THE ACTION MODES OF *LIPPIA SIDOIDES* (CHAM) ESSENTIAL OIL AS PENETRATION ENHANCERS ON SNAKE SKIN

M. B. Brito[1], Gabriela B. Barin[2], A. A. S. Araújo[1], D. P. de Sousa[1], S. C. H. Cavalcanti[1], Ana Amélia M. Lira[1] and Rogéria S. Nunes[1]*

[1]Departamento de Fisiologia, Universidade Federal de Sergipe, Av. Marechal Rondon, s/n, Cidade Universitária, 49100-000 São Cristóvão, Sergipe, Brazil
[2]Laboratório de Síntese e Aplicação de Materiais, Universidade Federal de Sergipe, Av. Marechal Rondon, s/n, Cidade Universitária, 49100-000, São Cristóvão, Sergipe, Brazil

The aim of this work was to study the effect of *Lippia sidoides* essential oil (LSEO) on stratum corneum lipids and the permeation of salicylic acid. DSC and FTIR spectroscopy were applied. LSEO 1% (v/v) significantly enhanced salicylic acid flux through snake skin. According to the DSC curves changes in the transition temperature of the lipids were observed indicating that LSEO can interact with stratum corneum. The IR spectrum of skin treated with LSEO showed a decrease in the peak intensity for CH_2 stretchings (2920–2850 cm^{-1}) however the peak positions did not alter suggesting the extraction of the lipids.

Keywords: *DSC, essential oil, FTIR, Lippia sidoides, penetration enhancers*

Introduction

Various studies have demonstrated that transdermal application may be a suitable alternative to the oral route in the administration of drugs with systemic activity [1]. However, the success of a transdermal drug delivery system depends on the ability of the drug penetration into the skin in sufficient amount to maintain a therapeutic level [2]. The primary barrier to transdermal diffusion is the stratum corneum (SC), the thin outermost layer of the skin, which is comprised of a regular array of protein-rich cells that are embedded in a multilamellar lipid domain [3].

The efficiency of the SC as a barrier for drug transport has led to the development of several strategies in order to overcome the low permeability of drugs through the skin and to increase the application of transdermal drug delivery [4–6]. A common approach is the use of chemical penetration enhancers which enhance the permeability of the SC. Ideally a penetration enhancer is a chemical compound, which reduces reversibly the barrier resistance of the SC without damaging the viable cells [2].

Recently, considerable attention is paid to the use of essential oils derived from natural products as penetration enhancers to improve drug permeation due to their high penetration enhancing abilities and low irritancy potential [7]. *Lippia sidoides* Cham (Verbenaceae) is very common in Northeast of Brazil. It is used as folk medicine to treat gastrointestinal disorders and presents antimicrobial [8] and larvicidal activity [9]. The major components in *L. sidoides* essential oil are thymol (59.65%), E-caryophyllene (10.60%) and p-cymene (9.08%) [10]. Terpenes such as thymol, menthol, carvone, and 1,8-cienole are reported to increase the permeation of various polar and non-polar drugs by interacting with the SC lipids or keratin, or by increasing the solubility of the drugs into SC lipids [3, 11–14]. These terpenes are structurally similar to those compounds which are present in *L. sidoides* essential oil.

Summarizing these considerations the aim of the present investigation was to evaluate the thermoanalytical profile and the effectiveness of *Lippia sidoides* essential oil to promote permeation of salicylic acid (SA).

Experimental

Materials

Salicylic acid (SA) was purchased from Henrifarma (São Paulo, Brazil). *Lippia sidoides* essential oil was donated by the Department of Agronomy at the Federal University of Sergipe and snake skin of the Boa constrictor species was donated by Butantã Institute. All other chemical reagents were of at least reagent grade and all materials were used as supplied.

* Author for correspondence: adriasa2001@yahoo.com.br

1388–6150/$20.00
© 2009 Akadémiai Kiadó, Budapest
Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

Preparation of stratum corneum samples

In order to study the effect of LSEO on SA permeation, the snake skin was cut into small circular discs with approximate diameters of 3 cm and hydrated over pH 7.4 phosphate buffer solution for 24 h before being submitted to permeation experiments. In order to study the effect of LSEO in the organization of SC, the skin was removed after the permeation experiments rinsed with distilled water then dried at ambient condition and kept under vacuum in desiccators to remove completely the free water. DSC and FTIR were used to characterize the samples. Untreated snake skin was used as control.

