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ZINC OXIDE AND ZINC OXIDE NANOPARTICLES AS ENHANCERS IN TOPICAL PHARMACEUTICAL AND COSMETIC PRODUCTS

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ABSTRACT

Objectives: Scientists have widely investigated the use of chemical enhancers to improve drug transport through the skin. In this study, ZnO and ZnO nanoparticles (ZnO-NPs) has been used as dermal absorption enhancers for Ibuprofen (IP).

Methods: Seven different formulations containing IP, ZnO, or ZnO-NPs were prepared. Dermal absorption experiments were performed at 32°C using a diffusion cell containing phosphate buffer saline (pH 7.4) and a slice of chicken skin. Cumulative amounts of skin permeated IP, ZnO or ZnO-NPs were plotted over time.

Results: After 60 minutes, 90, 8 and 81 mg ZnO, ZnO-NPs and IP were passed through the skin, respectively. This amount for IP was 105, 114, 131 and 183 mg in presence of 100 mg ZnO, 100 mg ZnO-NPs, 200 mg ZnO-NPs, and 500 mg ZnO-NPs, respectively.

Maximum amount of not-permeated IP was seen for formulation 1 (IP without enhancer) and minimum not-permeated IP was seen for formulation 5 (IP with 500 mg ZnO-NPs as enhancer).

Conclusion: ZnO and more strongly ZnO-NPs could act as enhancers for transdermal delivery of IP. Such effect was improved by increase in concentration of ZnO-NPs. Therefore, ZnO-NPs can be used as enhancer in dermal drug delivery formulations.

Key words: *Zinc Oxide; Zinc Oxide nanoparticles; Ibuprofen; Enhancer; skin permeation.*

INTRODUCTION

Transdermal drug administration possesses many advantages including decreased first-pass drug metabolism, no gastro-intestinal degradation, long term delivery (>24 hours) (especially for transdermal patches) and controlled delivery and termination[1]. The main barrier for transdermal drug delivery, is the skin's topmost layer, stratum corneum (SC). The SC must be altered to increase the advantages of transdermal drug administration. This has been the subject of research for pharmaceutical scientists over the last couple of decades[2]. Extensive research on chemical enhancers has been performed over the last 20 years which form the main component of formulation-based approaches for transdermal drug delivery[3]. It is now believed that formulation components influence extent and rate of passive transdermal absorption[4]. Permeation of a drug through the skin in the presence of an enhancer is related to chemical structure of enhancer and physico-chemical interactions of the enhancer with the drug or with the skin components[5-7]. More than 200 chemicals have been shown to enhance skin permeation of drugs. Chemical penetration enhancers (CPEs) should build blocks to make new skin microstructures without irritation[3].

Zinc is a relatively low price, biocompatible and non-toxic essential element for human health[8]. Forslind found that zinc could alter the physical structure of the skin layers without affected by metabolism in skin layers[9]. Parat et al. proved that zinc has antioxidant and cytoprotective effects on skin keratinocytes in cell (HaCaT) culture[10]. Zinc Oxide (ZnO) has been applied topically to heal wounds and for treatment of other skin disorders[7,11,12]. The zinc distribution in the skin showed a peak in the epidermal layer decreasing toward the SC, with an exception in the SC which was free of zinc[13].

Variety of nanoparticles (NPs) including elemental, hydrophobic or hydrophobic-polymeric NPs have been widely used for improving the delivery of pharmaceuticals across the skin[14,15]. In this study, ZnO and ZnO nanoparticles (ZnO-NPs) were used as dermal absorption enhancers for a model drug.

Since non-steroidal anti-inflammatory drugs (NSAIDs) are vastly used in dermal products and their skin

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penetration has been the subject of tremendous studies, Ibuprofen (IP) was used as the model drug[12]. In addition IP with a hydrophobic structure and a molecular weight of 206.29 g/mol can suitably resemble most of the topical drugs which have similar structure and molecular weight.

MATERIALS AND METHODS

ZnO (MW: 81.408 g/mol), Ibuprofen, potassium dihydrogen phosphate, Sodium hydroxide, zinc acetate, triethanolamine, ethanol, and acetic acid, were purchased from Sigma-Aldrich company, USA.

Preparation of ZnO-NPs

ZnO-NPs were prepared according to Vafaee et al. Briefly, 100 ml zinc acetate solution (2%), was added to 100 ml triethanolamine (TEA) solution (1%) in ethanol to produce ZnO-NPs at 50–60°C while stirring[16]. The resulting solution was kept under stirring at room temperature for one hour. The suspension was centrifuged and washed three times with deionized water (DW) and then three times with ethanol and freeze dried.

