Original Article
DOI: 10.4274/tjps.galenos.2021.63903

INFLUENCE OF VEHICLES AND PENETRATION ENHANCERS ON CINNARIZINE PERMEATION THROUGH THE SKIN

TAŞIYICILARIN VE PENETRASYON ARTTIRICILARIN SINARIZININ DERIDEN PERMEASYONUNA ETKISI

İngilizce Kısa Başlık: Permeation of Cinnarizine through the Skin
Türkçe Kısa Başlık: Sinarizinin Deriden Permeasyonu

Şükran Damgali[1], Samet Özdemir[2], Asli Barla Demirkoz[3], Asli Barla Demirkoz[4], Melike Üner[1*]
[1]Department of Pharmaceutical Technology, Istanbul University,Istanbul, Turkey
[2]Department of Pharmaceutical Technology, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkey
[3]Aromsa Besin Aroma ve Katkı Maddeleri Sanayi ve Ticaret Anonim Şirketi. Kocaeli
[4]Department of Nutrition and Dietetics, Faculty of Health Sciences, Halic University, Istanbul, Turkey

Corresponding Author Information
Melike Üner
melikeuner@yahoo.com
+90 212 440 00 00
https://orcid.org/0000-0003-2786-5947
28.11.2020
06.04.2021

ABSTRACT
INTRODUCTION: The aim this study was to determine influence of vehicles and penetration enhancers on penetration and permeation of cinnarizine through the skin.
METHODS: Topical formulations based on hydrogel, O/W emulsion and oleaginous cream formulations were prepared. After determination of physical properties of formulations, penetration and permeation of cinnarizine through the Stratum corneum and the skin was investigated by an ex vivo study.
RESULTS: The cumulative amount of cinnarizine permeated from the base hydrogel formulation was about 5 times higher than the base O/W emulsion and base oleaginous cream formulations. Incorporation of penetration enhancers to the base hydrogel and O/W emulsion formulations generally increased cinnarizine penetration through the skin. Transcutol® was confirmed to provide the highest penetration enhancement in the hydrogel formulation. Propylene glycol was found to be the most suitable penetration enhancer for cinnarizine in oleaginous cream. Glycerol and oleic acid displayed the highest effect in O/W emulsion.
DISCUSSION AND CONCLUSION: It was concluded that the hydrogel containing Transcutol® provided the highest penetration through the skin of all formulations and this formulation could be an alternative to the oral route in treatment of Ménière's disease and motion sickness. Thus, the risk of systemic side effects caused by the drug can be reduced or eliminated. Thus, the risk of systemic side effects caused by oral medication can be reduced or eliminated.
Keywords: Cinnarizine, Ménière's disease, motion sickness, penetration enhancers, skin permeation
ÖZ
GİRİŞ ve AMAÇ: Bu çalışmanın amacı taşıyıcıların ve penetrasyon arttırıcıların sinarizinin deriden penetrasyonu ve permeasyonu üzerindeki etkisini tayin etmektir.
YÖNTEM ve GEREÇLER: Hidrojel, Y/S emülsiyonu ve yağlı krem bazlı topikal formülasyonlar hazırlandı. Formülasyonların fiziksel özelliklerinin tayininden sonra, sinarizinin Stratum corneum ve deriye penetrasyon ve permeasyonu bir ex vivo çalışma ile incelendi.

BULGULAR: Baz hidrojel formülasyonundan nüfuz eden kümülatif sinarizin miktarı, baz Y/S emülsiyonu ve baz yağlı krem formülasyonlarından yaklaşık 5 kat daha yüksekti. Baz hidrojel ve Y/S emülsiyon formülasyonlarına penetrasyon artırıcıların ilavesi, genellikle sinarizinin deriden penetrasyonunu arttırdı. Transcutol®'un hidrojel formülasyonunda en yüksek penetrasyon artışını sağladığı doğrulandı. Yağlı kremde sinarizin için en uygun penetrasyon artırıcı propilen glikol olarak bulundu. Gliserol ve oleik asit Y/S emülsiyonunda en yüksek etkiyi gösterdi.
TARTIŞMA ve SONUÇ: Transcutol® içeren hidrojelin tüm formülasyonlar arasında deriden en yüksek sinarizin geçişi sağladığı ve bu formülasyonun Meniere hastalığı ile hareket hastalığının tedavisinde oral yola bir alternatif olabileceği sonucuna varıldı. Böylece ağızdan alınan ilacın neden olduğu sistemik yan etki riski azaltılabilir veya ortadan kaldırılabilir.
Anahtar Kelimeler: Sinarizin, Meniere hastalığı, hareket hastalığı, penetrasyon artırıcılar, deri geçirgenliği

