Journal of Pharmaceutical Research Vol. 14, No. 1, January - March 2015 : 1-6

USING A SYNERGISTIC COMBINATION OF TWO ENHANCERS FOR DERMAL DELIVERY OF COLLAGEN IN PHARMACEUTICAL AND COSMETIC PRODUCTS

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Received on: 25.11.2014 Revised: 04.02.2015 Accepted: 27.02.2015

ABSTRACT

Objectives: Dermal delivery is an excellent route of drug administration. In this study, Zinc Oxide nanoparticles(ZnO-NPs) and 6 solvents were used as enhancers to promote dermal permeation of collagen.

Methods: Formulations contained collagen, solvent and ZnO-NPs. Permeation of collagen was determined by an *ex vivo* (animal skin) method.

Results: Using sunflower oil, olive oil, coconut oil, liquid paraffin, dimethyl sulfoxide or tetrahydrofuran, lead to permeation of 46, 47, 60, 241, 56 and 35 mg collagen, respectively. Addition of 200 mg ZnO-NPs, resulted in permeation of 68, 61, 72, 286, 63 and 47 mg collagen, respectively. Using 300 or 400 mg ZnO-NPs and liquid paraffin, lead to permeation of 299 and 286 mg collagen, respectively.

Conclusion: Using the solvents or ZnO-NPs enhanced the permeation of collagen. The enhancement was more when a solvent and ZnO-NPs were used simultaneously. Among them, combination of liquid paraffin and ZnO-NPs, showed a synergistic enhancing effect leading to the maximum permeation observed in the study. This was explained with the completely different enhancing mechanisms of liquid paraffin and ZnO-NPs. Therefore, the study suggests using liquid paraffin and ZnO-NPs or other combinations of enhancers having distinct mechanisms to prominently improve the permeation of collagen.

Key words: *Collagen; ZnO nanoparticles; Organic solvents; Skin permeation.*

INTRODUCTION

Dermal and transdermal drug administration possess many advantages including decreased or loss of first-pass drug metabolism, no gastro-intestinal degradation, long time delivery (>24 hours especially for transdermal patches), controlled delivery and termination and bypassing problems related to GI administration (absorption process, long transit time, pH changes and enzyme effects)[1]. In addition, dermal administration of drugs is a safe, non-invasive, pain free and easy way of drug administration especially compared to injections beside that this way of drug administration shows significantly less risk of infections and irritations. Thus dermal administration is highly preferred by patients and considerably improves patients' compliance.

Main barrier for dermal 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 two latest decades[2]. Extensive research on chemical enhancers has been performed over the last 20 years which form the main strategy of formulation-design approaches for dermal and transdermal drug delivery[3]. It is now believed that formulation components can improve the quantity and rate of transdermal absorption[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 drugs skin permeation. Chemical penetration enhancers (CPEs) should build blocks to make new skin microstructures without irritation[3]. CPEs include aliphatic acids, fatty acids, esters, alcohols, oils, terpens and etc.[1,8,9].

Zinc is a relatively inexpensive, biocompatible and non-toxic essential vital element. Zinc has been shown to have no interactions with most drugs[10]. Parat et al. proved that Zinc has antioxidant and cytoprotective effects on skin keratinocytes in cell (HaCaT) culture[11]. Zinc oxide (ZnO) (MW: 81.408 g/mol) has been applied topically to heal wounds and for treatment of other skin disorders[10]. Zinc distribution showed its peak in the epidermal layer decreasing toward the SC, while exceptionally its concentration was high in the SC[12-14].

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Combination of enhancers for collagen delivery

One group of penetration enhancers are hydrophobic nanoparticles (NPs) including NPs made from lipids or hydrophobic polymers and inorganic NPs. The polymeric NPs should be evaluated in terms of safety, biocompatibility and especially degradation kinetics. Thus they should be accurately designed to become suitable for use in medications[15-17]. All the three types of NPs, entrap drug and lead the drug passing through the skin. But there is another method for inorganic NPs which is using them along with the free drug in the formulation without any drug entrapment. This method is employed in this study using biocompatible Zinc Oxide nanoparticles (ZnO-NPs) as enhancer. Fortunately, the used inorganic NPs, ZnO-NPs, usually do not show toxicity[7]. Moreover, they can not practically and easily pass the skin[10,11,13]. As a result, they have not the potential to cause toxicity. On the other hand, because the target organ is the skin bearing a permanent turnover, all materials accumulated in the skin (especially outer skin) will release out along with the skin within a few days.