Characterization

Three equal parts of the prepared ZnO-NPs (each 10 mg) were weighed and each was redispersed and mixed into 10 ml of DW and injected into the sample injector of a zetasizer (Malvern, UK) to obtain the size of ZnO-NPs. The reported particle size was the average size of the three samples ± the calculated standard deviation (SD) of them.

Infrared spectrum

An infrared (IR) spectrum of 0.05% IP solution in 0.1 N Sodium Hydroxide was obtained using a Nicolet Magna 550 spectrophotometer. Another IR spectrum was obtained for comparison from a mixture of 50 mg of IP and 50 mg of the prepared ZnO-NPs in 100 ml of DW (stirred for 2 hours at 32°C), which was filtered by 0.1 µ membrane filter to remove the NPs and then was added adequate amount of sodium hydroxide to reach the concentration of 0.1 N.

Preparation of formulations

Formulations 1-7 were prepared by mixing the appropriate amount of each substance with 2 ml of DW as dispersion to make a paste, using mechanical overhead mixer (Heidolph, RZR 2020, Germany). (Table 1)

Table 1: *Constituents of the formulations 1-7 and their quantitative amounts (mg or ml).*

Formulation number	IP (mg)	ZnO (mg)	ZnO-NPs (mg)	DW (mL)
1	----	200	----	2
2	----	----	200	2
3	200	----	----	2
4	200	100	----	2
5	200	----	100	2
6	200	----	200	2
7	200	----	500	2

Permeation test

The skin permeability experiments were carried out with a one chamber (acceptor compartment) diffusion cell and a capped effective diffusion area of 10 cm^2 at the top. 30 ml of phosphate buffered saline (PBS), pH 7.4, (medium) was poured into the chamber and a slice of skin of (3 months old) chicken was sandwiched between the top of the chamber and its cap and then each formulation (paste) was placed and spread on the skin before capping. The cell was placed in a shaker-incubator (Heidolph incubator 1000, Heidolph co., Germany) at 32°C for 140 minutes[3,17]. The use of *in vitro* models for screening is also supported by the fact that SC, the principle site of enhancer action, shows similar behavior *in vivo* and *in vitro* in most aspects[18]. Three milliliter samples were taken out from the medium at 0, 20, 40, 60, 80, 100, 120 and 140 minutes after placing the sample on the skin. To keep sink condition, 3 ml fresh PBS was replaced each time. The samples were analyzed for determination of the concentration (amount) of IP or Zn, as the permeated amounts of IP or ZnO-NPs, respectively. For determination of IP concentration in samples, 180 µl acetic acid was added and dissolved into the sample solutions and then absorbance of the samples were obtained at 264 nm using UV-Visible spectrophotometer (Perkin-Elmer-Lambda25, USA). For determination of Zn concentration in samples, an inhibitive enzyme assay for heavy metals has been employed using protease. Casein was used as a substrate and Coomassie dye was used to denote the completion of casein hydrolysis. In the absence of inhibitors, casein was hydrolyzed and the solution became brown, while in the presence of Zn, the hydrolysis of casein was inhibited and the solution remained blue[19]. Cumulative amounts of permeated IP or ZnO-NPs were calculated and plotted against time (0, 20, 40, 60, 80, 100, 120 and 140 minutes).

Statistical analysis

Each formulation was tested 3 times and the reported data is the mean ± 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. p-value <0.05 was considered to be significant.

RESULTS

Zetasizer presented an average particle size of 80.1 ± 5.0 nm for ZnO-NPs. As the chemical structure of IP (Figure 1) shows, there is no functional group in IP, having the potential to interact with double charged elements including Zn^{+2} (ZnO). In addition, according to

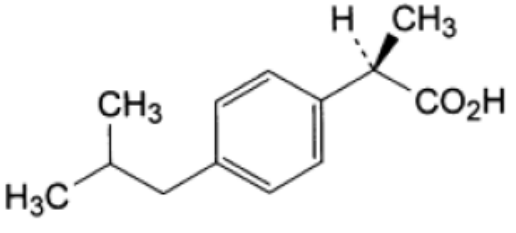

Fig. 1. *Chemical structure of Ibuprofen*

the present literature[20], no interactions could occur between IP and ZnO-NPs. For further confirmation, IR spectrums of IP were obtained. They showed no change before and after the exposure to ZnO-NPs (Figure 2).

