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Measurement of Hansen Solubility Parameters of third-degree burn eschar

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ABSTRACT

Topical drug therapy is one of the most effective approaches in third-degree burn wound treatments. To optimize and enhance drug permeation through burn eschar, we need to characterize this barrier, most importantly, its affinity to drugs; the subject of this investigation.

Hansen Solubility Parameters (HSP), as polarity and affinity scale, were measured here for human third-degree burn eschar through uptake studies using 19 solvents at 25 °C and 32 °C and two hydration levels by gravimetric method combined with thermal analysis and Karl Fischer titration. HSP parameters of dispersion (δ_D), bipolar (δ_P), and hydrogen bonding (δ_H) were calculated by HSPiP software.

Results showed δ_D, δ_P, and δ_H of 17.0, 12.5, 14.6 and 16.8, 12.4, 14.4 at 25 and 32 °C respectively for normally-hydrated samples. Full hydration increased HSP values to 17.2, 12.9, 15.3 (25 °C) and 17.1, 12.8, 15.1 (32 °C). Good correlations between solvents uptakes and HSP values were observed for all parameters; higher for δ_P. Increased temperature decreased them with more changes in δ_H. Relative Energy Differences (RED) were calculated and shown to be a good parameter for predicting drug-eschar affinity.

The obtained information is useful for drug selection and carrier design in drug delivery through burn eschar.

1. Introduction

Burn is one of the most common injuries of the skin and can disturb the skin's functions at a widespread level. After burns, the skin barrier properties are changed [1], and therefore, understanding the characteristics of this new barrier is important for effective management of burns wound treatment, including drug therapy.

Third-degree or full-thickness burns, the subject of the present investigation, cause destruction of the epidermis and

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dermis, and a tissue rich in denatured proteins and decomposed lipids called "eschar" is formed afterward [2]. This tissue is a very good environment for microbial colonization and proliferation and it can potentially cause morbidity and mortality in patients with burn [2,3].

There are two strategies to control burn wound infection: debridement surgery and systemic/topical antibiotic drug therapy [3]. Debridement surgery sometimes is not possible because the resources, staffing, and general condition of the individual burn unit will strongly influence this approach [3]. Furthermore, prompt burn wound excision and closure are feasible only for patients with less than about 40% of total burn surface area [3]. Systemic delivery of antibiotics needs a longer duration of treatment because of poor penetration into necrotic wounded tissue [3]. Therefore topical antibiotic therapy is an important strategy to control the growth of microorganisms and bacteria in the eschar tissue and accelerate the healing process [3].

It has been suggested that an appropriate topical antibiotics application controls many of the septic problems [4]. Despite the use of topical antibiotics, previous studies have shown that some antibiotics could not penetrate the burn eschar in therapeutic amounts [5,6]. To optimize drug delivery through this barrier, its barrier properties should be understood.

There are not much data available on required properties for good eschar penetration. Our group (Ghaffari et al. [6]) studied the permeation of lipophilic diazepam and hydrophilic clindamycin phosphate and showed that these drugs show permeability coefficients of 17.4×10^{-3} cm/h and 13.1×10^{-3} cm/h, respectively. We also showed that ethanol can decrease the permeation of these drugs due to its drying effect [6]. In another study, we showed that permeation enhancers such as glycine, water, saline, hexane: ethanol, and sodium lauryl sulfate can increase permeation of lipophilic nitroglycerin through burn eschar [7]. This study also showed that the permeation of sodium sulfadiazine through third-degree burn eschar can be increased by water, glycerin, ethyl acetate: ethanol, and hexane: ethanol [7]. Our group also has shown that liposomal formulation can decrease the permeation of clindamycin through burn eschar [8].

Different factors affect permeation of drugs through biological barriers including physicochemical properties of drug and barrier such as drug affinity to the membrane, drug interactions with the membrane, diffusivity and molecular weight [9]. For optimized delivery, different properties are required. For example, the ideal drug absorption through normal skin should have a low molecular weight of <600 Da [9], low melting point (<200 °C), and an elevated, but balanced, lipophilicity; logP of 1–3 [9]. Furthermore, it should have solubility in water and oils to achieve a high concentration gradient and increase permeation across the skin [9]. Such properties required for normal skin cannot be extrapolated to burned eschar, because the structure of eschar is significantly different from normal skin [10].