Biologic therapeutic molecules are developing fast, Pharmaceutical scientists are much more interested in using biologic drugs instead of other chemical drugs. This can be mainly attributed to the considerable efficacy and biocompatibility of the biologically active molecules.

Collagen is a natural (biologic) hydrophilic polypeptide with molecular weight of 100kDa(for β and α). Collagen fibers make the major content of the extracellular region supporting most tissues. Collagen is also found inside certain tissue cells. Collagen is responsible for skin elasticity and resistance. Collagen degradation is the reason for wrinkles resulting in aging. Collagen strengthens blood vessels and leads to tissue development. Because of such benefits, collagen has been widely used and has been an important constituent of recent topical cosmetic and pharmaceutical products[18]. Despite the wide use of collagen, its dermal penetration is still poor. Efficient delivery of collagen is necessary because of its important therapeutic effects in many skin defects such as aging and ulcers. Besides, the high cost of collagen makes its efficient delivery necessary. In this study, we aimed to improve the penetration of collagen into the skin using certain organic solvents and ZnO-NPs as combinations of dermal penetration enhancers[19].Using these two kinds of enhancers (solvent and NPs) in this study, was based on the fact that they have two completely different enhancing mechanisms. Their simultaneous use was considered to have the potential of a great enhancing effect. Using such a combination of two mechanistically different enhancers as an effective combination has not been studied until now. The solvents used in this study include sunflower oil, olive oil, coconut oil, liquid paraffin (mineral oil), dimethyl sulfoxide(DMSO) and tetrahydrofuran(THF). All these solvents have been widely used in different cosmetic and pharmaceutical products because of their enhancing effects and their other various advantages. The enhancing effects of these solvents have been clearly demonstrated in many published literature[3].

The enhancing effect of ZnO-NPs for dermal delivery of a simple hydrophobic low molecular weight drug, ibuprofen, was demonstrated for the first time in our previous study[19]. But collagen which was used as the active ingredient in this study, is a hydrophilic high molecular weight protein. Beside the therapeutic effects of collagen, it can be a model for other similar proteins for their dermal or transdermal delivery.

In this paper, skin permeation of collagen was determined using an *ex vivo* diffusion cell method considering the fact that SC is a dead layer and shows similar behaviors *in vivo and ex vivo*[20].

MATERIALS AND METHODS

Materials

ZnO nanoparticles (nominal average particle size of 100 nm), collagen, sunflower oil, olive oil, coconut oil, liquid paraffin, DMSO and THF were purchased from Sigma-Aldrich company, USA.

Preparation of formulations

Formulations numbered as 1-18 were prepared. The formulations and their constituents are listed in Table 1. Every formulation was prepared by mixing collagen (500 mg) and (0, 200, 300 or 400 mg) ZnO-NPs, in 2 ml of one of the solvents listed in Table 1. Such a concentration for the solvents, indeed is the maximum solvent concentration used in different topical preparations as enhancer. The maximum amount was chosen to obtain a maximum enhancing action[3]. Besides, such an amount for NPs was their optimum concentration as topical enhancer, obtained in our previous work[21]. Every formulation was mixed by a mechanical overhead mixer (Heidolph, RZR 2020, Germany) for 15 minutes to make a paste.

***Table 1** : Formulations 1-18 and their constituents.*

Formulation Number	Collagen (500 mg)	Solvent (2 ml)	ZnO-NPs (200 mg)
1	✓	DW	0
2	✓	Sunflower oil	0
3	✓	Olive oil	0
4	✓	Coconut oil	0
5	✓	Liquid paraffin	0
6	✓	DMSO	0
7	✓	THF	0
8	✓	DW	200
9	✓	Sunflower oil	200
10	✓	Olive oil	200
11	✓	Coconut oil	200
12	✓	Liquid paraffin	200
13	✓	DMSO	200
14	✓	THF	200
15	✓	DW	300
16	✓	Liquid paraffin	300
17	✓	DW	400
18	✓	Liquid paraffin	400

