 49.66±9.45% and for surfactants was 13.00±7.00%.

The experiments showed that the ZnO-NPs increased the dermal permeations of all the studied substances comparing the permeations without using the ZnO-NPs ($p<0.05$). Such increases ranged from 1% (for TEA)or 5% (for EG or glycine) to 56% (for tween80) or 42% (for glycerol). For oily solvents, such increases were 20-28% with a mean of 24.00%±4. For hydrophobic solvents, such increases were 14-39% with a mean of 29.25%±10.96. For hydrophilic solvents, such increases were 5-42% with a mean of 22.00%±12.50. Ultimately for surfactants, such increases were 1-56% with a mean of 18.80%±22.24.

Figure 1 (a) and (b), presents the permeated amounts of everyused substance through the skin, when used solely and when used simultaneously with the ZnO-NPs. Figure 1(c)gives the percentages of permeated substances in both conditions, without the ZnO-NPs and with the ZnO-NPs. Figure 1(d)gives the percentages of increased permeations for the same formulations with the averages shown at the top for each of the four groups. Figure 2(a), presents the mean permeated amounts (%) of each of the four groups of the substances in absence and in presence of ZnO-NPs and Figure 2(b) presents the differences between each pair of the means.

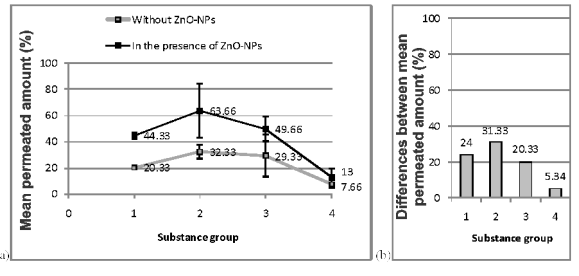

Fig. 2. *(a): Mean permeated amounts (%) of each group without the ZnO-NPs (□) and in the presence of the ZnO-NPs (□) (n=3), (b):Differences between each pair of the means ofpart (a), (Group 1: Oily solvents including sunflower oil, olive oil and coconut oil, Group 2: Hydrophobic solvents including LP, DMSO, THF and EA, Group 3: Hydrophilic solvents including PG, EG, glycerol, ethanol, IPA and acetic acid solution, Group 4: Surfactants including tween80, SLS, TEA, cholesterol and glycine).*

Figure 3(a), shows the relationship between the MWs of the oily solvents and their permeated amounts (%)(without ZnO-NPs). Figure 3 (b) shows the increased permeated amounts (%) of the oily solvents in presence of ZnO-NPs, versus their MW. Similarly, Figure 3(c) and (d) are related to the hydrophobic solvents, Figure 3(e) and (f) are related to the hydrophilic solvents and Figure 3(g) and (h) are related to the surfactants.

