Core–shell-type polymer–lipid nanoparticles for the transdermal delivery of daidzein

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Daidzein (DZ) is an ideal Chinese herb for treating cardiocerebrovascular disease. Despite its poor water solubility and limited application, DZ was successfully loaded, along with penetration enhancer azone, onto core–shell-type polymer–lipid nanoparticles through a two-step preparation method. The mean particle diameter, encapsulation efficiency, and drug-loading rate of DZ–polymer-azone–lipid nanoparticles (D-P-Azone-LNs) were 77.59 ± 2.26 nm, 79.91% ± 1.46%, and 1.50% ± 0.02%, respectively. In skin penetration experiment, the cumulative amount of DZ from D-P-Azone-LN reached 14.91 μg·cm^{-2} at 72 h. This amount was 1.44 and 6.01 times higher than those of DZ–polymer–lipid nanoparticles (D-P-LNs) and DZ solution, respectively. Confocal laser scanning microscopy was used to conduct a qualitative analysis. *In vivo* study, D-P-Azone-LN achieved better skin retention than those of D-P-LN and DZ. Furthermore, no significant irritation was observed in the skin irritation experiment. These results suggested that the core–shell polymer–lipid nanoparticles, which combined the properties of polymer and lipid nanoparticles, can offer a useful formulation for enhancing the skin permeation of drug for transdermal delivery.

1. Introduction: Daidzein (DZ), a natural isoflavone of Pueraria Lobata Ohwi, has been widely applied to treat hypertension and cardiocerebravascular diseases [1]. However, given its low water solubility and poor gastrointestinal absorption [2, 3], DZ exerts limited therapeutic efficacy and clinical application. For enhancing the absorption and improving the efficacy of DZ, a patent nanoparticle-based transdermal delivery system can be fabricated.

Nanoparticles composed of biodegradable polymers or lipids have attracted considerable attention because of their targeting delivery and significant therapeutic potential [4]. As the two main types of drug nanocarriers, liposomes and biodegradable polymeric nanoparticles share numerous similar advantages, such as biocompatibility, adjustable size and targeted delivery. Meanwhile, these nanocarriers exhibit their own unique advantages and limitations because of different properties. Core–shell-type polymer–lipid compounded carriers, a new type of nanoparticles, exhibit the advantages of both liposome and polymer nanoparticles. Chemical drugs, gene/protein drugs and water/oil-soluble drugs can all be loaded onto this improved hybrid nanocarrier and released temporally [5]. In this study, poly(lactic-co-glycolic acid) (PLGA) was selected because of its high-loading quality for hydrophobic drugs. Lecithin monolayer was used to form a shell and provided favourable biocompatibility [6].

Core–shell polymer–lipid nanoparticles are applied in various drug delivery applications, such as being desirable adjuvants for vaccine delivery [7], loading docetaxel to treat cancer [8] and providing a hybrid nanoparticle system for siRNA delivery [9]. Core–shell polymer–lipid nanoparticles are a promising platform for drug delivery because they can deliver multiple drugs, increase drug encapsulation, control drug release rates and exert therapeutic effects [8–10]. However, to our knowledge, transdermal applications of these tools have not been reported.

On the basis of the previous work, the entrapment of the penetration enhancer into drug carrier was an effective way for transdermal delivery [11]. Azone was thought to exert its effect as a useful penetration enhancer by interacting with structured lipids of the stratum corneum.

This work aims to (i) prepare DZ–polymer-azone–lipid nanoparticles (D-P-Azone-LNs); (ii) characterise and evaluate the physicochemical properties of D-P-Azone-LNs; and (iii) estimate the transdermal effect of D-P-Azone-LNs *in vitro* and *in vivo*.

2. Materials and methods

2.1. Chemicals and reagents: DZ (purity > 98%) was purchased from Qingze Co., Ltd. (Jiangsu, China). PLGA (Mn = 15,000 Da, GA/LA = 50:50) was supplied by Jinan Daigang Biomaterial Tech Co., Ltd. (Shandong, China). Azone, lecithin from eggs, and Tween-20 were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All solvents in use were of analytical reagent grade.

2.2. Animals: Sprague–Dawley (SD) male rats (200±20 g) were purchased from Shanghai Jie si Jie Experimental Animal Co. Ltd. The animals were reared in a room at 25±2°C and a relative humidity (RH) of 55±5%. All skin samples were obtained from animals by following the instructions of the Animal Ethics Committee of Shanghai Jiao Tong University.