Permeation test

The skin permeabilities of collagen were obtained using a diffusion cell. The cell had a donor compartment and a receiver compartment with an effective diffusion area of 10 cm^2 between the two compartments. The receiver chamber was filled with 30 ml of phosphate buffer solution (PBS) pH 7.4, as medium. A small piece of skin of (3 months old) chicken was fixed between the two chambers. Pieces of freshly killed chickens' skin were carefully selected and cut in order to be similar to each other in terms of their lipid layer thicknesses. Chicken skin was used because skins of mice and chickens are similar in terms of thickness. For further simulation of mouse skin, the piece of chicken skin was cut from chicken's abdomen which contains no feather. Another reason for using chicken skin was more respect for ethics, because unlike mice, chickens are routinely killed daily for human food. Otherwise, lots of mice would be killed just for their small piece of skin, which was not accepted by the ethics committee. Each of the formulations 1-18 (all the amount of every formulation at once) was applied and rubbed on the fixed skin by a swab before putting the cap[3,21]. Then the cell was placed in a shaker-incubator (Heidolph incubator 1000, Heidolph Co., Germany) with a constant temperature of 32°C for 1.5 hours[3,21]. Such a time was chosen because a topical semisolid formulation usually remains on the skin until about 1.5 hours. Therefore, after 1.5 hours, a sample from the PBS was taken out and analyzed for determination of collagen concentration. Then the total amount of collagen(mg) in the whole 30 ml PBS was calculated as the total skin permeated collagen.The experiment was performed for every one of the formulations 1-18 and the calculated amounts were plotted for all the formulations (Figures 1-4).

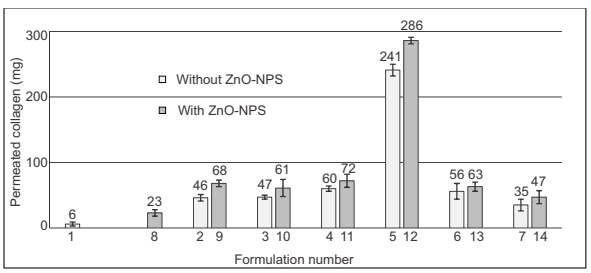

***Fig. 1**: Permeated amount (mg) of collagen for formulations1-14.(n=3)*

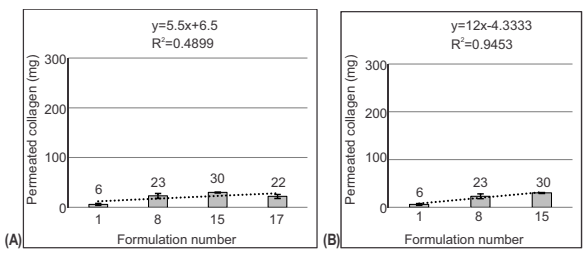

***Fig. 2:** Permeated amount (mg) of collagen for formulations 1, 8, 15 and 17 (A), and 1, 8 and 15 (B). (n=3)*

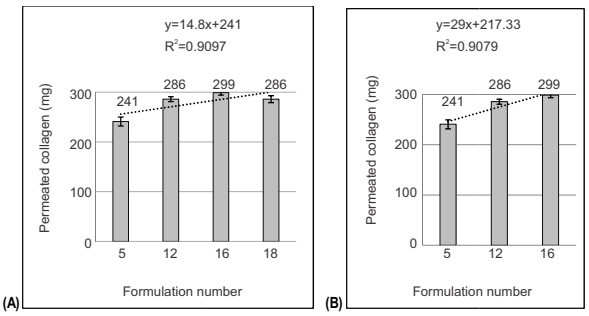

***Fig. 3 :** Permeated amount (mg) of collagen for formulations 5, 12, 16 and 18 (A), and 5, 12 and 16 (B). (n=3)*

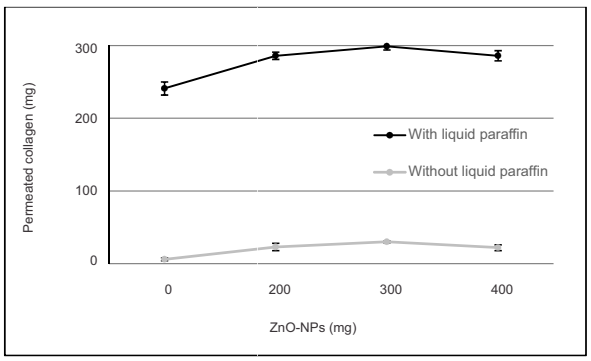

***Fig. 4 :** Permeated amount (mg) of collagen for formulations 5, 8, 15 and 17 (without liquid paraffin) and formulations 1,12, 16 and 18 (with liquid paraffin). (n=3)*