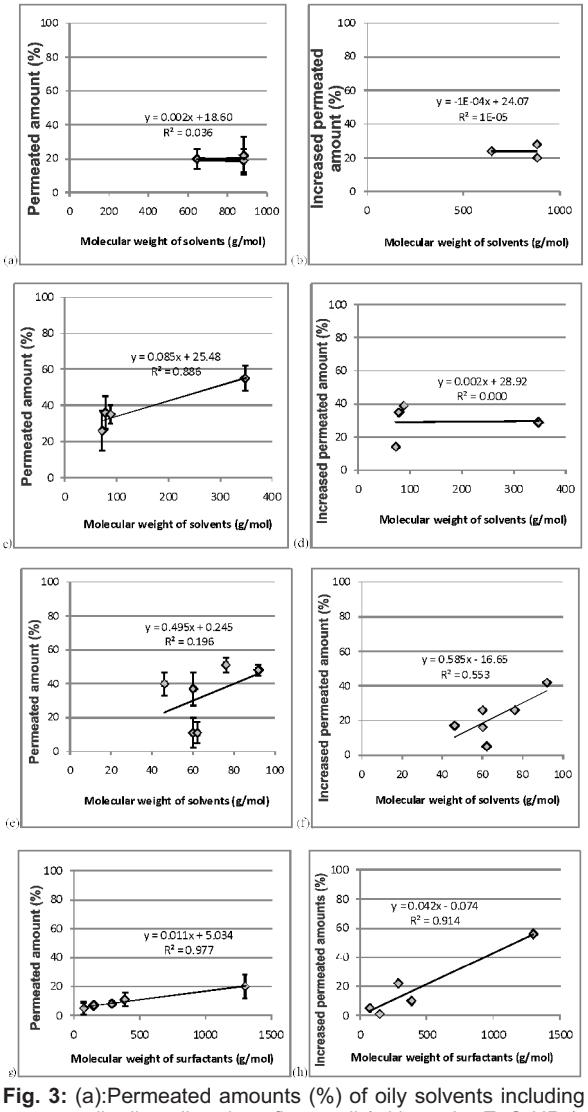

Fig. 3: (a):Permeated amounts (%) of oily solvents including coconut oil, olive oil and sunflower oil (without the ZnO-NPs), versus their molecular weights including 645.6, 882 and 882.9 g/mol, respectively(n=3). (b): Increased permeated amounts (%) of oily solvents (in the presence of the ZnO-NPs), versus their molecular weights (n=3). (c):Permeated amounts (%) of hydrophobic solvents including THF, DMSO, EA and LP (without the ZnO-NPs), versus their molecular weights including 72.11, 78.13, 88.11 and 348 g/mol, respectively (n=3). (d): Increased permeated amounts (%) of hydrophobic solvents (in the presence of the ZnO-NPs), versus their molecular weights (n=3). (e): Permeated amounts (%) of hydrophilic solvents including ethanol, acetic acid solution, IPA, EG, PG and glycerol (without the ZnO-NPs), versus their molecular weights including 46.07, 60.05, 60.10, 62.07, 76.09 and 92.09 g/mol, respectively (n=3). (f): Increased permeated amounts (%) of the hydrophilic solvents (in the presence of the ZnO-NPs), versus their molecular weights (n=3). (g): Permeated amounts (%) of surfactants including glycine, TEA, SLS, cholesterol and tween80 (without the ZnO-NPs), versus their molecular weights including 75.07, 149.20, 288.38, 386.65 and 1300 g/mol, respectively (n=3). (h): Increased permeated amounts (%) of surfactants (in the presence of the ZnO-NPs), versus their molecular weights (n=3).

DISCUSSION

Considering the first data series presented in the Figure 1, (a), (b) and © the used substance spossessed different permeations($p<0.05$) through the skin confirming previous works published by other researchers[26-31].

Among these substances, LP, PG and glycerol possessed the highest permeations, respectively ($p<0.05$). Such an excellent permeation for the LP could be attributed to its hydrophobicity and also its ability to dissolve the lipids of the skin layers. Such acceptable permeations for the PG and the glycerol could be attributed to their good solvent properties besides their ability to absorb and keep water. The permeations of the three oily solvents were near to each other ($p>0.05$). As mentioned, among the hydrophobic and hydrophilic solvents, the highest permeations belonged to LP and PG, respectively ($p<0.05$). Among the surfactants, the highest permeation belonged to tween80 ($p<0.05$). It could be attributed to its higher hydrophobicity than the other used surfactants, although it had a significantly higher MW than the other surfactants. Apparently, the surfactants positioned between the amphiphilic molecules of the skin layers because of the natural chemical structure of the surfactants. Therefore, they could just slightly pass and leak out the skin. In this order, the liquid state and the nonionic characteristic of the tween80 helped it to pass through the skin more than others.

Considering the second series of the Figure1 (a), (b) and (c), using the ZnO-NPs increased the permeations of all the substances($p<0.05$). These increases showed-dramatic variations between different substances. The data of the part (d) come in confirmation of the data of the previous parts showing only the percentages of those increases. Part (d) also shows the average of the increasesrelated to the substances of each group.

Figure 2(a), which compares the mean permeations (%) for each group without and with ZnO-NPs, better shows the effect of ZnO-NPs on the increase inpermeations of the substances($p<0.05$).Figure 2(b)presents the differences between each pair of the means which were24, 31.33, 20.33 and 5.34 % for the four groups, oily solvents, hydrophobic solvents, hydrophilic solvents and surfactants, respectively. These differences indicated the effect of ZnO-NPs on enhancement of permeation for each group. Although these four differences in Figure 2(b), were different from each other($p<0.05$), but there were no dramatic variations between them, leading to the similarity of the two curves of the Figure 2(a) and revealing the similarity of ZnO-NPs enhancement effect on the four groups. Considering the solvents (oily, hydrophobic or hydrophilic), the enhancement effect of the ZnO-NPs was found to be almost constant or in a narrow range. This range was 20.33 to 31.33% in this study.