INTRODUCTION

Cinnarizine (CNZ) is a piperazine derivative histamine H1-antagonist and a selective calcium channel blocker drug.[1,2] It is commonly prescribed for peripheral and cerebral disorders, vertigo, tinnitus, nistagmus, motion sickness and Ménière's disease. There is only oral formulations in the pharmaceutical market of CNZ. The oral bioavailability of CNZ is low and variable. Many side effects of CNZ have been reported. Side effects of CNZ range from mild to quite severe. Its more common side effects are drowsiness and blurred vision, sweating, dry mouth, headache, skin problems, lethargy, gastrointestinal irritation, hypersensitivity reactions, muscle rigidity and tremor. CNZ can easily pass blood-brain barrier and it displays sedative activity. Thus, its use by pilots and aircrew who must be dependably alert due to increased levels of drowsiness caused by the medication, is generally limited. Long-term CNZ therapy may cause weight gain, depressive conditions and several extrapyramidal syndromes including tremor, acute and chronic Parkinsonism. CNZ can cause a tardive dyskinesia similarly to neuroleptic agents.
An alternative route to oral administration could provide an effective drug therapy. The transdermal route can be stated as one of the most reliable routes of application. Transdermal dosage forms introduce an alternative for delivery of actives that have low oral bioavailability and systemic side effects. Moreover, the first pass metabolism is avoided by the transdermal route. There are various strategies to accelerate the drug passing through the skin. Thus, immediate and moderate action can also be observed. Penetration enhancers are usually required to enhance permeation through the skin by different penetration mechanisms for optimization of well-formulating topical products. Thus, to obtain an efficient treatment can be provided via the transdermal route. Penetration enhancers essentially improve transdermal delivery of both lipophilic and hydrophilic actives by decreasing barrier resistance of the skin.[3,4] Polyols, fatty acids and terpenes are commonly used as penetration enhancers. Diethyleneglycol monoethylether (Transcutol®) (Tc), propylene glycol (PG), glycerol (Gl), oleic acid (OA) and limonene (L) are some of the most generally used penetration enhancers. They are able to carry drug delivery further through the skin displaying different mechanisms, through upper layers of the skin, mainly the *Stratum corneum*. Tc and PG alter thermodynamic activity of permeants in their vehicles after permeating through tissues themselves at first. Afterwards, permeants diffuse into the skin by modification of driving forces for diffusion.[5-7] The activity of PG is also pronounced to result from solvatation of α-keratin within the *Stratum corneum*, herewith promoting permeation by reducing drug-tissue binding. L promotes the permeation of lipophilic and amphiphilic penetrants by increasing their diffusion in the *Stratum corneum*.[8,9] OA, a long chain fatty acid, enhances percutaneous drug absorption by decreasing the phase transition temperatures of the skin lipids. A polar head group attached to the alkyl chain of OA conducts its potential enhancement function. On the other hand, Gl displays its penetration enhancing effect when in combination with water.[10]
In this study, it was aimed to prepare topical formulations of CNZ to overcome side effects caused by oral administration of the drug and to provide an alternative therapy in Ménière's disease and motion sickness. For this purpose, effects of various traditional vehicles and penetration enhancers on the permeation of CNZ were investigated. Topical formulations based on a hydrogel (G), O/W emulsion (E) and oleaginous cream (OC) were prepared and their physical characteristics were determined. Penetration and permeation of CNZ through the *Stratum corneum* and skin were investigated by an *ex vivo* study. This study was performed on abdominal skin of Wistar albino rats since the rat skin could be used as a model for investigation of transdermal drug delivery through the human skin as reported in earlier studies. *In vivo* and *ex vivo* tests on rats have been demonstrated that can be used for searching properties required from actives and/or vehicles for skin delivery.[11-14]

MATERIAL AND METHODS

Cinnarizine (CNZ) was kindly provided from Nobel İlaç San. ve Tic. A.Ş., Turkey. Hydroxypropyl methylcellulose (Methocel™ K15M) (HPMC) was thankfully presented by Colorcon (Turkey). Propylene glycol (PG), oleic acid

(OA), polyethylene glycol (PEG 400), glycerine (Gl) and Tween® 80 were purchased from Merck (Germany). Polyvinyl pyrrolidone (PVP® K90), cetyl alcohol and liquid paraffine were purchased from Sigma-Aldrich (Germany). Stearic acid and glyceryl monostearate were purchased from Doğa İlaç Hammaddeleri Tic. Ltd. Şti. (Turkey). Transcutol® (Tc) was provided by Gattéfosse (France). All other reagents and chemicals were of analytical grade.

Preparation of topical formulations

Compositions of base CNZ formulations (G, E and OC) are presented in Table 1. Penetration enhancers (Tc, PG, Gl and OA) were added to those formulations (Table 2). L was also added to base formulations at the rate of 5%. However, formulations went to the phase separation or they lost their homogenity within one week. Thus, those formulations were excluded from the study and constituents of formulations with L were not entered to Table 2. As an addition, Tc was confirmed to be incompatible with base formulation OC.

Quantification of CNZ

High performance liquid chromatography (HPLC) method was verified for analytical quantification of the drug in samples obtained during experiments. ICH guideline for the method validation procedure was considered for this purpose.[15] Linearity, intra-day and inter-day precision, accuracy, recovery and specifity were determined for verification of the method. Each verification analysis was replicated 6 times.

For this purpose, a HPLC apparatus (Shimadzu LC-20AT) was equipped with an UV/VIS detector (Shimadzu SPD-20A) and autosampler (Shimadzu SIL-20A HT). Separation was carried out using a TC-C18 column (5 µm, 4.6 x 250 mm) (Agilent Tech, Germany) at 40 ºC. Samples were detected under 1 mL/min flow rate of acetonitrile:ammonium phosphate monobasic solution (pH 4.5) (6:4, v/v) as the mobile phase at 253 nm. 0.24 mg/mL stock standard solution of CNZ in methanol was prepared to evaluate linearity of the method under the selected conditions. Drug determination was carried out at six concentrations (4-24 µg/mL) for providing the calibration curve.

Solubility of CNZ in various mediums

The solubility of CNZ was determined in various mediums according to the method reported in USP XIX.[16] 15 mL of the dissolution medium was placed in four 25-mL flasks for this purpose. A quantity of CNZ was added to each flask which was greater than the quantity expected to dissolve in the medium. Flasks were closed and they were fixed up in a constant temperature water bath (Daihan Scientific, Korea) adjusted to 25 ± 1 ºC. The apparatus was maintained under 200 rpm continuous agitation. Dispersions were filtered through S & S[5893] blue ribbon papers (2 µm pore size, Schleicher & Schuell, Germany) after 24 h agitation. Measured portions of clear supernatants were removed and the solubility of CNZ in the dissolution medium was determined by HPLC.

Partition coefficient

The partition coefficient of CNZ between isopropyl myristate and distilled water was determined using the shake flask method, following the guidelines of the European Chemical Bureau (European-Chemical-Bureau, Dir 92/69/EEC).

In vitro drug release of formulations

0.45 µm cellulose acetate membranes (Sartorius, Germany) were kept in the receptor phase, a physiological saline solution:PEG 400 mixture (PSS:PEG 400) (6:4, v/v) over night. Membranes were placed between two halves of Franz-type diffusion cells with 3.15 cm^2 surface area and 33.2 mL volume containing the receptor phase. 1 g topical formulation was placed on to the membrane in the donor phase. The receptor phase was kept at 37 ± 0.5 °C constant temperature during this study for 6 h. Samples were taken from the receptor phase at certain time intervals. Cumulative amounts of CNZ released (mg/cm^2) determined by HPLC after samples were filtered through S & S[5893] blue ribbon papers. Six replicates were conducted for each formulation. Drug release profiles were obtained by plotting cumulative amounts of the drug as the function of time and release profiles were evaluated by using different kinetic models (zero order, first order and Higuchi square-root model).[17-18] The exponent value (n) of Korsmeyer-Peppas kinetic model were considered for specifying drug release mechanisms well un-known or for more than one type of release mechanisms comprised.