Determination of collagen concentration

A modified Bergman and Loxley rapid procedure was used for determination of collagen concentration. Indeed since collagen molecule is full of hydroxyproline groups, determination of these groups was the goal of this method. Briefly, 2 mL of isopropanol was added to 1 ml of sample in a tube and the tube was placed on a vortexat room temperature. Then 1 mL of oxidant solution (H_2O_2) was added and again the tube was placed on a vortex at room temperature. Then 2 mL of Erlich's reagent was added under perchloric acid hood followed by a vortex (with capping for evaporation limitation). Tubes were heated for 25 minutes in a water bath at 60°C. Ultimately the samples were cooled down and their absorbances were measured at 558 nm against a blank (PBS) immediately (20 minutes maximum) using a UV-Visible spectrophotometer (Perkin-Elmer-Lambda25, USA)[22].

Statistical analysis

The permeation test was performed three times for every formulation. The reported data in this paper are mean±SD (n=3). One-way analysis of variance (ANOVA) was used for comparing the means. SPSS for Windows (release 11.5.0) was employed for statistical analysis. p-value <0.05 was considered as indication of significant difference between the means.

RESULTS

Total dermal permeated amounts (mg) of collagen were obtained for all the formulations from the permeation experiments and are plotted in Figures 1-4.

Figure 1 shows the permeated amounts of collagen for formulations 1-14. Formulations 1, 2, 3, 4, 5, 6 and 7 contained DW, sunflower oil, olive oil, coconut oil, liquid paraffin, DMSO and THF, as solvent, respectively. As pictured in the Figure, in the absence of any enhancer, little amounts of collagen, 6 mg, permeated the skin (formulation 1). Oils (or fatty acid containing oils) including sunflower oil, olive oil and coconut oil significantly ($p<0.05$) increased the permeated amounts (46, 47 and 60 mg, respectively) compared to formulation 1. Among these oils, coconut oil enhanced the permeation (60 mg) more than the two others ($p<0.05$). Formulation 5, containing liquid paraffin, sharply increased the permeation compared to all other solvents ($p<0.05$).The permeation related to formulation 6 (56 mg) containing DMSO, was close to that for formulation 4 containing coconut oil. Formulation 7 containing THF, enhanced the permeation (35 mg) but this enhancement was less than others ($p<0.05$).

Formulations 8, 9, 10, 11, 12, 13 and 14 each contained 200 mg ZnO-NPs and DW, sunflower oil, olive oil, coconut oil, liquid paraffin, DMSO or THF, respectively. According to Figure 1, formulation 8 slightly increased the permeation (23 mg) compared to formulation 1($p<0.05$).The permeated collagen obtained from formulations 9, 10, 11, 12, 13 and 14 were 68, 61, 72, 286, 63 and 47 mg, respectively. This demonstrated a significant increase in permeation compared to their corresponding formulations without ZnO-NPs (formulations 2-7) with no exception ($p<0.05$ for each pair of formulations).

Among all the formulations 1-14, formulation 12 (containing both liquid paraffin and ZnO-NPs) showed the most permeated collagen (286 mg)($p<0.05$).

Figure 2 presents the permeated amounts (mg) of collagen for formulations 1, 8, 15 and 17, each containing 2 mL DW in addition to 0, 200, 300 and 400 mg ZnO-NPs, respectively. They showed 6, 23, 30 and 22 mg permeated collagen, respectively. Such results revealed that increasing the amount of ZnO-NPs from 0 to 200 and from 200 to 300 mg, increased the permeated collagen (23 and 30 mg, respectively) ($p<0.05$). But increasing the amount of ZnO-NPs from 300 to 400 mg, did not increased the permeated collagen (22 mg) anymore and also slightly decreased the permeated collagen ($p<0.05$).