2.3. Quantitative analysis: High-performance liquid chromatography (HPLC) with an SPD-20A UV–vis detector and LC-20A system (Shimadzu, Japan) was used to determine drug concentration. The analysis was conducted on a Hypersil ODS_2 column (150 mm × 4.6 mm ID, 5 μm). The flow phase consisted of acetonitrile and deionised water at a 30:70 ratio by volume (v/v). This phase was eluted at a rate of 1.0 ml · min^{-1}. Then, 20 μl of the sample was injected each time. Detection and analysis were carried out by the LC Solution software.

2.4. Preparation of D-P-Azone-LN core–shell nanoparticles: D-P-Azone-LNs were prepared from PLGA, azone, and egg lecithin by using a two-step emulsification method as published previously [12]. Preparation and formulation were optimised through single-factor experiment [13]. The final optimal formulation and process are described below. DZ (1 mg) and 5 mg of PLGA were dissolved in 2 ml organic solvent (acetone: ethanol = 7:3 by volume), whereas 0.2 g Tween-20 (emulsifier) was dissolved in 20 ml of deionised water. The two phases were mixed and magnetically stirred at 25°C (1000 rpm for 2 h) to form the polymeric core. Meanwhile, 2 mg of egg lecithin and 0.2 g of azone were dissolved in 5 ml of chloroform. The chloroform was removed by rotary evaporation to obtain a thin lipid film [14]. Dissolving PLGA core nanoparticles in the container of the dry lipid film, the mixed solution was finally homogenised by ultrasonic agitation (Fig. 1). Nanoparticles for

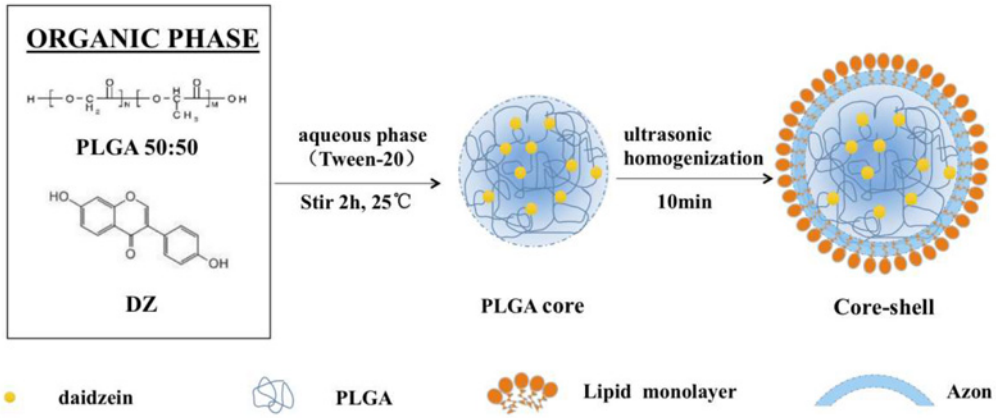

Fig. 1 *Preparation of core–shell D-P-Azone-LNs*

immediate use were stored at 4°C, and those for later use were freeze dried at −20°C.

2.5. Characterisation of D-P-Azone-LNs: The average size, polydispersity index (PDI), and zeta potential of D-P-Azone-LNs were measured by a Zeta sizer (Malvern, UK) at 25°C. Transmission electron microscopy (TEM) (Tokyo, Japan) was then conducted to observe the morphology of D-P-Azone-LNs. Furthermore, the nanoparticles were collected by ultracentrifugation and freeze dried to measure drug loading (DL) and entrapment efficiency (EE) capacity (see Table 1). The amount of DZ embedded in the nanoparticles was determined by HPLC

$$\mathrm{EE}\% = W_1/W_2 \times 100\% \quad (1)$$

$$\mathrm{DL}\% = W_1/W_3 \times 100\% \quad (2)$$

where W_1 is the actual amount of drug encapsulated in the nanoparticles, W_2 is the initial amount of drug used in the fabrication of nanoparticles, and W_3 is the total weight of the nanoparticles.