Ex vivo skin penetration and permeation studies

2.5-3 months aged male Wistar albino rats (200-250 g) were provided from Aziz Sancar Institute of Experimental Medicine. The experimental protocol was approved by the Local Ethical Committee of Animal Experiments (17.12.2013, No: 2013/131). Animals were housed in plastic cages at 22 ± 1 °C and 60 ± 1% humidity under 12 h light-dark cycle. They were given standard laboratory diet and tap water *ad libitum*. Precisely shaved full-thickness abdominal rat skins were taken after they were sacrificed for *ex vivo* skin penetration and permeation assessments. The underlying fatty tissue was removed by blunt dissection without damaging the epidermal surface. Skins were placed between two halves of Franz-type diffusion cells. 1 g formulations were applied on the skin in the donor chamber of cells. PSS:PEG 400 (6:4, v/v), was used as the receptor phase. This study was continued for 6 hours at 37

± 1 ºC constant temperature. Cumulative amount of CNZ permeated was verified in samples collected from the receptor phase at predetermined time intervals by HPLC. Three replicates were conducted for each formulation. The cumulative amount (Q_n, mcg/cm^2) of CNZ permeated through the skin was calculated and cumulative drug amounts were plotted as the function of time (t, h).[19-21] The steady state flux of the drug (J_s, mcg/cm^2/h) was ascertained from the slope of linear part of plot using the linear regression analysis ($r > 0.99$) and then the efficiency of the penetration enhancers were determined.

Penetration of CNZ was assayed through the skin. For this purpose, abdominal rat skins over receptor chambers of Franz-type diffusion cells were used. Excess formulation in contact with the *Stratum corneum* was carefully expunged using cotton swabs.[22] Circular PVC adhesive tape pieces in 1 cm semidiameter (Ve-ge®, İzmir, Turkey) were applied with a light pressure over the diffusion area. Then, it was removed with forceps. The first two strips were thrown away, because they collected residue of the formulation within crevices of the skin surface. The next 10 adhesive tape pieces were then applied using the uniform firm pressure to obtain the formulation residue deposited within the skin tissue. They were then removed with uniform force rapidly using forceps. All adhesive tape pieces were collected in a 25 mL flask for extracting the drug content. For extracting CNZ, 10 mL ethanol was added to flasks and all flasks were tightly closed. They were fixed in the water bath at 25 ± 1 ºC. The apparatus was adjusted to 160 rpm continuous agitation for 24 h. Flasks contents were then filtered through S & S^{5893} blue ribbon papers. Measured portions of clear supernatants were removed from each flask for determination of CNZ content by HPLC. Subsequently, the solubility constraint (σ_{sc}) of CNZ in the *Stratum corneum* was also calculated [$\log \sigma_{sc} = 1.911(10^3/\text{melting point as Kelvin}) - 2.956$].[23] The melting point of CNZ was obtained by differential scanning calorimetry (DSC) analysis. For this purpose, a DSC apparatus (Universal V4.5A TA Instruments, U.S.A.) was employed. 9.7 mg of sample was weighted into aluminum pans of the apparatus and heated with 10 ºC/min heating rate under 50 mL/min N_2 flow. Thermogram of the sample was obtained indicating its melting point and enthalpy.

Data treatment and statistics

Drug release and permeation profiles of formulations obtained from *in vitro* and *ex vivo* experiments and data obtained from tape stripping experiment were compared using one way analysis of variance (ANOVA) test and the subsequent Tukey post hoc pairwise test. The Minitab® 18 Statistical Software was used for this purpose by setting the significance level as $\alpha = 0.05$.

RESULTS AND DISCUSSION

Analytical quantification

Analytical quantification of CNZ by HPLC was verified according to the instructions of the ICH Harmonised Tripartite Guideline.[15] The representative linear equation was A = aC + b, where A is the absorbance, a is the slope, C is the concentration and b is the intercept. The regression equation was A = 81417.9C + 3405.3 (correlation coefficient, r = 0.9999). The retention time of CNZ was found as 5.8 min (Figure 1A). Limits of detection (LOD) and quantification (LOQ) of the quantification method were determined as 5.929 ng/mL and 17.968 ng/mL, respectively. Relative standard deviations for accuracy, intra-day and inter-day precision of the methods were below 2%. Recovery of CNZ was found to be 99.87 ± 0.06 - 100.74 ± 0.03%. Chromatograms of the receptor phase and placebo base formulations demonstrated the specifity of the method (Figure 1B-E). Chromatograms of the formulations containing penetration enhancers (TC, PG, GL and OA, individually at the 5% rate in the formulations) were also verified the specifity of the method (they are not presented). Peaks of ingredients in the formulations were observed not to interfere the drug peak.

Solubility of CNZ

The solubility of CNZ in various mediums at 25 ºC are represented in Table 3. PSS:PEG 400 mixture (6:4, v/v) was decided to be used as the receptor phase since the solubility of CNZ was found to be the highest in it.

In vitro drug release of formulations

To achieve the sink condition, the receptor phase must have a high capacity to dissolve or carry away the drug. An acceptable sink condition has been reported to be one where the maximum concentration of the drug in the receptor phase reached during the experiment does not exceed 30% of its maximum solubility in the receptor phase.[24] It is provided in a volume of medium that is at least 3-10 times the saturation volume. The solubility of CNZ at 37 ± 1 ºC was also determined and it was found to be 2.352 ± 0.012 mg/mL. Thus, the volume of the receptor phase allowed to maintain the sink condition.

Permeation characteristics of a drug through the skin can't be judged with *in vitro* drug release experiments. But it avails researchers to reckon some reasons of low drug penetration rate including affinity of the drug to the vehicle.[25,26] CNZ was confirmed to display the highest affinity to the base OC compared to base G and E ($p<0.05$). The emulsion formulation E was confirmed to display the highest drug release rate among the base formulations up to 6th hours ($p<0.05$) (Figure 2 and Table 4). The base hydrogel (G) (0.293 mg/cm^2/h) and oleaginous cream (OC) (0.151 mg/cm^2/h) formulations respectively followed E (0.326 mg/cm^2/h). It was determined that incorporation of

penetration enhancers to the base formulations led to a significant increase in drug release rate of the vehicles in the case of formulations E and G ($p<0.05$) while an opposite situation was occurring for OC with all penetration enhancers. Tc and Gl addition to formulation E and PG addition to formulation G provided the highest release rate of drug from vehicles ($p>0.05$). Due to the lipophilic character of CNZ resulted in its retention in base OC formulation.[27] Moreover, incorporation of penetration enhancers (PG, Gl and OA) to OC statistically insignificantly changed the drug release rate and slower drug release profiles were obtained from formulations OC-OA, OC-Gl and OC-PG ($p>0.05$) compared to the emulsion and hydrogel formulations. Thus, it was affirmed that increase in the solubility of the drug in the vehicle resulted in slower drug release.[25] E and G formulations displayed anomalous transport of drug release as the result of kinetic modelling (Table 4). This phenomen can be attributed to two mechanisms conducted drug release, diffusion and polymer relaxation in hydrogel formulations or drug release free from concentration in emulsion formulations.