Methods

Differential scanning calorimetry (DSC)

DSC curves were recorded on a module supplied by TA Instruments in the 25–900°C temperature range. The heating rate was 10°C min^{-1}, dynamic nitrogen atmosphere (50 mL min^{-1}) and platinum crucibles with ~4.0 mg of samples were used. The DSC was calibrated with indium (m.p. 156.6°C; ΔH_{fus}= 28.54 J g^{-1}), conforming to ASTM standard [12].

Fourier transform infrared spectroscopy (FTIR)

The snake skin structure was evaluated and the infrared absorption data was obtained in the range of 4000–400 cm^{-1} using Bomen FTIR spectrophotometer, model MB-120, at room temperature.

In vitro permeation studies

Franz-type diffusion cells with a diffusional area of 1.726 cm^{-2} were used in the in vitro permeation studies. The previously hydrated stratum corneum was mounted between donor and receptor compartments. Two milliliters of drug solution (2 mg mL^{-1}) in propylene glycol: phosphate buffer pH 7.4 (PG:PB, 1:1) with or without 1% LSEO was placed on the skin surface in the donor compartment. The receptor compartment was filled with 14 mL of phosphate buffer pH 7.4. During the experiments the solution in the receptor phase was maintained at 37°C and stirred at 500 rpm. Aliquots of 1 mL were collected from the receptor side at designated time intervals (0.5, 1, 2, 3, 4, 6 and 8 h) and 1 mL of the phosphate buffer was added into the receptor side immediately after each sample collection [15]. The drug concentration in the collected samples was determined by HPLC equipment with an UV detector. The column was C18 (250×4.6 mm, 5 μm). Elution was carried out at room temperature with the mobile phase consisting of acetonitrile, Milli-Q water, and pH 2.5 phosphate buffer (35:25:40, v/v/v) at a flow rate of 1 mL min^{-1} when detection was carried out at 230 nm. The assay was linear (r^2=0.999) in the concentration range of 2–50 μg mL^{-1}, with the lowest detection limit at 0.05 μg mL^{-1} of SA. The method was validated in terms of accuracy and precision. The results are the mean and standard deviations of at least three determinations [16].

Data analysis

The cumulative amount of the drug that permeated through 1 cm^2 of stratum corneum was plotted as a function of time. The flux (*Jss*) was determined from the slope of the steady-state portion of the amount of the drug permeated (μg cm^{-2}), *vs.* time. The permeability coefficient (K_p) was calculated from the following equation [11]:

$$K_p = Jss/C_v$$

where C_v is the total donor concentration of the salicylic acid.

To compare the permeation enhancement capacities of the permeation enhancer, the enhancement ratio was calculated as follows [17]:

$$ER = K_p \text{ with enhancer}/K_p \text{ without enhancer}$$

The reported values are mean ratios from a minimum of three replicates.

Results and discussion

DSC studies

DSC curves of snake skin treated with pH 7.4 phosphate buffer (control), 1% LSEO in PG:PB, and PG:PB are illustrated in Fig. 1. Thermal analysis has been used for three decades to study the physicochemical properties of skin in order to find correlations between the endothermic transitions and the nature of the components of stratum corneum [18]. The DSC curve of PG-PB shows endothermic event between 25–93°C. In the temperatures at 36.7 and 57.8°C endothermic transitions due to the melting of lipids can be seen. Untreated skin samples showed three endothermic peaks at 33, 56 and 62°C, the latter two being attributed to phase transitions of the stratum corneum lipids, according to Leopold and Lippold, who investigated human stratum corneum [19].