Figure 3 represents the permeated amounts (mg) of collagen for formulations 5, 12, 16 and 18, each containing 2 ml liquid paraffin in addition to 0, 200, 300 and 400 mg ZnO-NPs, respectively. They showed 241, 286, 299 and 286 mg permeated collagen, respectively. Such results revealed that increasing the amount of ZnO-NPs from 0 to 200 and from 200 to 300 mg, increased the permeated collagen (286 and 299 mg, respectively)($p<0.05$). But increasing the amount of ZnO-NPs from 300 to 400 mg, did not increase the permeated collagen (286 mg) any more and also slightly decreased the permeated collagen ($p<0.05$). Indeed, among these four formulations (formulations 5, 12, 16 and 18), the most permeated collagen was observed when 300 mg ZnO-NPs was used (formulation 16) ($p<0.05$) while the permeated collagen related to formulations 12 and 18 were equal ($p>0.05$).

The results showed in all the three Figures, proved that the most permeated amount of collagen in this study was 299±5 mg obtained for formulation 16 which contained 2 ml liquid paraffin and 300 mg ZnO-NPs.

Figure 4 includes the permeated amounts (mg) of collagen for formulations 1, 5, 8, 12, 15, 16, 17 and 18. This Figure, presents the permeated collagen when 0, 200, 300 or 400 mg ZnO-NPs were used, in presence of liquid paraffin or in absence of liquid paraffin. In fact, this Figure is a comparison of Figures 2 (A) and 3 (A).

DISCUSSION

Since collagen is a hydrophilic compound, lipids of SC limit its skin permeation. In this study liquid paraffin dramatically improved the permeation of collagen. Liquid paraffin consists of several hydrophobic hydrocarbon chains. It can penetrate the SC and deposit between the hydrophobic chains of SC lipids. This phenomenon decreases the SC lipid consistency and increases the permeability of SC lipid layer. On the other hand, Liquid paraffin is an excellent solvent for most hydrophobic substances and also for SC lipids. It can dissolve the SC lipids and therefore mix them with the skin water content generating a low viscosity liquid, suitable for penetration of collagen.

Sunflower oil is an unsaturated mixture of mostly oleic acid (omega-9) and linoleic acid (omega-6) and about 10% terpenes. Olive oil is composed mainly of the mixed triglyceride esters of oleic acid (55-83%) and palmitic acid. Coconut oil consists of 91% saturated fatty acids. Such fatty acids, terpenes and other constituents of these oils are from main groups of dermal CPEs. Subsequently, results showed that these oils (sunflower oil, olive oil and coconut oil) increased permeation of collagen compared to that in the absence of enhancers. Their mechanism is similar to that explained for liquid paraffin but weaker than liquid paraffin.

During the experiments it was observed that DMSO rapidly penetrated the skin. DMSO is a good solvent for both polar and nonpolar compounds and is miscible with organic solvents as well as water in wide ratios. Therefore, it increased the permeation. Liquid paraffin is a better solvent than DMSO for SC lipids. Thus DMSO increased the permeation less than liquid paraffin.

THF is a polar water-miscible organic solvent with a low viscosity. The ability of THF to dissolve lipids is not high. On the other hand, it does not absorb and entrap much water and therefore cannot increase the water content of the dermis (the mechanism of hydrophilic enhancers). Thus THF slightly increased the permeation.

Combination of enhancers for collagen delivery

The increased permeation caused by ZnO-NPs can be referred to their crystal structure as well as their nanometer size. Their nanometer size leads to their penetration into the skin without rapid exit from the skin. Therefore, they remain and deposit in the skin layers for a period of time. Such a deposition of nanosize crystal shape particles, disorders the normal arrangement of skin layers[23]. This phenomenon makes the skin more permeable for outer molecules like collagen.

On the other hand, such a skin structure disordering, can increase the permeation of the used liquid (solvent) enhancers proved in our previous study[24].This procedure is responsible for the synergistic enhancing effects using ZnO-NPs and liquid paraffin together. Indeed, the increased permeation of solvents (caused by ZnO-NPs) raised the permeation of collagen. Thus simultaneous use of a liquid (solvent) enhancer such as liquid paraffin and inorganic NPs such as ZnO-NPs, acted as an effective combination of CPEs and lead to a synergistic enhancing effect on collagen permeation. On the other hand, such a synergistic effect was attributed to the completely different enhancing mechanisms of liquid paraffin as a liquid (solvent) enhancer and ZnO-NPs as a NP type enhancer. This means that the two enhancers do not compete with each other in their enhancing procedure and actually they complete the enhancing effect of each other. Thus these two groups of enhancers synergistically strengthen their enhancing effects. It means that the permeation caused by combination of the two enhancers including liquid paraffin and ZnO-NPs (synergistic), was more than the permeation caused by liquid paraffin plus the permeation caused by ZnO-NPs (additive).