Ex vivo skin permeation and penetration studies

CNZ permeation from the topical formulations through the rat skin was ascertained to involve in the polarity of the formulations and the type of the penetration enhancers as reported earlier studies conducted on skin permeation of lipophilic drugs.[27] Polar hydrogel structure provided the highest drug permeation rates among other vehicles (Figure 3 and Table 5). This can be attributed to the high partition coefficient of CNZ (log $P = 5.74 \pm 0.03$ in isopropyl myristate/water). G significantly displayed the highest permeation rate followed by E and OC ($p<0.05$), respectively. Tc was found as the most effective penetration enhancer compared to PG, GL and OA for G formulations ($p<0.05$). The highest drug permeation rate was obtained in formulation G-Tc (0.110 ± 0 mg/cm^2/h) followed by G-Gl (0.062 ± 0.002 mg/cm^2/h), G-PG (0.057 ± 0.001 mg/cm^2/h) and G-OA (0.050 ± 0.001 mg/cm^2/h). Although E formulations followed G formulations in the same penetration enhancer order, differences between E and OC formulations were insignificant ($p>0.05$). Tc and PG possibly contributed their own permeation through tissues and modified the thermodynamic activity of CNZ prior to modification of driving forces for drug diffusion as reported earlier.[19,28] Solvatation of *a*-keratin within the *Stratum corneum* by PG might additionally be claimed to improve permeation of CNZ by reducing drug-tissue binding. OA and Gl enhanced percutaneous absorption of CNZ by decreasing phase transition temperatures of skin lipids and displaying occlusion effect on the skin, respectively.[19,29] All formulations were affirmed to reach the steady-state flux (*Js*) at the 1st hour except for base formulation G (3rd hour). Amounts CNZ % in the *Stratum corneum* and the receptor phase that were determined for each formulation at the 6th hour are presented in Figure 4. It was found that amounts of CNZ accumulated in the *Stratum corneum* were significantly higher than determined in the receptor compartment. As can be seen in the figure, G-Tc displayed the highest skin penetration of the drug followed by formulations G-Gl, G-OA, E, G-PG, G, E-PG, E-Gl, OC, E-Tc, E-L, OC-OA and OC-GL, respectively ($p<0.05$). Formulations displayed high drug accumulation in the *Stratum corneum* were expected to exhibit continuous drug permeation of most of the retained drug by steady state flux in time. Melting point of CNZ was found as 121.94 ºC (121.16 ºC onset melting temperature and 98.18 J/g melting enthalpy) according to its DSC thermogram indicating a sharp melting peak (Figure 5). The solubility constraint (σ_{sc}) of CNZ was calculated as 1.88 in the *Stratum corneum* indicating the potential of this compound which may form a reservoir in the *Stratum corneum*. Organic substances with high melting points and enthalpies have lower aqueous solubility in general since solvents can't pass into the crystalline structure of such molecules to dissolve them.[30,31] Thus, an indirect relationship has been reported to exist between the melting point and the solubility of a drug.[32] In other words, a decrease in the melting point of a drug would led to an increase in its solubility in the *Stratum corneum* and consequently its penetration and then permeation through the skin.

CONCLUSION

Formulations that were studied in this research introduce various advantages over many transdermal drug delivery systems. They are suitable for large scale production and cost-effective dosage forms produced with excipients used in pharmaceuticals and cosmetics for years. *Ex vivo* study performed on rats gave information about influence of the polarity of vehicles and penetration enhancer on skin penetration of CNZ. It was concluded that skin penetration increased as the lipophilicity of the vehicle decreased. The hydrogel formulation without a penetration enhancer provided about five times higher drug permeation compared to an o/w emulsion and oleaginous cream. Furthermore, when Transcutol® was introduced to HPMC hydrogel, it displayed the highest penetration enhancer activity. As a result, HPMC hydrogel containing Transcutol® could be suggested as a suitable carrier for CNZ intended to be used topically for relief of various conditions.

ACKNOWLEDGEMENTS

This study was supported by The Research Fund of Istanbul University (Project number: 40188) and TUBITAK (The Scientific and Technological Research Council of Turkey) (Grant Number TEYDEB 1649B031305845).
A part of this study was presented as a poster in International Multidisciplinary Symposium on Drug Research and Development in Erzurum, Turkey in 5-7 October 2017.

Conflicts of interest: No conflict of interest was declared by the authors. The authors alone are responsible for the content and writing of the paper.