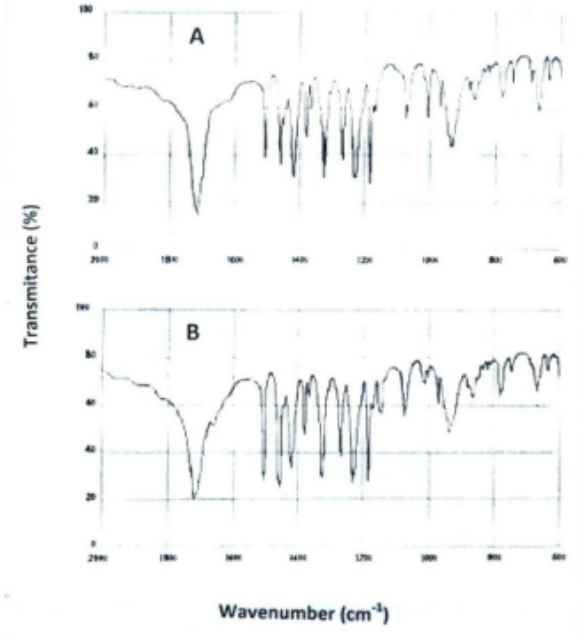

Fig. 2. *IR spectrums of IP before (A) and after (B) exposure to ZnO-NPs.*

The permeation test was performed to obtain amounts of permeated substance through the skin after 0, 20, 40, 60, 80, 100, 120 and 140 minutes using a diffusion cell. Figures 3 and 4 show the amounts (mg) of substances permeated through the skin versus time. According to Figure 3, after 60 minutes, 90 mg ZnO was penetrated the skin while just 8 mg ZnO-NPs were penetrated. The total permeated amount of ZnO (after 140 minutes) (115 mg) was significantly more than that for ZnO-NPs (33 mg) ($p<0.05$) and the permeation rate of ZnO (0.82 mg/min) was much more than permeation rate of ZnO-NPs (0.23 mg/min) ($p<0.05$). Figures 5 and 6 show the amounts of substances remained in the skin layers (not permeated), indicating portion that would pass through the skin in a relatively slow kinetic. Such a remained amount was near 2 times higher for ZnO-NPs in comparison with the ZnO (Figure 5). According to Figures 3 and 4, IP was permeated through the skin by a kinetic similar to the ZnO, but the total permeated IP (142 mg) was more than ZnO (115 mg), and significantly much more than ZnO-NPs (33 mg). Figure 4 shows that the total permeated amounts of IP in presence of 100 mg ZnO, 100 mg ZnO-NPs, 200 mg ZnO-NPs, and 500 mg ZnO-NPs, were 178, 189, 191 and 209 mg, respectively.

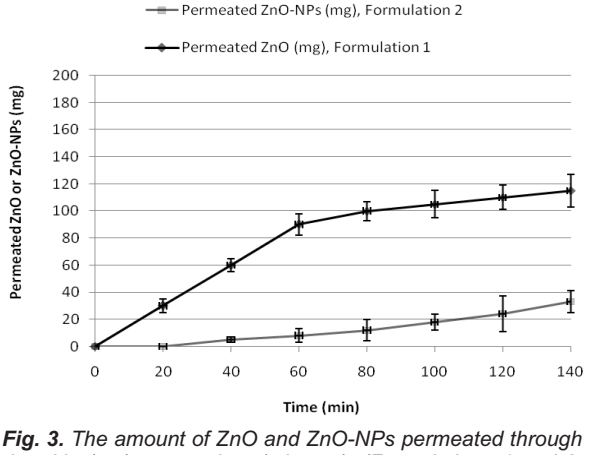

Fig. 3. *The amount of ZnO and ZnO-NPs permeated through the skin (mg) versus time (minutes), (Formulations 1 and 2, Table 1).*