2.6. *In vitro* skin penetration: After all hair was carefully shaved off by an electric shaver, the abdominal intact skin was excised, and excess fat was removed. Vertical Franz-type diffusion apparatus (RTJ-12B, Huanghai) equipped with 12 cells (2.3 cm^2 cross-sectional area and 7.0 ml volume) was used to study the permeability of D-P-Azone-LN core–shell nanoparticles. The stripped skin was fixed between the donor and receptor compartments of the cells, with the stratum corneum positioned towards the donor compartment.

DZ and prepared nanoparticles were placed into the donor compartments. Meanwhile, each receptor compartment was filled with phosphate-buffered saline (PBS) (pH = 7.4) and maintained at 37±0.5°C with magnetic stirring at 300 rpm [15]. During the experiment, bubbles were avoided as much as possible in the receptor compartments [16]. At appropriate time intervals (1, 2, 4, 18, 21, 28, 40, 48, 63, and 72 h), 1 ml of the receptor fluid was withdrawn and instantly replenished with the same volume of PBS. Then, the samples were filtered through a 0.45 µm polytetrafluoroethylene membrane for pretreatment and measured by HPLC.

The cumulative permeation quantity of DZ (Q_n) that permeated the skin was plotted versus time (t). The steady-state flux rate J_{ss} (µg·cm^{-2}·h^{-1}) was obtained as follows:

$$J_{ss} = \mathrm{d}Q_n/A \times \mathrm{d}t \quad (3)$$

where A is the skin surface area.

2.7. Confocal laser scanning microscopy (CLSM) studies: To visualise the transdermal effects, we employed a confocal microscope (Wetzlar, Germany) to observe the skin permeability of D-P-Azone-LNs loaded with 6-coumarin dye in their core. Images were captured by a Leica TCS SP5II laser scan confocal microscope under a laser excitation wavelength of 488 nm. Skin samples were obtained as described in Section 2.6.

The skins permeated by DZ–polymer–lipid nanoparticles (D-P-LNs) and D-P-Azone-LNs were removed from Franz-type diffusion cells after 6 and 48 h, respectively. After fixing with paraformaldehyde and dehydrating, we sectioned the frozen skin vertically and horizontally to a thickness of 8 µm.

2.8. *In vivo* skin retention: SD rats fasted overnight with free access to water before drug treatment. Abdominal skin hair was shaved off cleanly and carefully without skin injury. Male rats were randomly assigned to one of three groups (six rats for each group) and treated with DZ, D-P-LNs, or D-P-Azone-LNs (15 mg DZ/kg) on the skin (3 × 4 cm^2). Each administration patch was covered with a medical adhesive bandage. At the time of separation at 12 and 24 h, three rats in each group were sacrificed by cervical dislocation, and further processing skin was ready for HPLC detection.

2.9. Evaluation of skin irritant effects: Twelve rats were randomly divided into four groups, and nine of these rats (three groups) were treated similarly to that in Section 2.8. All rats were sacrificed after 24 h, and their skin (3 × 4 cm^2) was removed and fixed in 4% paraformaldehyde. After fixation and dehydration, the skin samples were infiltrated and embedded in paraffin. Cooling-solidified paraffin blocks were cut into 4 µm-thick sections and then stained by hematoxylin–eosin for histologic examination.

Table 1 Particle size and zeta potential of D-PNs and D-P-Azone-LNs (mean ± SD, $n = 3$)

Sample	Average size, nm	Zeta potential, mv	PDI	EE, %
D-PNs	67.21 ± 2.32	−31.5 ± 1.26	0.21 ± 0.11	—
D-P-Azone-LNs	77.59 ± 2.26	−2.95 ± 1.31	0.22 ± 0.02	79.91 ± 1.46

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2.10. Statistical analysis: All data processing in this Letter was repeated for three times, and the results were expressed as means ± standard deviations. Student's t-test was performed to evaluate the statistical significance of differences between two groups, $p < 0.05$ was considered significant.

3. Results and discussion

3.1. Characterisation of D-P-Azone-LNs: On the basis of optimal formulation, D-P-Azone-LNs achieved a spherical shape (Fig. 2).

Although the surface charge plays a considerable role in maintaining the nanoparticle stability, the zeta potential of D-P-Azone-LNs was only −2.95 ± 1.3 mV. The lipid-containing shell potentially serves as a surfactant to maintain nanoparticle stability [17]. Meanwhile, the surface charge of the DZ-loaded PLGA nanoparticles (D-PNs) was determined as −31.5 ± 1.26 mV. The potential difference confirms that the PLGA core was covered by lecithin film. The narrow diameter distribution and size of the nanoparticles were suitable for transdermal delivery.