Among these parameters, eschar drug affinity is very important for proper drug and vehicle selection. This property that is related to the polarities of drug and membrane is usually expressed by partition coefficient. Alternatively, such a concept can be better defined and characterized by the Hansen Solubility Parameter (HSP). Solubility parameter was originally proposed by Hildebrand [11] and was optimized by Hansen after recognizing its shortcomings [12]. HSP is based on cohesive energy that consists of three different parameters of dispersion (atomic) forces (δ_D), permanent molecular bipolar forces (δ_P) and, hydrogen bonding (δ_H), called Hansen Solubility Parameters (HSPs) [12]. The sum of the squares of these three parameters gives the square of the total (Hildebrand) solubility parameter (δ_T) or total cohesion energy (Eq. (1)) [12]:

$$\delta^2_T = \delta^2_D + \delta^2_P + \delta^2_H \quad (1)$$

The degree of affinity of compounds to each other would be known by comparing the HSP values of the systems. If the HSPs of two systems are the same or close, they can be dissolved in each other [12]; this is usually expressed as "like dissolves like". Quantitatively, the correlation between the HSP values of two systems is measured as R_a (HSP distance) (Eq. (2)), developed by Skaarup and Hansen [13]. Similar systems show smaller R_a.

$$(R_a)^2 = 4(\delta_{D2} - \delta_{D1})^2 + (\delta_{P2} - \delta_{P1})^2 + (\delta_{H2} - \delta_{H1})^2 \quad (2)$$

The HSP parameter initially began as an attempt to understand the solubility of polymers in solvents and solvent mixtures. However, this concept can be used for solubility or permeability of pigments, gloves, nanoparticles, DNA, human skin, and so on [12–15].

There are no experimentally measured data available for eschar's HSP. However, there are three HSP values available for human skin. The first attempt to determine the HSP of human skin that is reported by Hansen [12], involves measurement of psoriatic scales swelling upon contact with various solvents [15]. The other HSP value of normal human epidermis was evaluated by Hansen [12] using permeation data from Ursin et al. [16]. Furthermore, Abbott [17] has reported another HSP value for human skin, which is an estimated value. Finally, the last value that is obtained experimentally is for the human stratum corneum (SC), the main barrier for transdermal drug delivery, measured by our group [18]. The data are discussed later. The present study aims to measure HSP parameters for third-degree burn eschar for the first time.

2. Materials and methods

2.1. Chemicals

Methylene dichloride, methanol, ethanol, ethyl acetate, acetone, chloroform, 1-butanol, di butyl amine, 1-propanol, 2-propanol, polyethylene glycol, and tetrahydrofuran (THF) were obtained from Merck (Darmstadt, Germany). Dimethyl sulfoxide (DMSO) and diethyl ether were obtained from Chem LAB (Zedelgem, Belgium). Acetonitrile and 1-pentanol were obtained from Carlo Erba (Milano, Italy) and Fluka Chemie AG (Buchs, Switzerland), respectively.

2.2. Eschar samples

Eschar Samples were obtained from "Shahid Mottahari" Burn Injuries Hospital, Tehran, Iran. These samples were collected from 13 burn patients (8 men and 5 women; 33 ± 18 years, mean ± SD) after normal debridement of necrotic tissue. The cause of

burning in all patients was flame. Eschar samples were from abdomen, thighs and arms of the patients and their thickness was measured to be 1.5 ± 0.5 mm (mean ± SD, n = 13). Statistical analysis using Shapiro–Wilk test for burn eschar samples showed that the thickness distribution of eschar samples was normal (P = 0.05). Our studies using clindamycin phosphate (hydrophilic) and diazepam (lipophilic) have revealed that differences in eschar barrier performance among lower limb, upper limb and trunk of all genders are negligible under the age of 60 years.

The present investigation is performed under the supervision of Shahid Beheshti University of Medical Science (SBMU) Ethics Committee and all protocols were reviewed and approved by this committee.

Obtained samples were first soaked in distilled water for 2 h to help cleaning the attached debris. The samples were then cleaned and placed on an aluminum foil in laboratory condition for about 5 h. The samples were then cut into circles with a diameter of approximately 1.5 cm, placed in a nylon bag, and stored at −20 °C until use. Previous studies have shown that the barrier properties of eschar do not change during the mentioned storage conditions [2].

During each experiment, the frozen samples were put at ambient laboratory conditions (about 24–26 °C and 30 ± 2 RH) to get defrosted and equilibrated with the ambient condition. In this study, eschar samples were used at two hydration levels of normally-hydrated, which are the samples as prepared above, and, fully-hydrated level, which was obtained after submerging eschar samples in water for 24 h [10].

2.3. Eschar HSP measurement

Hansen solubility parameters (HSP) of the eschar were measured by gravimetric method. For this purpose, eschar samples were submerged in selected solvents with different HSP values for 12 h (Table 1). The solvent uptakes% was then measured and HSP values were calculated, as described below.