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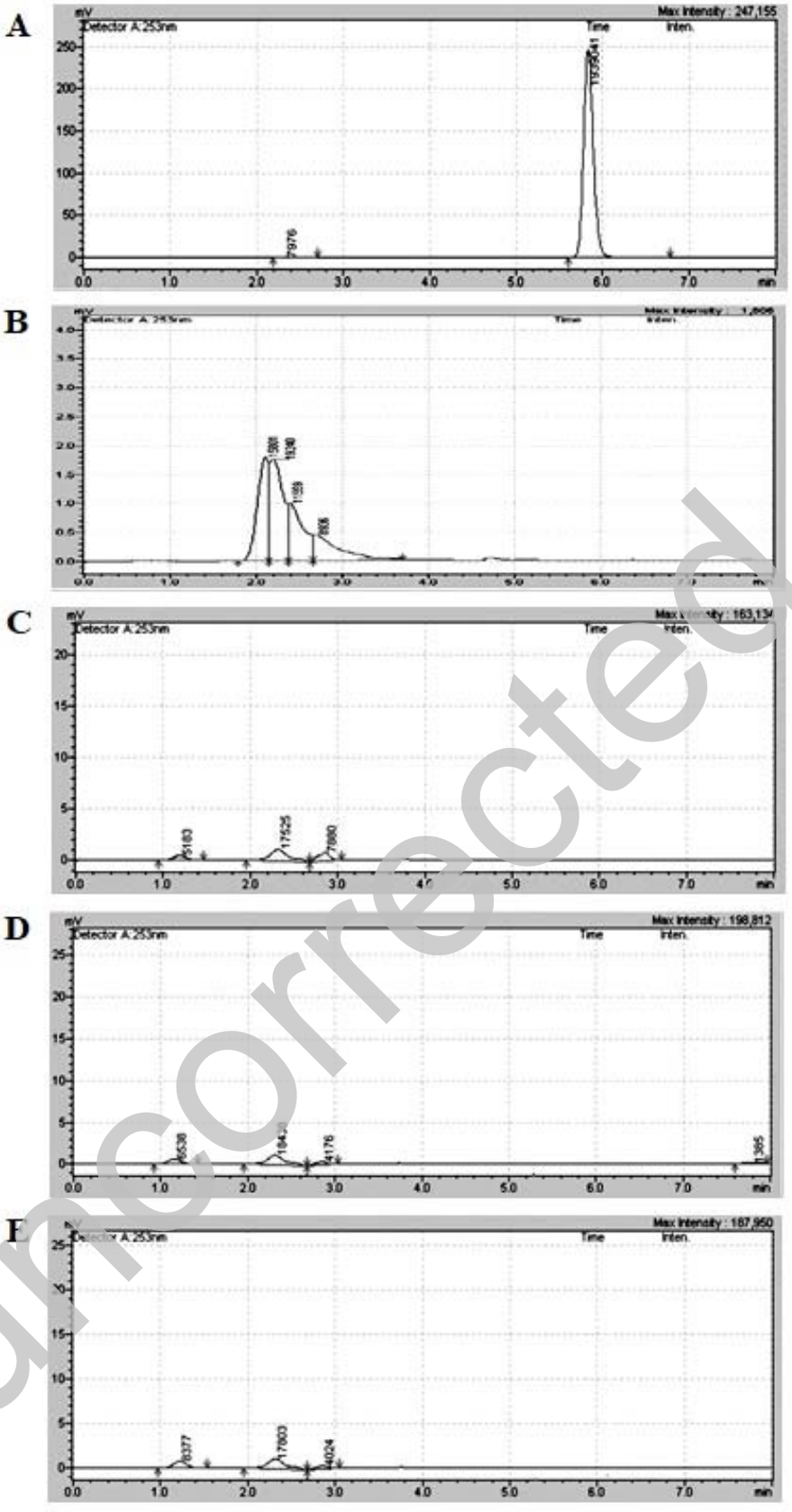

Figure 1. HPLC chromatograms - (a) CNZ, (b) receptor phase and placebo base formulations [(c) base G formulation, (d) base E formulation and (e) base OC formulation]

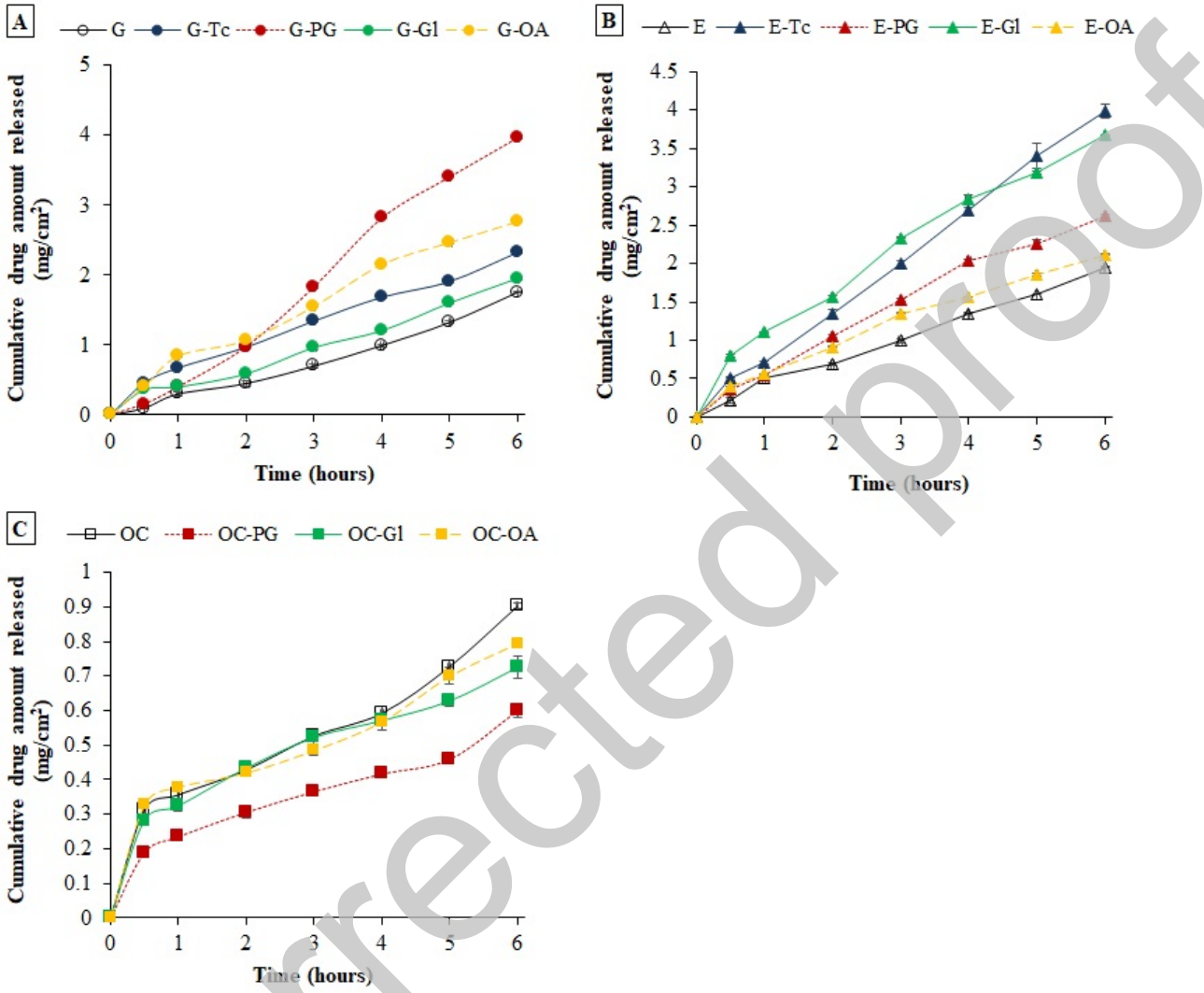

Figure 2. CNZ release profiles of topical formulations in PSS:PEG 400 (6:4, v/v)