3.2. *In vitro* skin penetration: Skin permeation profiles of DZ are displayed in Fig. 3 and Table 2. Data showed continuous rising penetration of D-P-Azone-LNs, which achieved the highest drug permeation rates. The following sequence was observed: D-P-Azone-LNs > D-P-LNs > DZ.

Several causes can explain the enhancement of skin permeation of D-P-Azone-LNs. *In vitro*, azone released first when the lipid shell disintegrated and promoted drug permeability [18]. As a commonly used penetration enhancer, azone exerts a hydration effect on the stratum corneum and reduced the drug diffusion resistance [19]. Given its narrow size distribution (<100 nm) and large available surface area, nanoparticles easily penetrated the skin and achieved sustained drug release.

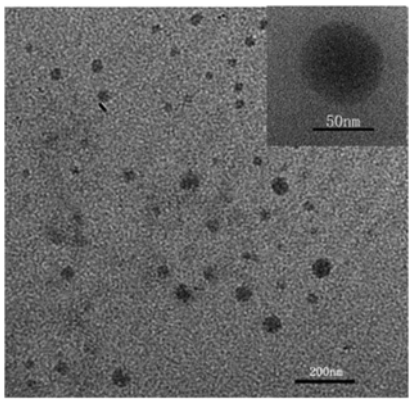

Fig. 2 *TEM photographs of D-P-Azone-LNs*

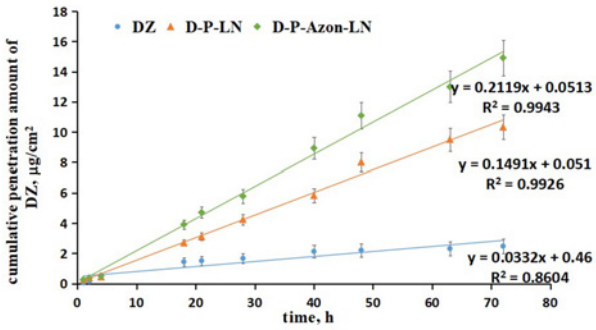

Fig. 3 *In vitro cumulative penetration amount of DZ: DZ, D-P-LNs, and D-P-Azone-LNs. Each value represents the mean ± SD (n = 3)*

Table 2 Penetration effect of DZ from DZ, D-P-LNs, and D-P-Azone-LNs

Formulation	J_{ss}, μg·cm^{-2}h^{-1}	$Q_{72}h$, μg cm^{-2}	ER
DZ	0.034 ± 0.001	2.48 ± 0.50	—
D-P-LN	0.143 ± 0.013**	10.33 ± 0.93**	4.17
D-P-Azone-LN	0.207 ± 0.017**	14.91 ± 1.19**	6.01

J_{ss}, the steady-state flux rate; $Q_{72}h$, the cumulative amount of drug permeated per unit area at 72 h; ER, the enhancement ratio, and ER = $Q_{72}h$ (formulation)/$Q_{72}h$ (DZ).
*$p < 0.05$, **$p < 0.01$ versus DZ. Each value represents the mean ± SD ($n = 3$).

3.3. CLSM studies: Images of the longitudinal and cross sections of the skin treated with C_6-D-P-LNs and C_6-D-P-Azone-LNs are shown in Fig. 4.

With a prolonged time of drug action from 6 to 48 h, the fluorescence intensity increased in both groups. The depth-dependent distribution of fluorescence confirmed the increasing amount of drugs that permeated into the skin.

In vitro skin permeation study showed that D-P-Azone-LNs and D-P-LNs raised the cumulative release amount of DZ. CLSM (visual and qualitative) further proved that the skin permeability of D-P-Azone-LNs was higher than that of D-P-LNs.

3.4. *In vivo* skin retention: The amount of DZ remaining in the skin after 12 and 24 h was measured (Fig. 5).