2.4. Solvent selection

As there is no data available for burn eschar, the solvent selection was based on HSP data available for human skin [12] and the stratum corneum (SC) [18] that are 17.6, 12.5, 11.0 and 16.5, 12, 7.7 for δ_D, δ_P, and δ_H, respectively. 19 common solvents/mixtures were selected with known HSP values of close, higher, and lower than the mentioned HSP parameters (Table 1). To obtain HSP of mixtures, Eq. (3) was used where δ is any of the Hansen solubility parameters and *f* is the weight fraction of solvent in the mixture [12]:

$$\delta_{mixture} = \delta_1 f_1 + \delta_2 f_2 + \ldots + \delta_n f_n \quad (3)$$

Solvents were selected to give the range of 14.5–18.4 for δ_D, 3.1–18 for δ_P, and 4.2–22.3 for δ_H. To reduce the effects of diffusional differences in uptake studies, molar volumes of all solvents were chosen to be close and as low as possible. The molar volumes of selected solvents for the present study were 40.6–170.7 cm^3/mol (Table 1).

2.5. Eschar water content measurement

The water contents of eschar samples were measured by thermogravimetric analysis (TGA) apparatus (TGA50, Shimadzu Company, Japan). Eschar samples with weights of approximately 5 mg were placed in aluminum pans of TGA. Samples were heated to 200 °C at 10 °C/min under a nitrogen atmosphere. The weight changes were recorded considering that weight changes are due to water evaporation. The hydration level was then calculated by Eq. (4) using eschar initial sample weight (*ISW*) and final sample weight (*FSW*). Higher temperatures were not used as there is a risk of eschar

Table 1 – Solvents used to obtain HSP of eschar in the present investigation. HSP and molar volume data are obtained from the HSP user's Handbook [12] and HSPiP software.

Solvent	Molar volume (cm^3/mol)	HSPs ($MPa^{1/2}$)		
		δ_D	δ_P	δ_H
DMSO	71.3	18.4	16.4	11.3
1-Butanol	91.5	15.9	6.3	15.2
Ethanol	58.6	15.8	8.8	19.4
1-Propanol	75.1	16.0	6.8	17.4
Acetonitrile	52.9	15.3	18.0	6.1
Di butylamine	170.7	15.7	2.6	4.2
Methylene dichloride	64.4	17.4	8.8	8.6
Methanol	40.6	14.7	12.3	22.3
Acetone	73.8	15.5	10.4	7.0
Chloroform	80.5	17.8	3.1	5.7
Tetrahydrofuran	81.9	16.7	4.9	5.5
Di ethyl ether	104.7	14.5	2.9	4.6
Ethyl acetate	98.6	15.8	5.3	7.2
DMSO:1-butanol (60:40)	71.3:91.5	17.4	12.1	12.4
DMSO:1-butanol (50:50)	71.3:91.5	17.2	11.3	13.3
DMSO:1-butanol (70:30)	71.3:91.5	17.7	13.2	11.9
1-Pentanol	108.6	15.9	5.9	13.9
2-Propanol	76.9	15.8	6.1	16.4
Propylene glycol	73.7	16.8	10.4	21.3

decomposition and weight changes due to the evaporation of material other than water.

$$\text{Hydration (\%)} = (ISW - FSW)/FSW \times 100 \quad (4)$$

2.6. Uptake studies

Gravimetric method was used to measure the uptake of solvents by eschar. Eschar samples were weighted and then placed in a 10 ml vial containing 2 ml of each solvent, separately. The vials were then left for 12 h at 25 °C to allow solvent uptake. After this time, samples were weighted again and solvent uptakes% were calculated by Eq. (5) using eschar initial weight (*IW*) and final weight (*FW*). The experiments were repeated 6 times (eschar samples of 6 patients) for each solvent.

$$\text{Uptake (\%)} = (FW - IW)/IW \times 100 \quad (5)$$

TGA was used to ensure that if the lipids of the eschar were extracted by solvents. For this purpose, the solvents were analyzed by TGA before and after contact with eschar (uptake studies) for any residual material.

Karl Fischer apparatus (type DL77, Mettler-Toledo Co, Switzerland) was used to measure water extraction from eschar by solvents. The solvents were placed in Karl Fischer apparatus to obtain any possible amount of water extracted from eschar by solvents.

2.7. HSP calculation

HSPiP software (4th Edition 4.1.7.0) was used to calculate the HSP parameters of eschar here. To obtain these parameters, the solvents should be ranked according to the extent of their uptakes by eschar. Two approaches to solvents classification are usually used: the first approach that was employed here is to rank the solvents in graded groups of up to 6 based on their uptakes. Ranking begins with group 1, which includes good solvents with highest (best) uptakes, and ends in the last group (group 6) that shows the lowest (worst) uptakes. Other groups are located between these two groups, correspondingly. In the present investigation, we used a one-way ANOVA analysis of variance followed by the least significant difference (LSD) post hoc test to determine solvents with similar uptakes. This method gave us four groups as described later. In the second approach, solvents are divided into two groups of good solvents (rank 1) and bad ones (rank 0).

The uptake scores then are input into the HSPiP software to get HSP values. This software plots a 3-dimensional sphere based on solvent scores, as shown later in the results. Each dimension is assigned to one parameter of HSP, i.e. δ_D, δ_P, or δ_H. Good solvents are located in the sphere and other ones are placed outside the sphere. The center of the sphere represents the three-dimensional Hansen Solubility Parameters of eschar (δ_D, δ_P, and δ_H), as described and shown later.