Use of 200 mg ZnO-NPs resulted in more permeation of collagen compared to without ZnO-NPs. Also using 300 mg ZnO-NPs resulted in more permeation of collagen compared to that for 200 mg ZnO-NPs. Such a dose dependent mechanism is shown in Figures 2 (B) and 3 (B). This observation may be due to the fact that ZnO-NPs could cause more irregularity in the skin layers structure leading a more penetration of collagen to skin. Despite this observation, no increase in the permeation was obtained using 400 mg ZnO-NPs. Also surprisingly the permeation was less using 400 mg ZnO-NPs compared to that for 300 mg ZnO-NPs. Indeed the permeation was equal to use of 200 or 400 mg ZnO-NPs. These results suggest a saturation in enhancing mechanism of ZnO-NPs at the dose of 400 mg (Figures 2 (A) and 3 (A)). The reason can be saturation of skin by ZnO-NPs leaving no physical voids in the skin for the permeation of rest of collagen. Such results also demonstrated the entrapment of the NPs in the skin layers. Figures 2 (A) and 3 (A) give the linear regression properties which match a non-linear condition as a saturation mechanism. While the saturation points are omitted in the Figures 2 (B) and 3 (B), they show a linear relation between the amount of ZnO-NPs and the permeated amounts of collagen.

CONCLUSION

In this study, all the used solvent(liquid) enhancers increased skin permeation of collagen. These solvents include sunflower oil, olive oil, coconut oil, liquid paraffin, DMSO and THF. Among them, maximum increase was related to liquid paraffin. ZnO-NPs increased the permeation of collagen too. Maximum increase was obtained in case of 300 mg of ZnO-NPs. Simultaneous use of each of the solvents with ZnO-NPs caused much more increase in the permeation compared to using them (solvent or NPs) alone. Among them, simultaneous use of liquid paraffin and ZnO-NPs revealed a synergistic enhancing effect leading to maximum permeation of collagen.The study suggests using combination of liquid paraffin and ZnO-NPs the permeation of collagen or other proteins in the formulations of topical pharmaceutical and cosmetic products can be increased. In addition, the study suggests using combination of other enhancers with completely different mechanisms a considerable increase in the permeation of proteins in such formulations may be achieved.

ACKNOWLEDGEMENT

The research was supported by Ardabil University of Medical Sciences.

CONFLICT OF INTEREST

The authors report there are no conflicts of interests.

REFERENCES

1. Barry BW. Breaching the skin's barrier to drugs. Nat Biotechnol. 2004; 22(2):165-7.
2. Prausnitz M, Mitragotri S, Langer R. Current status and future potential of transdermal drug delivery. Nature Reviews Drug Discovery. 2004; 3:115-124.
3. Smith EW, Maibach HI. Penetration enhancer classification. In: Barry BW, editor. Percutaneous Penetration Enhancers. London, New York: Taylor & Francis, ISBN 0-8493-2152-2; 2006. P. 4-14.
4. Karadzovska D, Brooks J, Monteiro-Riviere N, Riviere J. Predicting skin permeability from complex vahicles. Advanced drug delivery reviews. 2013; 65(2):265-277.
5. Lee CK, Uchida T, Noguchi E, Kim NS, Goto S. Skin permeation enhancement of tegafur by ethanol/panasate 800 or ethanol/water binary vehicle and combined effects of fatty acids and fatty alcohols. J Pharm Sci. 1993; 82:1155-9.
6. Szasz G, Takacs-Novak K, Kokosi J. Planar chromatography. J Pl Chrom. 1999; 12(1):46-50.
7. Bae YS, Hill N, Bibi Y, Dreiher J, Cohen A.Innovative Uses for Zinc in Dermatology. Dermatologic Clinics. 2010; 28(3):587–597.