DSC curve of LSEO in PG-PB shows two endothermic events at 25 and 112°C. In this temperature range endothermic transitions were observed at 36.7 and 49.2°C due to the melting of the lipids. Then, changes in the transition temperatures for the lipids were observed when PG-PB and LSEO in

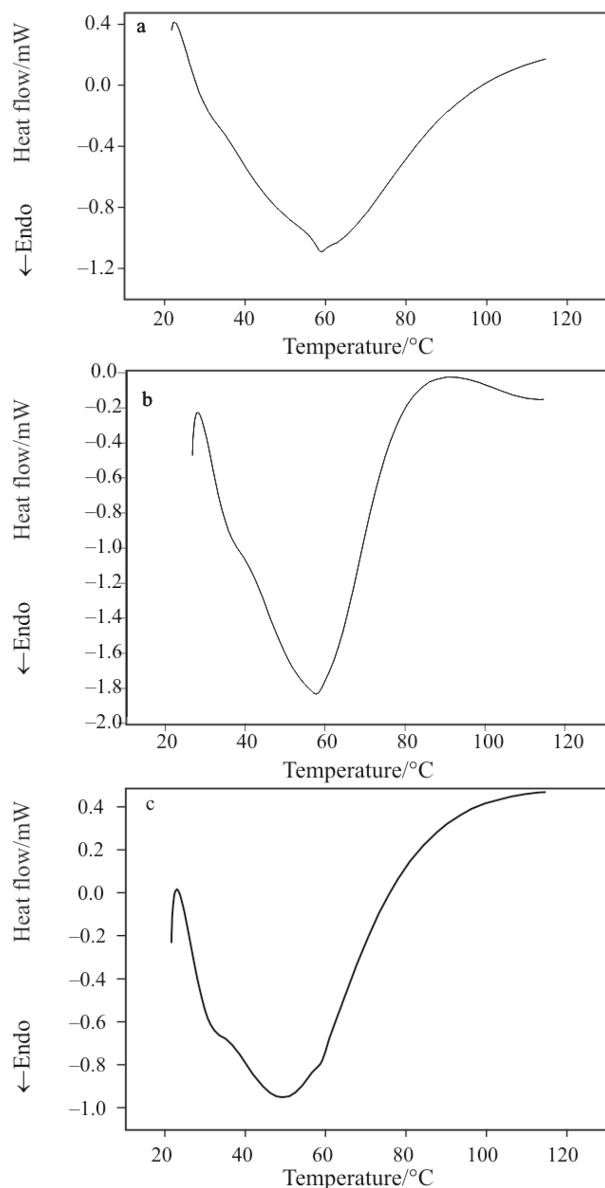

Fig. 1 DSC curve of a – SC treated with pH 7.4 phosphate buffer, b – pH 7.4 phosphate buffer and propylene glycol, c – pH 7.4 phosphate buffer, propylene glycol and 1% LSEO

PG-PB were compared indicating that LSEO can interact with stratum corneum lipids.

FTIR studies

The FTIR spectra of SC treated with pH 7.4 phosphate buffer, PG:PB, and PG:PB in LSEO1% (A) (4000–400 cm^{-1}) and (3000–2800 cm^{-1}) are given in Fig. 2.

Many of the IR spectra bands of SC can be attributed to the lipid or protein molecular vibrations. The hydrocarbon chains of lipids give asymmetric and symmetric vibrations of CH_2 at 2920 and 2850 cm^{-1}, respectively [20]. The heights and areas of these two peaks are proportional to the amount of lipids present in SC. So, any extraction of the lipids by enhancer results decrease in the peak height and area [17]. Some enhancers may also fluidize the SC lipids, which can be noted from the shift of CH_2 stretching peaks to a higher wavenumber, or the peak area reduction could be due to the extraction of lipids [18]. The IR spectra of SC treated with solutions of PG:PB with 1% LSEO showed a slight decrease in the height and area of the lipid peak compared to the IR spectra of SC without treatment (control) but did not show shift of these peaks to higher wavenumbers. This observation indicates that PG:PB with LSEO extracted the lipids, but did not fluidize them.