2.8. Influence of hydration and temperature on eschar's HSP

To evaluate the effect of hydration level on solvent uptakes by eschar, fully-hydrated eschar samples were also used here. To obtain fully-hydrated samples, normally-hydrated samples were submerged in water for 24 h [10]. Water uptakes by the eschar samples were measured at 6, 12, 18, and 24 h hydration using TGA as explained earlier.

To investigate the effect of temperature on solvent uptakes, HSP values of normally and fully-hydrated eschar samples were studied at ambient temperature and also 32 °C, the surface temperature of the skin.

2.9. Influence of eschar thickness on HSP

Over the years of studying on ecshar permeation, we have received eschar samples ranging in thickness from below 1 to slightly above 2 mm. Drug permeation through eschar is passive and according to Fick's law, drug permeation flux through this barrier is expected to change by thickness. This has been shown in our previous studies for permeation of clindamycin phosphate through thick and thin eschar samples [19]. For uptake, and therefore HSP studies, the situation is different as uptake is a polarity matter and is not affected by thickness as far as the structure remains the same throughout the eschar and enough time is given for uptake. To confirm this, we studied the uptakes of solvents by thin (0.83 ± 0.06 mm) and thick (1.53 ± 0.15 mm) eschar samples (data are mean ± SD, n = 3) at 25 and 32 °C using normally-hydrated eschars and calculated HSP parameters of thin and thick samples.

3. Results

3.1. Water uptake by eschar

Eschar water content after 6, 12, 18, and 24 h contact with water was measured at 25 °C and 32 °C. The samples were subjected to thermogravimetric analysis (TGA) (Fig. 1) and weight loss was used to determine hydration level.

As shown in Fig. 1, only one weight loss peak is observed in TGA studies, and this was attributed to water loss. The wide range of the thermogram also indicates the gradual evaporation of water from the inner layers of the eschar. Similar patterns were obtained at 32 °C for normally and fully-hydrated eschar samples.

Normally-hydrated eschar showed a moisture content of 14.8% ± 3.8 (w/w) at ambient temperature. Fully-hydrated eschar (hydration period of 24 h) showed a moisture content of 139.3% ± 12.8 (w/w) and 125.7% ± 13.7 (w/w) at 25 and 32 °C, respectively. Results (Fig. 2) also showed that, during hydration, there was a rapid increase in water content up to 6 h followed by a plateau and there was no significant increase in the eschar water content up to 24 h. These data show that 24 h is enough for obtaining fully-hydrated eschar samples. Although it seems that the hydration level at 32 °C tends to be lower than that of ambient temperature (Fig. 2), statistical analysis shows that, except for 6 h of treatment, there is no statistically significant difference ($P < 0.05$) in water content of eschar samples between ambient temperature and 32 °C.

In a study reported by Shah et al. [20], similar results were obtained for hydration over 48 h for rat stratum corneum at 37 °C, which indicates rapid hydration in the first 6 h similar to the hydration pattern of eschar. It was also shown that there was