8. Shokri N, Akbari Javar H, Ghadermazi R. Effects of skin penetration enhancers in topical antiaging products containing α-Hydroxy acids and hyaluronic acid. Avicenna J Med Biochem. 2014; 2(2):1-7.
9. Shokri N, Akbari Javar H, Khalaj A. Effect of hydrophilic solvents on enhancing transdermal delivery of albumin. IJPSR. 2014; 5(8):3175-82.
10. Forslind B. The skin barrier: analysis of physiologically important elements and trace elements. Acta Dermato-Venereologica. 2000; 208:46-52.
11. Parat M, Richard M, Pollet S, Hadjur Ch, Favier A, Beani J. Zinc and DNA fragmentation in keratinocyte apoptosis: its inhibitory effect in UVB irradiated cells. Journal of Photochemistry and Photobiology. 1997; 37(1-2):101-106.
12. Song CX, Szulwach KE, Fu Y, Dai Q, Yi C, Li X, Li Y, Chen CH, Zhang W, Jian X, Wang J, Zhang L, Looney TJ, Zhang B, Godley LA, Hicks LM, Lahn BT, Jin P, He C. Selective chemical labeling reveals the genome-wide distribution of 5-hydroxymethylcytosine. Nat Biotechnol. 2011; 29(1):68-72.
13. Desouza E, AbuAtiya I, Al-Ebraheem A, Wainman B, Fleming D, McNeill F, Farquharson M. Characterization of the depth distribution of Ca,Fe and Zn in skin samples, using synchrotron micro-x-ray fluorescence (SmXRF) to help quantify in-vivo measurements of elements in the skin. Applied Radiation and Isotopes. 2013; 77:68–75.
14. Trevor A, Katzung B, Masters S, Knuidering-Hall M. Katzung & Trevor's Pharmacology Examination and Board Review. 2013; Tenth edition:Part VI:Drugs with Important Actions on Blood, Inflammation, and Gout. 317-25.
15. Shokri N, Akbari Javar H, Fouladdel Sh, Khalaj A, Khoshayand MR, Dinarvand R, Atyabi F, Nomani A, Azizi E, Preparation and evaluation of poly(caprolactone fumarate) nanoparticles containing doxorubicin HCl, Daru. 2011;19 (1):12-22.
16. Shokri N, Akbari Javar H, Fouladdel Sh, Khalaj A, Dinarvand R, Azizi E, Preparation and characterization of crosslinked and non-crosslinked polycaprolactone fumarate (PCLF) NPs as carriers for doxorubicin HCl, African Journal of Pharmacy and Pharmacology. 2011;5(7):797-805.
17. Shokri N, Azizi E, Akbari Javar H, Fouladdel Sh, Khalaj A, Dinarvand R, In vitro and in vivo evaluation of poly(caprolactone fumarate) nanoparticles, IJPSR. 2012;3(9):3106-3115.
18. Rnjak-Kovacina J, Wise S, Li Z, Maitz P, Young C, Wang Y, Weiss A, Electrospun synthetic human elastin:collagen composite scaffolds for dermal tissue engineering. Acta Biomaterialia. 2012; 8:3714–22.
19. Shokri N, Goodarzi MT, Akbari Javar H, Soltani Y, Zinc Oxide and Zinc Oxide nanoparticles as enhancers in topical pharmaceutical and cosmetic products. Journal of Pharmaceutical Research. 2014; 13(2):40-44.
20. Warner KS, Li SK, He N, Suhonen TM, Chantasart D, Bolikal D, Higuchi WI, Structure –activityrelationship for chemical skin permeation enhancers: probing the chemical microenvironmentof the site of action. J Pharm Sci. 2003; 92, 1305–22.
21. Warner R, Bush R, Ruebusch N,Corneocytes Undergo Systematic Changes in Element Concentrations Across the Human Inner Stratum Corneum. Journal of Investigative Dermatology. 1995; 104(4):530-36.
22. Bergman I, Loxley R, Two improved and simplified methods for thespectrophotometric determination of hydroxyproline. Anal Chem. 2002; 35(12):1961-65.
23. Rao S, Shekhawat G, Toxicity of ZnO engineered nanoparticles and evaluation of their effect on growth, metabolism and tissue specific accumulation in Brassica juncea, Journal of Environmental Chemical Engineering. 2014; 2:105–14.
24. Shokri N, Akbari Javar H. Zinc oxide nano-particles as skin permeation enhancer for solvents and surfactants, Journal of Pharmaceutical Research. 2014; 13(3):85-91.