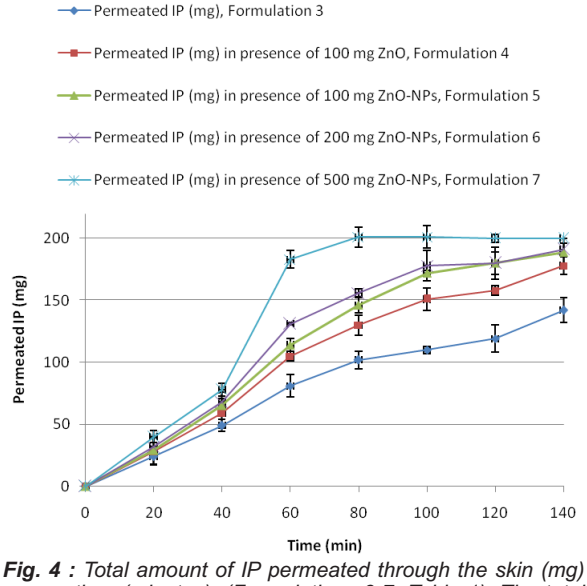

Fig. 4 : *Total amount of IP permeated through the skin (mg) versus time (minutes), (Formulations 3-7, Table 1). The total permeated IP (after 140 minutes) for formulations 3, 4, 5, 6 and 7 were 142, 178, 189, 191 and 200 mg, respectively.*

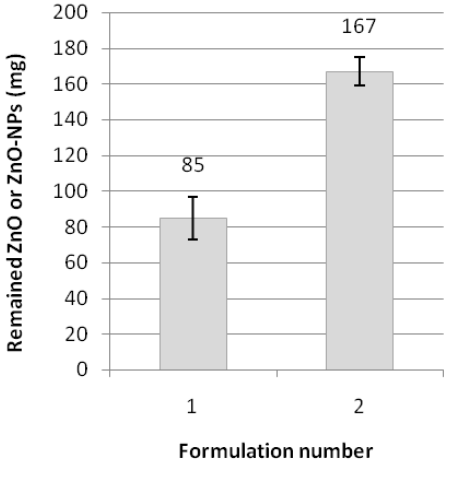

Fig. 5 : *Amounts of ZnO (1) and ZnO-NPs (2) remained in the skin (not permeated) after 140 minutes (mg).*

Which means the total permeated amounts of IP in presence of ZnO or ZnO-NPs, were significantly higher than that without ZnO or ZnO-NPs (142 mg) ($p<0.05$ for all four pairs). In addition, the permeated IP in presence of 100 mg ZnO-NPs (189 mg) was slightly higher than that in presence of 100 mg ZnO (178 mg) ($p<0.05$). Moreover, a linear relation was observed between amount of ZnO-NPs and total permeated IP, and between amount of ZnO-NPs and permeation rate of IP (Figure 7 and 8) which means that higher permeated IP was seen in presence of higher amounts of ZnO-NPs. Subsequently, the remained IP in the skin, (Figure 6), was lower in presence of ZnO-NPs in comparison with the presence of ZnO, as confirmation for the data in previous paragraphs. In addition the remained IP was decreased by increasing ZnO-NPs.

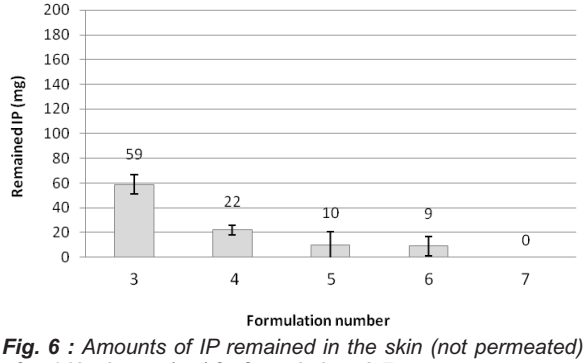

Fig. 6 : *Amounts of IP remained in the skin (not permeated) after 140 minutes (mg) for formulations 3-7.*

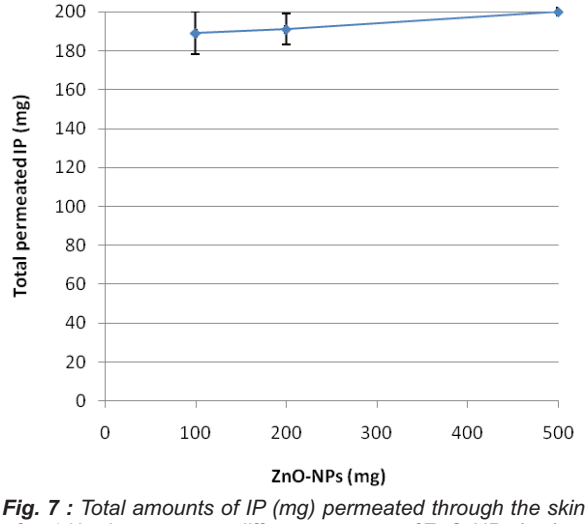

Fig. 7 : *Total amounts of IP (mg) permeated through the skin after 140 minutes versus different amounts of ZnO-NPs (mg) as enhancer (Formulations 5-7, Table 1).*