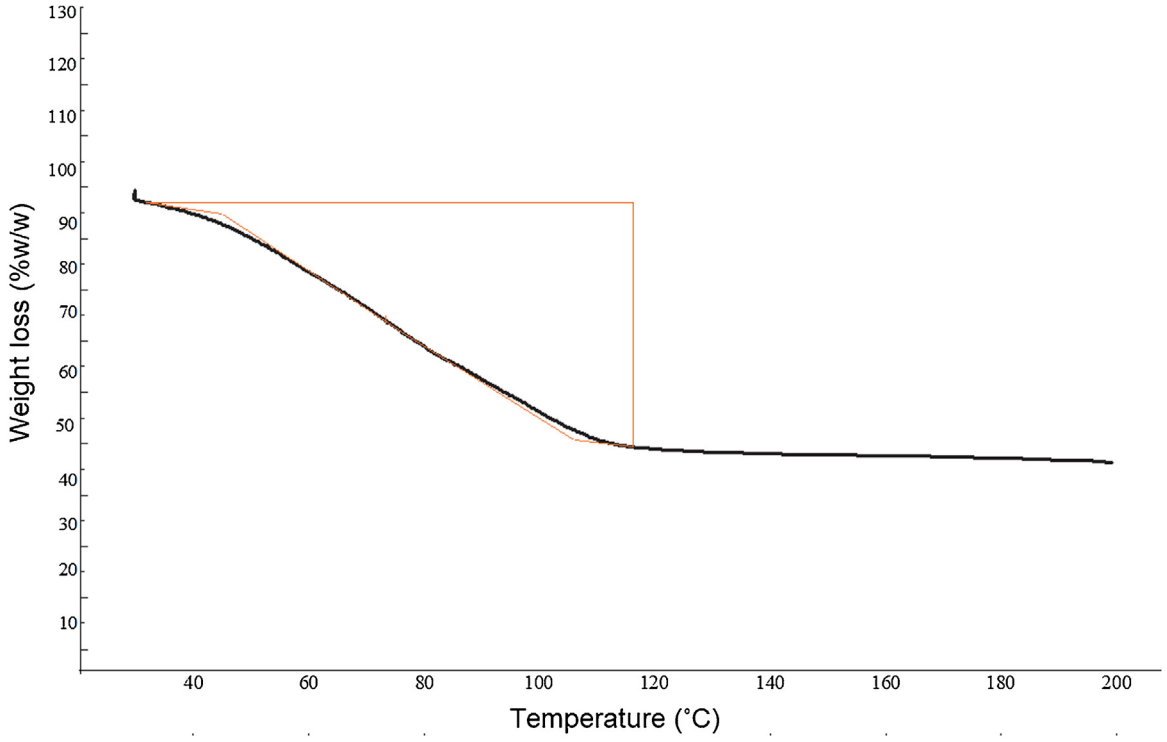

Fig. 1 – Sample TGA thermogram of eschar heated from ambient temperature to 200 °C obtained for fully-hydrated eschar samples hydrated at 25 °C.

an increase in the bound water content of the skin upon hydration. Makhmalzadeh et al. [10] investigated the effect of hydration on barrier properties of human third-degree burn eschar using 24 h pretreatment to achieve fully-hydrated samples.

3.2. *Water extraction by solvents*

Evaluation of water extraction from fully-hydrated eschar by solvents using Karl Fischer titration method showed that almost all solvents were able to extract water from fully-

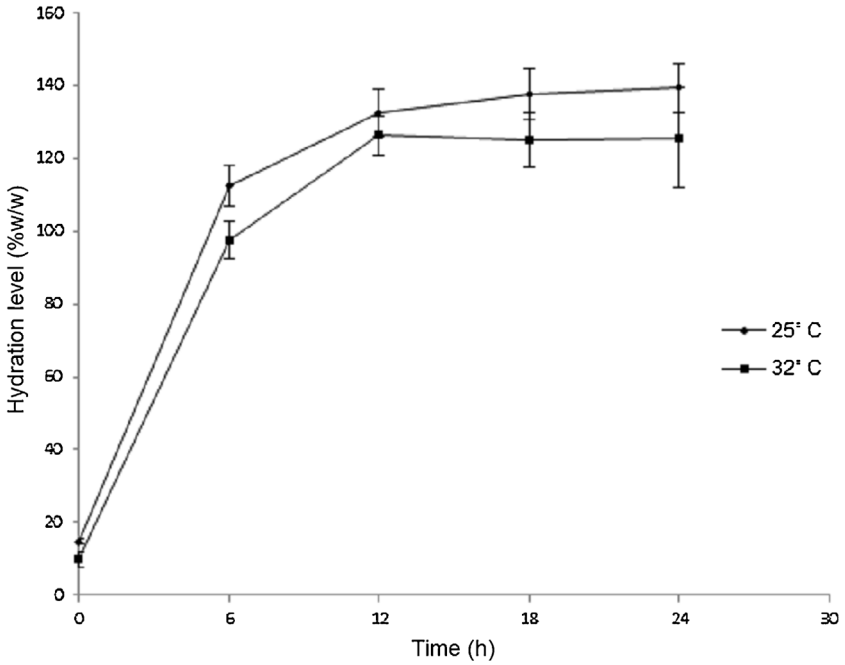

Fig. 2 – Hydration level of fully-hydrated eschar samples after 6, 12, 18, and 24 h treatment with water at 25 and 32 °C, data are mean ± SD, n = 3.

hydrated eschar, and indicated that polar and water-miscible solvents such as ethanol, acetone, DMSO (and its mixtures with 1-butanol), propylene glycol, 1-propanol, and 2-propanol were able to extract more water from the eschar than other solvents at both 25 and 32 °C (Table 2). These data were used for correction of uptake results and HSP calculation using Eq. (5), as explained earlier. However, no water was detected in solvents that had been in contact with normally-hydrated eschar samples. This might show that the water in normally-hydrated eschar samples is either not easily available for extraction (i.e. bound water) or the amount of extracted water is lower than the detection limit of the apparatus (less than 2 mg/mL). Considering these and the initial weight of eschar samples (250–550 mg), these results show that the extracted water from normally-hydrated samples (if any) is negligible in comparison to eschar/solvent weight and the data can be considered completely reliable.

3.3. *Solvents uptakes*

Tables 3 and 4 show the results of solvents uptakes by eschar at 25 °C and 32 °C for normally and fully-hydrated eschar samples. These data show that polar solvents such as DMSO and its mixtures with 1-butanol show more uptake than nonpolar solvents such as diethyl ether and THF. A previous study of the current group on the intact skin stratum corneum also have shown similar patterns [18].