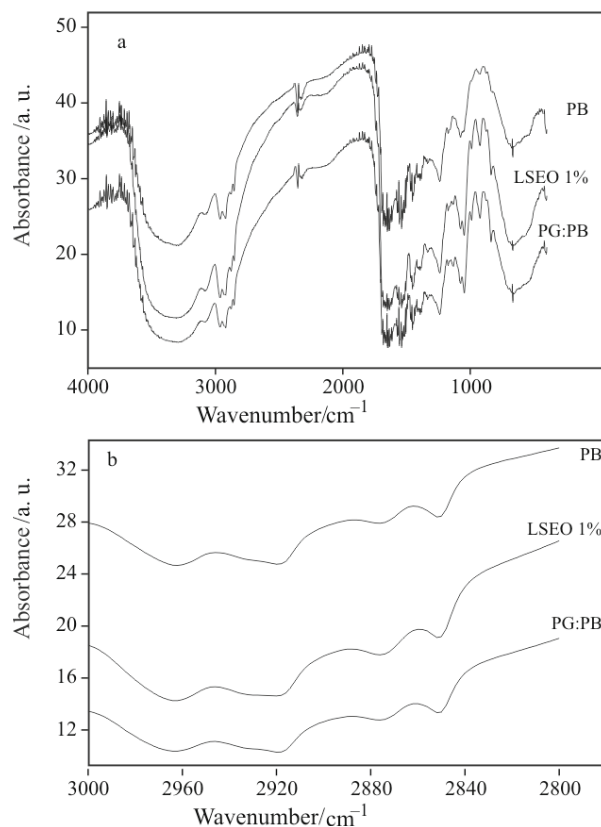

Fig. 2 FTIR spectra of SC treated with pH 7.4 phosphate buffer (PB), pH 7.4 phosphate buffer and propylene glycol (PG:PB) and PG:PB in 1% LSEO

In vitro permeation studies

The effect of LSEO on the *in vitro* permeation profiles of SA through snake skin is shown in Fig. 3. 1% LSEO in combination with PG:PB (1:1) increased the *in vitro* transport of SA as compared to the control. The steady-state flux, permeability coefficient and enhancement ratios of SA through the SC are summarized in Table 1.

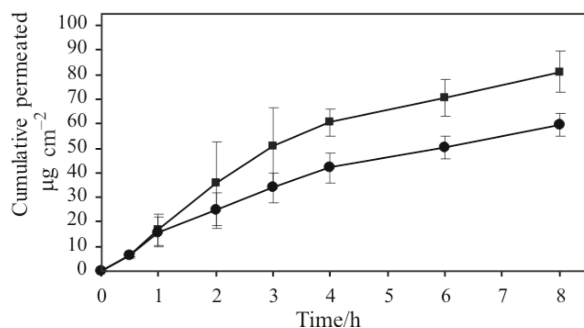

Fig. 3 Permeation profiles of SA across of snake skin (mean ±S.D., n=3–4). Key: • – without; ■ – with LSEO 1% (v/v)

Table 1 Permeation parameters of SA across snake skin (mean ±S.D., n=3–4)

Essential oil/ v/v%	Flux steady-state/µg cm^{-2}	K_p (10^{-3} cm)	ER_p/ k_{pa} k_{pb}^{-1}
0	4.906±1.63	2.45	1.0
1	7.089±2.07	3.55	1.45

The flux and permeability coefficients of SA in the presence of LSEO were significantly greater than the control. This argument can be further supported by the DSC findings of skin treated by LSEO causing a reduction in lipid transition temperature with more broadened phase transition of the SC lipids in comparison to that of the vehicle. That can indicate a lipid bilayer disruption in the SC caused by LSEO. FTIR also confirmed the permeation enhancer effect of LSEO. The IR spectra showed that skin treated with LSEO decreased slightly in height and area of the lipid peak suggesting an extraction of these skin lipids.

An enhancer increases the permeation of a drug through the epidermis, either by increasing the permeability coefficient of the drug in SC or decreasing the tortuous intercellular pathway in SC, or both [21, 22]. In this study, the essential oil probably disrupted the SC bilayer and extracted SC lipids.

PG may be used as solvent for transdermal formulations but it can also have permeation enhancing properties [23]. On the other hand, it had been shown previously by Takeuchi *et al.* [24] that fatty acids increase PG penetration into skin, too. Summarizing these findings, the mutual permeation enhancement of PG and LSEO is due firstly to the enhanced solubility of LSEO in and its penetration into the stratum corneum, both being caused by PG. Secondly it is due to enhanced PG permeability, which is caused by LSEO insertion between the intercellular lipids of the stratum corneum. Therefore, in this enhancer combination LSEO acts as an ‘enhancer’ and PG as a ‘co-solvent’, according to the terminology introduced by Barry [25].