DISCUSSION

Results of permeation experiments proved much lower transdermal absorption of ZnO-NPs in comparison with ZnO, that is a very large portion of ZnO-NPs still remained in the skin layers after 140 minutes. Such a significant difference ($p< 0.05$) is attributed to two characteristics of ZnO-NPs. First is the larger particle size of ZnO-NPs, 80 nm, than ZnO molecules (4 nm)[21].

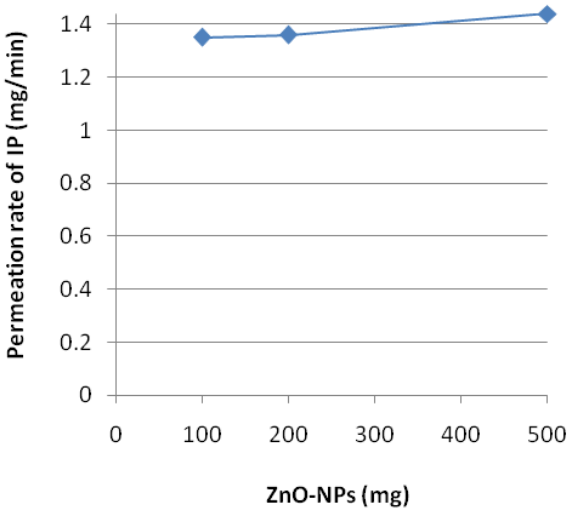

Fig. 8 : *Permeation rates of IP (mg) through the skin during 140 minutes versus different amounts of ZnO-NPs (mg) as enhancer (Formulations 5-7, Table 1).*

Second is the crystalline trigonal morphology of ZnO-NPs compared to the crystalline hexagonal morphology of ZnO molecules[21] which means that ZnO is more similar to spherical shape than ZnO-NPs. Therefore, the smaller size and the more spherical shape of ZnO (than ZnO-NPs) lead to a more transfer of ZnO across skin in comparison to the ZnO-NPs.

While a more permeation could be predicted for ZnO than IP (because of the lower molecular weight of ZnO than IP), a similar permeations happened for ZnO and IP. It can be as a result of interactions between Zn and skin components, which also cause the remained ZnO to be more than remained IP after 140 minutes. Thus, ZnO and ZnO-NPs showed lower skin permeation compared to IP and also ZnO-NPs showed lower permeation compared to ZnO.

In case of IP transdermal absorption (skin permeation), the increased permeated IP in presence of ZnO or ZnO-NPs, can be as a result of the skin microstructure alteration by ZnO and ZnO-NPs. In addition, it can be as a result of relatively high residence time of ZnO and ZnO-NPs in skin layers which is much more for ZnO-NPs than ZnO. This fact makes the ZnO and especially ZnO-NPs, potent enhancers for increasing the transdermal absorption of drugs. Because of the unique crystalline structure of ZnO-NPs (trigonal)[21], they could deposit in the skin and alter the skin microstructure much more than ZnO, and therefore, enhance the IP absorption more than ZnO. The other reason for such strong enhancer effect of ZnO-NPs, can be attributed to the long time remaining of the NPs in the skin. Such a phenomenon gives the drug (IP) enough time for passing through the skin. Moreover, such a mechanism alters the skin structure leading an enhancement in IP transport through the skin. Such deposition of zinc and its salts in skin layers has been proved by other researchers earlier where they showed that zinc and its derivatives, mostly deposited and remained in dermis and SC compared to epidermis (after a few hours)[13,22].

The increase in IP enhanced permeation by increase in the amount of ZnO-NPs, can be due to increase in modified skin microstructure and therefore increase in passing pathways suitable for IP absorption. In conclusion, ZnO-NPs were used successfully for enhancement of IP permeation through skin.

CONCLUSION

ZnO and ZnO-NPs increased the model drug, IP, percutaneous absorption and therefore they can be used as transdermal enhancers in pharmaceutical or cosmetic topical products. In this order ZnO-NPs appeared as the stronger enhancer, increasing not only the total absorbed drug, but also the drug absorption rate.

ACKNOWLEDGMENT

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CONFLICT OF INTEREST

The authors report there are no conflicts of interests.

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