Comparison of solvent uptakes between 25 and 32 °C showed that uptakes were increased at 32 °C in comparison to 25 °C for almost all solvents for both hydration levels (Tables 3 and 4). This increase was significant for polar solvents such as ethanol, DMSO and DMSO/1-butanol mixtures for normally-hydrated eschar ($P < 0.05$) (Table 3). In the case of fully-hydrated eschar, DMSO (and its 60:40 & 50:50 mixtures with 1-butanol), ethanol, propylene glycol, 2-propanol and acetonitrile showed significant increase (Table 4). This should be due to the higher solubility of solvents in eschar samples at the higher temperature, a well-known thermodynamic phenomenon.

Comparison of solvent uptakes by normally-hydrated and fully-hydrated eschar samples showed that polar solvents such as ethanol, propylene glycol, 1-propanol, 2-propanol, acetone and 70:30 mixture of DMSO:1-butanol at 25 °C and propylene glycol, acetonitrile, 1-propanol, 2-propanol and acetone at 32 °C, showed significantly higher uptake by fully-hydrated eschar than normally-hydrated eschar samples (one-way ANOVA, post hoc LSD, $P < 0.05$) (Tables 3 and 4), that is reasonable considering the higher hydrophilic environment of fully-hydrated samples. Besides this, the previous finding for the effect of hydration on eschar barrier function indicated that hydration level affects the permeability of eschar [10], so fully-hydrated eschar is more permeable than normally and semi-hydrated eschar due to opening the compact structure of tissue [10]. This might also have helped higher uptakes of polar solvents, observed here. Finally, results revealed that nonpolar solvent uptakes do not change much after hydration ($P < 0.05$, Tables 3 and 4).

Table 2 – Water extraction by solvents from fully-hydrated eschar samples at 25 and 32 °C as measured by Karl Fischer titration. Data are mean ± SD, n = 6.

Solvent	Water extraction by solvent (% w/w)	
	25 °C	32 °C
DMSO	12.2 ± 4.2	10.2 ± 2.9
DMSO:1-butanol (70:30)	10.5 ± 2.3	11.6 ± 1.6
DMSO:1-butanol (60:40)	10.9 ± 1.7	12.5 ± 2.1
DMSO:1-butanol (50:50)	9.7 ± 1.7	10.7 ± 2.2
Ethanol	11.7 ± 3.4	14.5 ± 4.9
Propylene glycol	10.4 ± 1.5	10.9 ± 1.2
1-Propanol	10.2 ± 0.9	11.3 ± 1.6
2-Propanol	10.1 ± 1.2	10.7 ± 2.6
Methanol	8.4 ± 4.2	5.7 ± 1.6
1-Butanol	6.7 ± 1.6	5.1 ± 3.3
Acetonitrile	9.6 ± 3.2	6.1 ± 1.1
Tetrahydrofuran	5.8 ± 1.5	6.5 ± 1.3
Chloroform	1.2 ± 0.3	1.1 ± 0.8
Ethyl acetate	4.1 ± 3.8	2.9 ± 0.6
Di butylamine	5.2 ± 0.8	4.2 ± 2.1
Methylene dichloride	1.2 ± 0.7	2.5 ± 1.4
1-Pentanol	4.3 ± 1.1	4.9 ± 2.1
Acetone	10.8 ± 0.6	11.2 ± 2.8
Di ethyl ether	2.3 ± 1.5	1.2 ± 0.1

3.4. *Calculation of HSP parameters of normally and fully-hydrated eschar*

One-way ANOVA and post hoc LSD on the extent of solvents uptake by normally-hydrated eschar at 25 °C and 32 °C showed that solvents can be grouped in descending order of uptake as follows: (i) DMSO, DMSO:1-butanol (70:30), DMSO:1-butanol (60:40), DMSO:1-butanol (50:50) and ethanol; (ii) propylene glycol, 2-propanol and 1-propanol; (iii) 1-butanol, methanol and acetonitrile; (iv) THF, ethyl acetate, dibutyl amine, methylene dichloride, 1-pentanol, chloroform, acetone and diethyl ether (Table 3). Applying the same statistical analysis for fully-hydrated eschar samples showed a minor difference and accordingly, propylene glycol was placed in a group (i) and acetone was placed in a group (iii) for fully-hydrated samples at both temperatures. The classification for the rest of the solvents was as those of normally-hydrated samples (Table 4).

The solvents and their rankings were then put into the HSPiP software to obtain the HSP sphere (Fig. 3) and calculate the HSP parameters and radius of the interaction sphere (R_0) (Table 5). Fit 1 was obtained for all calculations that show that there is a perfect separation of solvents based on their uptakes by eschar samples at all used conditions.