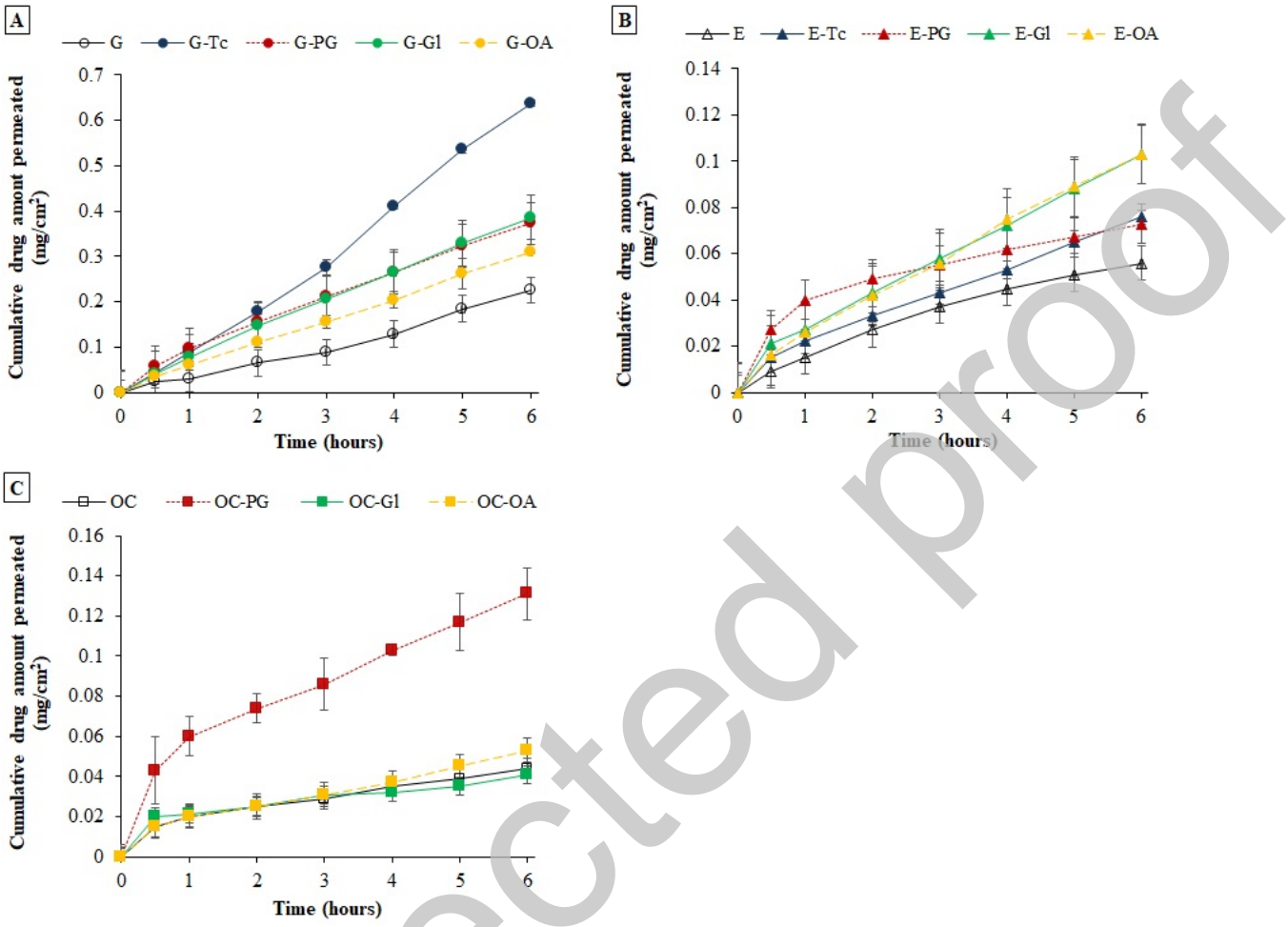

Figure 3. Permeation profiles of CNZ through the rat skin

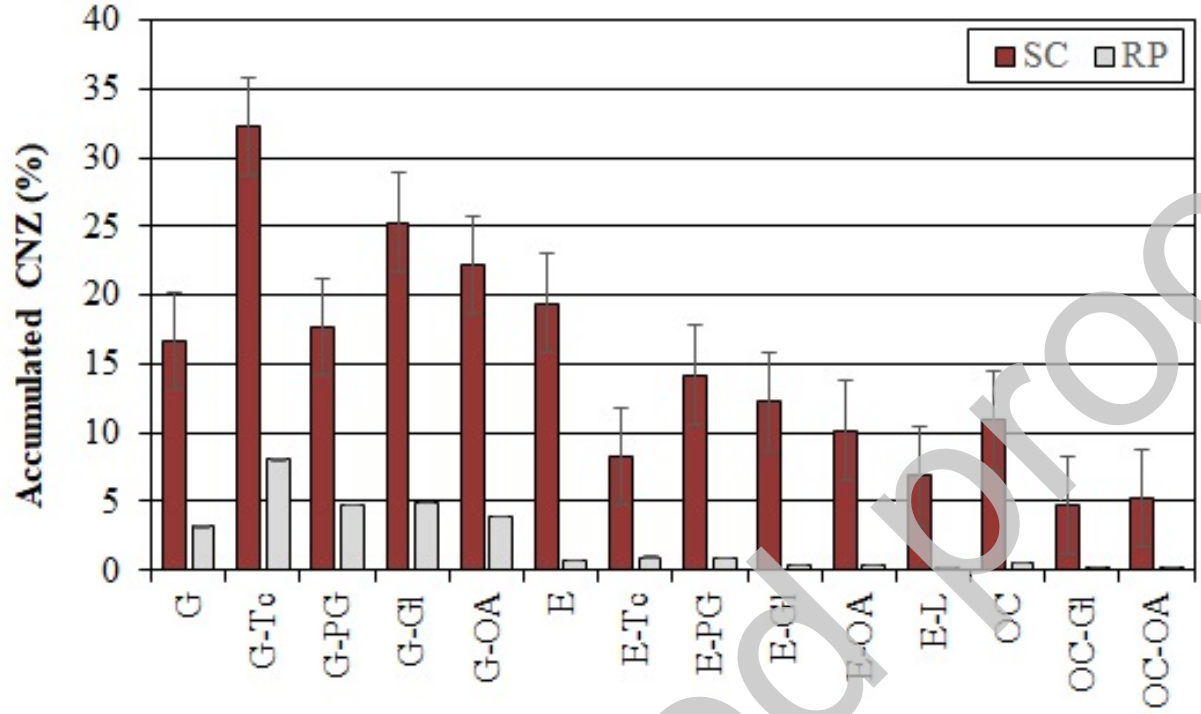

Figure 4. Cumulative amount of CNZ (%) retained in the *Stratum corneum* (SC) of rat skins and remained in the receptor compartment (RC) after 6 h application of the formulations