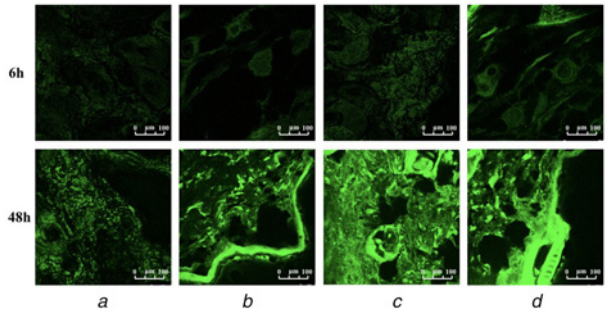

Fig. 4 *Confocal images of the skin longitudinal and cross section obtained at 6/48 h after drug treatment*
a Transection and
b Vertical section of rat's abdominal skin from C_6-D-P-LNs
c Transection and
d Vertical section of the rat's abdominal skin from C_6-D-P-Azone-LNs in 6 and 48 h, respectively

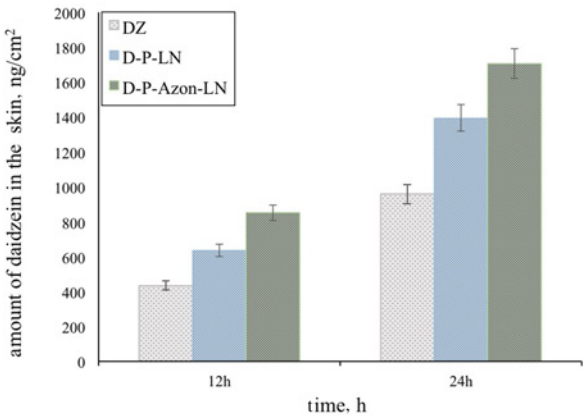

Fig. 5 *Amount of DZ form pure DZ, D-P-LNs, and D-P-Azone-LNs in vivo rat skin at 12 and 24 h. Each value represents the mean ± SD (n = 3)*

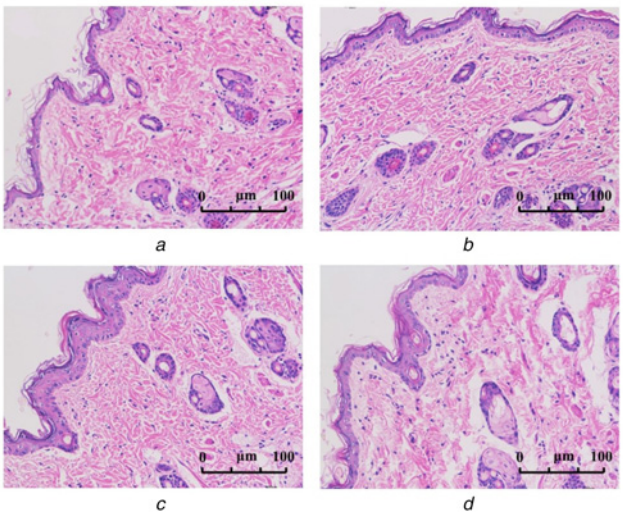

Fig. 6 *Histological section of rat's skin under haematoxylin–eosin staining after drug treatment of 24 h*
a Blank
b Pure DZ
c D-P-LNs
d D-P-Azone-LNs

As expected, the drug retention of D-P-Azone-LNs was the highest at the same concentration and time. Additional azone was believed to be the primary one, contributing to these results.

3.5. Skin irritation: The evaluation of the skin irritant effects of D-P-Azone-LNs is displayed in Fig. 6. Microscopic examination of the treated skin indicated no degeneration or necrosis and no inflammatory cell relative to those of blank skin (Fig. 6*a*). However, intercellular space dilatation was noted in the D-P-Azone-LN treatment group (Fig. 6*d*). In the study of Singh [20], augmented skin lipid fluidity and epidermal intercellular space dilatation were observed after azone was used. Specifically, azone caused fluidisation of the skin lipid of the stratum corneum but with no significant harmful effect.

4. Conclusion: Novel core–shell polymer–lipid nanoparticles containing the penetration enhancer azone was successfully prepared for the transdermal delivery of DZ. The fabricated nanoparticles were verified to improve skin permeation and drug retention. The nanoparticles were evaluated by *in vitro/in vivo* study. Compared with the traditionally designed D-P-LNs, the newly formulated D-P-Azone-LNs showed better percutaneous permeability and substantially enhanced drug accumulation in the skin. Furthermore, the modified formulation yielded no sign of skin irritation.