3.5. *Effect of eschar thickness on Hansen solubility parameters*

Investigation of the effect of eschar thickness on solvents uptakes and HSP showed that there is no significant difference ($P > 0.05$) in uptake% of solvents between thin and thick eschar samples for both 25 and 32 °C (see Fig. 4 as an example). There were also no differences in classification of solvents according to their uptakes% between thick and thin samples. Accordingly, calculation of HSP values showed HSP values of $\delta_D = 17.0$, $\delta_P = 12.5$ and $\delta_H = 14.6$ for both thin and thick samples at 25 °C and $\delta_D = 16.8$, $\delta_P = 12.5$ and $\delta_H = 14.4$ for both thin and thick samples at 32 °C.

Table 3 – Solvents uptakes (%w/w) into normally-hydrated eschar samples at 25 and 32 °C with their rank according to HSPiP software solvent classification. Data are mean ± SD, n = 6.

Solvent	25 °C		32 °C		P-value[a]
	Uptake	Rank	Uptake	Rank	
DMSO	46.3 ± 3.7	1	54.7 ± 3.7	1	0.000
DMSO:1-butanol (70:30)	42.6 ± 4.6	1	52.8 ± 3.6	1	0.000
DMSO:1-butanol (60:40)	43.3 ± 1.5	1	50.6 ± 4.5	1	0.001
DMSO:1-butanol (50:50)	44.7 ± 3.5	1	50.3 ± 4.8	1	0.016
Ethanol	41.7 ± 2.5	1	50.2 ± 3.7	1	0.000
Propylene glycol	25.3 ± 3.8	2	29.2 ± 5.9	2	0.076
1-Propanol	24.7 ± 1.5	2	27.8 ± 3.5	2	0.121
2-Propanol	25.3 ± 2.5	2	27.6 ± 2.7	2	0.299
Methanol	16.5 ± 0.7	3	17.5 ± 1.6	3	0.701
1-butanol	15.7 ± 2.5	3	15.5 ± 2.8	3	0.951
Acetonitrile	14.9 ± 1.7	3	16.3 ± 1.7	3	0.215
Tetrahydrofuran	6.3 ± 0.9	4	8.6 ± 2.1	4	0.292
Chloroform	6.1 ± 1.7	4	6.4 ± 0.7	4	0.878
Ethyl acetate	5.7 ± 1.9	4	8.3 ± 0.8	4	0.234
Di butylamine	4.9 ± 2.3	4	5.9 ± 0.7	4	0.646
Methylene dichloride	4.8 ± 1.2	4	7.5 ± 1.6	4	0.228
1-Pentanol	4.7 ± 1.8	4	5.9 ± 1.2	4	0.550
Acetone	4.3 ± 2.7	4	5.6 ± 1.8	4	0.530
Di ethyl ether	3.3 ± 1.1	4	5.1 ± 1.2	4	0.400

[a] Comparison between 25 and 32 °C using one-way ANOVA followed by LSD post hoc test.

Table 4 – Solvents uptakes (% w/w) into fully-hydrated eschar samples at 25 and 32 °C with their rank according to HSPiP software solvent classification. Data are mean ± SD, n = 6.

Solvent	25 °C		32 °C		P-value[a]
	Uptake	Rank	Uptake	Rank	
DMSO	46.5 ± 5.5	1	55.1 ± 8.7	1	0.015
DMSO:1-butanol (70:30)	49.3 ± 2.2	1	55.8 ± 10.1	1	0.060
DMSO:1-butanol (60:40)	44.8 ± 7.7	1	55.2 ± 2.7	1	0.003
DMSO:1-butanol (50:50)	47.1 ± 4.6	1	52.2 ± 7.5	1	0.049
Ethanol	46.8 ± 3.4	1	55.6 ± 4.2	1	0.014
Propylene glycol	45.7 ± 4.8	1	53.5 ± 7.7	1	0.025
1-Propanol	33.7 ± 3.4	2	38.5 ± 3.6	2	0.357
2-Propanol	30.9 ± 4.3	2	38.4 ± 3.7	2	0.033
Methanol	18.1 ± 2.8	3	23.6 ± 3.8	3	0.111
1-Butanol	17.7 ± 2.2	3	19.5 ± 2.2	3	0.600
Acetonitrile	17.3 ± 1.8	3	26.6 ± 1.7	3	0.008
Tetrahydrofuran	7.8 ± 1.2	4	8.9 ± 1.5	4	0.734
Chloroform	5.7 ± 2.3	4	7.5 ± 4.4	4	0.586
Ethyl acetate	6.2 ± 2.7	4	7.4 ± 1.4	4	0.719
Di butylamine	4.4 ± 0.6	4	3.4 ± 2.2	4	0.779
Methylene dichloride	7.5 ± 1.6	4	8.1 ± 2.1	4	0.861
1-Pentanol	3.3 ± 0.8	4	6.8 ± 3.3	4	0.300
Acetone	15.1 ± 2.6	3	19 ± 4.1	3	0.249
Di ethyl ether	2.6 ± 0.9	4	4.7 ± 2.7	4	0.528

[a] Comparison between 25 and 32 °C using one-way ANOVA followed by LSD post hoc test.