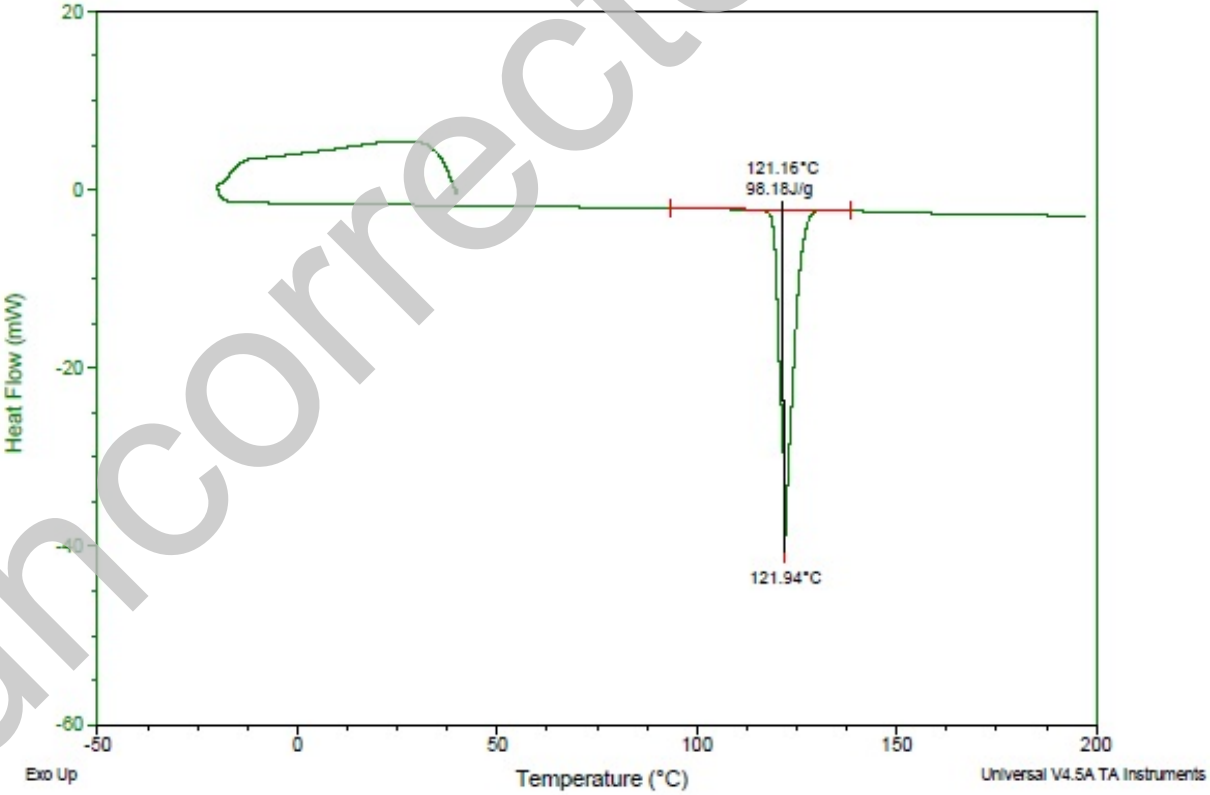

Figure 5. DSC thermogram of the drug (CNZ)

TABLES

Table 1. Constituents of the base formulations

Constituents (%, w/w)	**G**	**E**	**OC**
CNZ	2.5	2.5	2.5
HPMC	3.15	-	-
PVP K90	0.35	-	-
PEG 400	15	-	-
Vaseline	-	8	75
Liquid paraffine	-	19	12.5
Stearic acid	-	2	10
Cetyl alcohol	-	2	-
Tween® 80	-	4	-
GMS	-	1	-
Saline	22.5	-	-
Distilled water	56.5	61.5	-
Methylparaben	0.15	0.15	-
Propylparaben	-	0.15	0.15

G, hydrogel; E, o/w emulsion; OC, oleaginous cream

Table 2. Formulations containing penetration enhancers

Formulations	**Enhancers (%)**			
	Tc	**PG**	**Gl**	**OA**
G*	-	-	-	-
G-Tc	5	-	-	-
G-PG	-	5	-	-
G-Gl	-	-	5	-
G-OA	-	-	-	2
E*	-	-	-	-
E-Tc	5	-	-	-
E-PG	-	5	-	-
E-Gl	-	-	5	-
E-OA	-	-	-	5
OC*	-	-	-	-
OC-PG	-	5	-	-
OC-Gl	-	-	5	-
OC-OA	-	-	-	5

*G, E and OC are base formulations. Tc, Transcutol®; PG, propylene glycol; Gl, glycerole; OA, oleic acid.

Table 3. Solubility of CNZ in different mediums at 25 ± 1 ºC

Mediums	**Solubility (mg/mL)**	
pH 7.4 PBS	0.017 ± 0.004	pi
pH 6.8 PBS	0.027 ± 0.008	pi
5% bovine serum albumin in PSS (w/v)	0.024 ± 0.006	pi
PSS:PEG 400 (8:2)	0.601 ± 0.013	vss
Water	0.749 ± 0.005	vss
PSS:PEG 400 (6:4)	1.406 ± 0.010	ss

According to the European and the United States Pharmacopeias-pi, practically in soluble; vss, very slightly soluble; ss, slightly soluble.