5 References

[1] Rimbach G.: 'Dietary isoflavones in the prevention of cardiovascular disease – a molecular perspective', *Food Chem. Toxicol.*, 2008, **46**, (4), pp. 1308–1319
[2] Lo F.H.: 'Studies on the anti-tumour activities of the soy isoflavone daidzein on murine neuroblastoma cells', *Biomed. Pharmacother.*, 2007, **61**, (9), pp. 591–595
[3] Huang Z.R.: 'In vitro and in vivo evaluation of topical delivery and potential dermal use of soy isoflavones genistein and daidzein', *Int. J. Pharm.*, 2008, **364**, (1), pp. 36–44
[4] Wang A.Z.: 'Chemorad nanoparticles: a novel multifunctional nanoparticle platform for targeted delivery of concurrent chemoradiation', *Nanomedicine*, 2010, **5**, (3), pp. 361–368
[5] Chan J.M.: 'PLGA-lecithin-PEG core–shell nanoparticles for controlled drug delivery', *Biomaterials*, 2009, **30**, (8), pp. 1627–1634
[6] Gershkovich P.: 'A review of the application of lipid-based systems in systemic, dermal/transdermal, and ocular drug delivery', *Crit. Rev. Ther. Drug Carrier Syst.*, 2008, **25**, (6), p. 545
[7] Bershteyn A.: 'Lipid-coated biodegradable particles as 'synthetic pathogens' for vaccine engineering'. IEEE Northeast Bioengineering Conf., Boston, MA, USA, 2009, pp. 1–2, doi: 10.1109/NEBC.2009.4967679
[8] Lee K.S.: 'Multicenter phase II trial of Genexol-PM, a Cremophor-free, polymeric micelle formulation of paclitaxel, in patients with metastatic breast cancer', *Breast Cancer Res. Treat.*, 2008, **108**, (2), pp. 241–250
[9] Shi J.: 'Differentially charged hollow core/shell lipid-polymer–lipid hybrid nanoparticles for small interfering RNA delivery', *Angew. Chem., Int. Ed. Engl.*, 2011, **50**, (31), pp. 7027–7031
[10] Moon J.: 'Antigen-displaying lipid-enveloped PLGA nanoparticles as delivery agents for a Plasmodium vivax malaria vaccine', *PloS One*, 2012, **7**, (2), p. e31472
[11] Harrison J.E.: 'The relative effect of azone and transcutol on permeant diffusivity and solubility in human stratum corneum', *Pharm. Res.*, 1996, **13**, (4), p. 542
[12] Mandal B.: 'Core–shell-type lipid-polymer hybrid nanoparticles as a drug delivery platform', *Nanomedicine*, 2013, **9**, (4), pp. 474–491
[13] Wang L.: 'Application of response surface methodology to optimize the production of antimicrobial metabolites by Micromonospora Y15', *Biotechnol. Biotec. Equip.*, 2017, **41**, (5), pp. 1016–1025
[14] Barani H.: 'A review on applications of liposomes in textile processing', *J. Liposome Res.*, 2008, **18**, (3), pp. 249–262
[15] Song J.: 'Daidzein-loaded nanostructured lipid carriers-PLGA nanofibers for transdermal delivery', *Int. J. Pharm.*, 2016, **501**, (1-2), pp. 245–252
[16] Li J.C.: 'Self-assembled cubic liquid crystalline nanoparticles for transdermal delivery of paeonol', *Med. Sci. Monit.*, 2015, **21**, pp. 3298–3310
[17] He P.: 'Phospholipid-assisted synthesis of size-controlled gold nanoparticles', *Mater. Res. Bull.*, 2007, **42**, (7), pp. 1310–1315
[18] Hadinoto K.: 'Lipid–polymer hybrid nanoparticles as a new generation therapeutic delivery platform: a review', *Eur. J. Pharm. Biopharm.*, 2013, **85**, (3), pp. 427–443
[19] Alexander A.: 'Approaches for breaking the barriers of drug permeation through transdermal drug delivery', *J. Control. Release*, 2012, **164**, (1), pp. 26–40
[20] Singh B.N.: 'Effects of ionization and penetration enhancers on the transdermal delivery of 5-flurouracil through excised human stratum corneum', *Int. J. Pharm.*, 2005, **298**, (1), pp. 98–107

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