Although the ZnO-NPs enhanced the permeations of all the substances (all the four groups), the highest difference was found to be for the hydrophobic solvents as 31.33%($p<0.05$) and the lowest for the surfactants as 5.34%($p<0.05$) (Figure 2(b)). In addition, the permeation

enhancement effect of the ZnO-NPs was higher for oily orhydrophobic solvents as 24 and 31.33%, respectively, than for hydrophilic solvents as 20.33%($p<0.05$) (Figure 2(b)). Since without using the ZnO-NPs, the permeations of the oily or the hydrophobic solvents were higher than those for the hydrophilic substances ($p<0.05$) or the surfactants ($p<0.05$)(first series of the Figure 1(a), (b) and (c)), it can be stated that ZnO-NPs enhanced the permeation of the high permeated substances (oily or hydrophobic solvents) more than those of the low permeated substances (hydrophilic substances or surfactants). In other words the enhancement effect of ZnO-NPs was dependent to the substances permeation abilities in the absence of ZnO-NPs. Such a phenomenon can be attributed to the enhancement mechanism of ZnO-NPs which is positioning in the skin layers and disordering the natural microstructure of the layers, causing more permeation of the substances through the skin. This mechanism seems to be almost independent to the chemical structure of the permeant molecule.

Figure 3 (c), (e) and (g) present in the absence of ZnO-NPs, the permeated amounts (%) of the hydrophobic solvents, hydrophilic solvents and surfactants were increased by increasing their MW, showing R^2 = 0.886, 0.196 and 0.977, respectively. There was one exception for this fact, related to the oily solvents, (a), where there were almost no change in the permeated amounts (%) by increasing their MW (R^2 = 0.036). Such a relationship between the permeation and the MW can be reasonable because generally, the hydrophobicity of the substances increases by the increase in their MW, where the MW still is not large enough to prevent the permeation.

Figure 3(b), (d), (f) and (h) present, the increased permeated amount (%) almost did not change by increasing the MW for group 1 and 2, but did change and increased for the groups 3 and 4.

Thus, effect of the ZnO-NPs on the permeations of the group 1 and 2 (oily and hydrophobic solvents, respectively), was not dependent on their MW(R^2~ 0, for both) (Figure 3 (b) and (d)). One reason could be that all of the substances of group 1 and 2 were hydrophobic and therefore they had good permeations almost without any rate-limiting factor. The other reason could be that their MW varied from 645.6 to 882.9 g/mol for oily solvents and from 72.11 to 348 g/mol for hydrophobic solvents, which were not wide ranges and therefore did not make considerable differences in the effects of the ZnO-NPs.

In contrast, effect of the ZnO-NPs on the permeations of group 3 and 4 (hydrophilic solvents and surfactants, respectively), was dependent on their MW(R^2 = 0.553 and 0.914, respectively)(Figure 3 (f) and (h)). About group 3, the hydrophilic solvents, their permeation were limited by their hydrophilicity. Therefore, not only their permeations were increased by increasing in their MW(Figure 3 (e)), but their increased permeations in the presence of the ZnO-NPs, were also increased by increasing in their MWs (Figure 3 (f)).

About group 4, the surfactants, not only their permeation were increased by increasing in their MW (Figure 3 (g)), their increased permeations in the presence of ZnO-NPs, were dramatically increased by increasing in their MWs (Figure 3 (h)), apparently because of the increase in their hydrophobicity by increase in their MWs.

There are other studies proving that NPs enhanced the permeations of different substances[32-37]. The studies includedlipid NPs, polymeric NPs, inorganic NPs, etc. The present work opened a door to the enhancement effects of ZnO-NPs which were not studied before and correlated with the enhancement effects of the reported NPs in the literature.

CONCLUSION

The skin permeations of the different substances used in this study were different from each other. Among them, LP, PG and glycerol possessed the highest permeations, respectively. Using ZnO-NPs enhanced and increased the permeation of all the substances.The ZnO-NPs showed higher effects on the hydrophobic and the oily solvents than the hydrophilic solvents and the surfactants. Such enhancement effects were not dependent to the MW of the oily or the hydrophobic solvents but were directly dependent to the MW of the hydrophilic solvents or the surfactants.In conclusion, the ZnO-NPs are suggested to be used properly in topical pharmaceutical or cosmetic products for enhancing the skin permeations of solvents or surfactants (as enhancer excipient).This action potentially can improve the absorption of active ingredient, when percutaneous absorption is intended. On the other hand, cautions should be noticed when the ZnO-NPs are present in topical products not intended for percutaneous drug absorption. Since the ZnO-NPs can enhance the drug absorption by enhancing the permeations of solvents or surfactants present in different formulations.

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CONFLICT OF INTEREST

The authors report there are no conflicts of interests.

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