Table 4. Release parameters of CNZ from the formulations for 6 hours and kinetic modelling of release profiles

			Zero order $[Q_t=Q_0 + k_0t]$		First order $[Q_t=Q_\infty (1 - e^{-kt})]$		Higuchi model $[Q_t=Q_0 + k_H t^{1/2}]$		Korsmeyer-Peppas model $[\log [Q_t/Q_\infty] = \log k + n\log t]$	
Formulations	***Q*** **(mg/cm²)**	**Release rate (mg/cm²/h)**	**r**	k_o	**r**	k_1	**r**	**D**	**r**	**n, Dominant release mechanism**
G	1.754 ± 0.004	0.293 ± 0.001	0.9910	0.286	0.9468	0.456	0.9642	0.887	0.9915	1.08 (non-Fickian), An.T.
G-Tc	2.325 ± 0.010	0.388 ± 0.002	0.9984	0.331	0.9715	0.279	0.9895	1.046	0.9957	0.65 (non-Fickian), An.T.
G-PG	3.966 ± 0.024	0.662 ± 0.004	0.9962	0.729	0.9395	0.555	0.9824	2.294	0.9986	1.33 (non-Fickian), An.T.
G-Gl	1.952 ± 0.022	0.326 ± 0.004	0.9918	0.296	0.9914	0.322	0.9624	0.916	0.9650	0.71 (non-Fickian), An.T.
G-OA	2.771 ± 0.013	0.463 ± 0.002	0.9932	0.430	0.9472	0.317	0.9880	1.363	0.9911	0.75 (non-Fickian), An.T.
E	1.951 ± 0.011	0.326 ± 0.002	0.9966	0.303	0.9427	0.353	0.9852	0.956	0.9914	0.84 (non-Fickian), An.T.
E-Tc	3.988 ± 0.087	0.666 ± 0.015	0.9991	0.649	0.9726	0.372	0.9831	2.036	0.9931	0.86 (non-Fickian), An.T.
E-PG	2.616 ± 0.021	0.437 ± 0.004	0.9945	0.422	0.9503	0.354	0.9942	1.346	0.9970	0.83 (non-Fickian), An.T.
E-Gl	3.674 ± 0.011	0.614 ± 0.002	0.9953	0.530	0.9683	0.271	0.9919	1.684	0.9929	0.63 (non-Fickian), An.T.
E-OA	2.113 ± 0.012	0.353 ± 0.002	0.9956	0.316	0.9592	0.297	0.9949	1.008	0.9967	0.70 (non-Fickian), An.T.
OC	0.902 ± 0.008	0.151 ± 0.001	0.9860	0.101	0.9979	0.186	0.9554	0.313	0.9547	0.40 (Fickian), Diffusion
OC-PG	0.600 ± 0.021	0.100 ± 0.004	0.9869	0.068	0.9832	0.191	0.9714	0.213	0.9816	0.43 (Fickian), Diffusion
OC-Gl	0.725 ± 0.034	0.121 ± 0.006	0.9934	0.078	0.9752	0.166	0.9942	0.249	0.9906	0.38 (Fickian), Diffusion
OC-OA	0.796 ± 0.010	0.133 ± 0.002	0.9874	0.083	0.9965	0.158	0.9567	0.258	0.9450	0.34 (Fickian), Diffusion

Q: cumulative amount of drug released; Q_t *and* Q_0 quantity of drug released at time t and in the release medium at t=0, respectively; r: correlation coefficient; k_1, k_0, and k_H rate constants of the Zero order, First order and Higuchi kinetic models, respectively; Q_t/Q_∞: fractional release of drug; *k* and *n*: kinetic constant and diffusion exponent of the release mechanism (slope) according to Korsmeyer-Peppas model; An.T.: Anomalous transport.

Table 5. Permeation parameters of CNZ through the skin

Formulations	Q_n (mg/cm^2)	J_s (mg/cm^2/h)	K_p (cm/h)	r	*ER*
G	0.250 ± 0.002	0.046 ± 0.002	1.86 x 10^{-3} ± 8.00 x 10^{-5}	0.9976	-
G-Tc	0.638 ± 0.007	0.113 ± 0.002	4.52 x 10^{-3} ± 6.63 x 10^{-5}	0.9976	2.46
G-PG	0.373 ± 0.011	0.056 ± 0.001	2.22 x 10^{-3} ± 0.87 x 10^{-5}	0.9991	1.22
G-Gl	0.386 ± 0.028	0.061 ± 0.001	2.45 x 10^{-3} ± 3.52 x 10^{-5}	0.9993	1.33
G-OA	0.309 ± 0.013	0.050 ± 0.001	2.00 x 10^{-3} ± 1.69 x 10^{-5}	0.9995	1.09
E	0.056 ± 0.021	0.008 ± 0.004	0.33 x 10^{-3} ± 15.48 x 10^{-5}	0.9879	-
E-Tc	0.076 ± 0.003	0.011 ± 0.002	0.43 x 10^{-3} ± 7.25 x 10^{-5}	0.9994	1.38
E-PG	0.073 ± 0.013	0.006 ± 0.002	0.26 x 10^{-3} ± 9.49 x 10^{-5}	0.9961	0.75
E-Gl	0.103 ± 0.012	0.015 ± 0.002	0.60 x 10^{-3} ± 6.06 x 10^{-5}	0.9998	1.88
E-OA	0.103 ± 0.017	0.016 ± 0.003	0.62 x 10^{-3} ± 11.72 x 10^{-5}	0.9991	2.00
OC	0.044 ± 0.005	0.005 ± 0.001	0.20 x 10^{-3} ± 3.01 x 10^{-5}	0.9962	-
OC-PG	0.131± 0.013	0.014 ± 0.001	0.57 x 10^{-3} ± 2.97 x 10^{-5}	0.9992	2.80
OC-Gl	0.041 ± 0.004	0.004 ± 0.001	0.15 x 10^{-3} ± 1.59 x 10^{-5}	0.9889	0.80
OC-OA	0.053 ± 0.003	0.007 ± 0	0.27 x 10^{-3} ± 0.36 x 10^{-5}	0.9970	1.40

Eqs. $Q_n = \frac{C_n V_0 + \sum_{i=1}^{n-1} C_i V_i}{A}$ $J_s = C_0 \frac{KD}{L}$ $K_p = \frac{J_s}{C_0}$ $ER = \frac{J_s \text{ (with the enhancer)}}{J_s \text{ (without the enhancer)}}$

Q_n: cumulative amount of the drug permeated; C_n: drug concentration in the receptor phase at the *n*th sampling interval; *A*: effective diffusion area (surface of the sample cell); V_0 and V_i: volumes of the receptor phase in the individual Franz cell and the sample, respectively; $\sum_{i=1}^{n-1} C_i$: sum of drug concentration determined at sampling intervals 1 through n−1; J_s: steady state flux of the drug; C_0: constant drug concentration in the donor phase; *D*: diffusion coefficient; *L*: thickness of the membrane; *K*: partition coefficient of the drug and the vehicle; K_p: permeability coefficient; r: correlation coefficient; ER: the enhancement ratio.