Conclusions

The present study showed that the essential oil decreased the efficiency of the SC as a barrier to SA transport. Understanding the mechanism of action of LSEO at the molecular level is important for its judicious inclusion in transdermal formulations to enhance permeation of drugs across skin reaching therapeutically effective blood concentrations. A high flux value was obtained when LSEO was used as permeation enhancer. DSC and FTIR studies contributed to the elucidation of the mechanism of permeation enhancement by LSEO. The essential oil probably disrupted the SC bilayer and extracted SC lipids. Other mechanisms of action of LSEO as a permeation enhancer might exist and will be evaluated in future studies.

Acknowledgements

The authors acknowledge to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de Sergipe (FAPITEC-SE) for financial support.

References

1 R. C. Wester and H. I. Maibach. Clin. Pharmacokinet., 23 (1992) 235.
2 B. W. Barry, Drug Pharmaceut. Sci., 18 (1983) 480.
3 S. Gao and J. Singh, J. Controlled Release, 51 (1998) 193.
4 R. Schiller, A. P. Funke and C. Günther. J. Therm. Anal. Cal., 77 (2004) 497.
5 K. Bhasi, A. T. Riga and K. S. Alexander, J. Therm. Anal. Cal., 75 (2004) 269.
6 L. Kang, P. C. Ho and S. Y. Chan. J. Therm. Anal. Cal., 83 (2006) 27.
7 L. Zhang, J. Hu, L. Li, L. Gao, Q. Zhu, Z. Li, Z. Wang and D. Su, Biol. Pharm. Bull., 29 (2006) 1217.
8 R. S. Nunes, A. A. M. Lira, E. Ximenes, J. A. da Silva and D. P. de Santana, Rev. Bras. Farm., 86 (2005) 80.
9 W. J. Silva, G. A. A. Dória, R. T. Maria, R. S. Nunes, G. A. Carvalho, A. F. Blank, P. B. Alves, R. M. Marçal and S. C. H. Cavalcanti, Bioresour. Technol., 99 (2008) 3254.
10 L. M. A. Macambira, C. H. S. Andrade, A. A. Craveiro, F. J. A. Matos and R. Braz Filho, Acta Amazon., 18 (1986) 449.
11 H. K. Vaddi, P. C. Ho, Y. W. Chan and S. Y. Chan, J. Controlled Release, 10 (2002) 121.
12 A. Nokhodchi, K. Sharabiani, M. R. Rashidi and T. Ghafourian, Int. J. Pharm., 335 (2007) 97.
13 S. T. K. Narishetty and R. Panchagnula, J. Controlled Release, 102 (2005) 59.
14 M. A. Yamane, A. C. Williams and B. W. Barry, J. Pharm. Pharmacol., 47 (1994) 978.

15 The American Society for Testing and Materials, Annual Book of ASTM Standards, 14 (1993) 1582.
16 A. F. El-Kattan, C. S. Asbill, N. Kim and B. B. Michniaka, Int. J. Pharm., 215 (2001) 229.
17 A. C. Williams and B. W. Barry, Int. J. Pharm., 57 (1989) 7.
18 H. Tanojo, J. A. Bouwstra, H. E. Junginger and H. E. Boddé. J. Therm. Anal. Cal., 57 (1999) 313.
19 C. S. Leopold and B. C. Lippold, J. Pharm. Pharmacol., 47 (1995) 276.
20 R. N. A. H. Lewis and R. N. McEelhaney, Infrared Spectroscopy of Biomocules, Wiley-Liss, New York 1996, pp. 159–202.
21 A. K. Levang, K. Zhao, P. C. Ho and S.Y. Chan, Int. J. Pharm., 181 (1999) 255.
22 M. Almirall, J. Montana, E. Escribano, R. Obach and J. D. Berrozpe, Drug Res., 46 (1996) 676.
23 B. Bendas, U. Schmalfuß and R. Neubert, Int. J. Pharm., 116 (1995) 19.
24 H.-J. Oh, Y.-K. Oh and C.-K. Kim, Int. J. Pharm., 212 (2001) 63.
25 B. W. Barry, J. Contolled Release, 15 (1991) 237.

ICTAC 2008

DOI: 10.1007/s10973-008-9669-8