4. Discussion

4.1. *Effect of temperature on eschar Hansen solubility parameters*

According to Hansen user's handbook [12], higher temperature means a general increase in the rate of solubility/diffusion/permeation, as well as larger solubility parameter spheres. Therefore, we expect that δ_D, δ_P, and δ_H parameters decrease with increasing temperature [12]. The relationship between Hansen solubility parameters of solvents and temperature (T) is given by Eqs. (6)–(8) [12]:

$$d\delta_D/dT = -1.25\alpha\ \delta_D \quad (6)$$

$$d\delta_P/dT = -0.5\alpha\ \delta_P \quad (7)$$

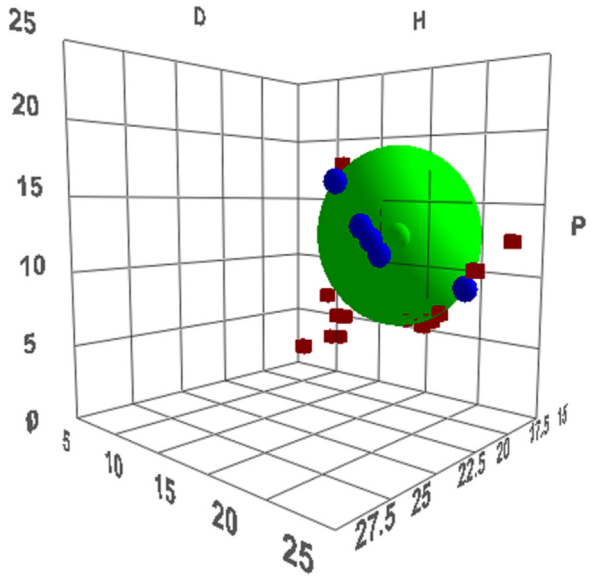

Fig. 3 – 3D sample solubility bodies of the normally-hydrated eschar at 32 °C provided as a sphere diagram and the position of solvents (●: inside the sphere and ■: outside the sphere) obtained from HSPiP software. The center of the spheres shows the HSP values for the eschar (see Table 5 for the results). D: dispersion (atomic) forces (δ_D), P: permanent molecular bipolar forces (δ_P) and H: hydrogen bonding (δ_H).

Table 5 – HSP values of dispersion (δ_D), polar (δ_P), hydrogen bond (δ_H) and total (δ_T) solubility parameters and the radius of interaction sphere (R_0) for normally and fully-hydrated eschar at 25 °C and 32 °C obtained in the present investigation.

Hydration level	Temperature (°C)	HSPs ($MPa^{1/2}$)				R_0
		δ_D	δ_P	δ_H	δ_T	
Normally-hydrated	25	17.0	12.5	14.6	25.6	6.6
Normally-hydrated	32	16.8	12.4	14.4	25.3	6.5
Fully-hydrated	25	17.2	12.9	15.3	26.4	6.6
Fully-hydrated	32	17.1	12.8	15.1	26.2	6.5

$$d\delta_H/dT = -\delta_H\,(1.22 \times 10^{-3} + 0.5\alpha) \quad (8)$$

Eqs. (6)–(8) show that there is a negative slope between temperature and all three parameters, and, that the δ_H parameter is the most affected by increasing temperature [12]. Our results indicate that by increasing the temperature from 25 °C to 32 °C, HSP parameters decrease for both hydration levels (Table 5). δ_D, δ_P, and δ_H parameters of normally-hydrated eschar were decreased about 0.2, 0.1 and, 0.2 units, respectively. For fully-hydrated eschar, δ_D, δ_P, and δ_H parameters were also decreased about 0.1, 0.1 and 0.2 units respectively, and as seen, δ_H showed more reduction than other two parameters for fully-hydrated eschar, in agreement with Eqs. (6)–(8) [12], that means increased temperature reduces hydrogen binding capacity of the eschar, as is expected.

4.2. *Effect of hydration level on eschar Hansen solubility parameters*

HSP parameters comparison of two hydration levels at 25 °C (Table 5), indicate that all three parameters of eschars were increased after 24 h eschar hydration: 0.2, 0.4 and 0.7 units for δ_D, δ_p and δ_H, respectively (Table 5). The increment was observed to be 0.3, 0.4 and 0.7 for δ_D, δp and δ_H, respectively at 32 °C (Table 5). These data show that hydrogen bonding parameter is most affected by eschar hydration at both temperatures. This might indicate increased hydrogen binding capacity by increased hydration level, an indication of increased free water in the system.

4.3. *Eschar thickness and Hansen solubility parameters*

The effect of eschar thickness on drug permeation through third-degree burn eschar has been previously investigated by our group to show that permeation of clindamycin phosphate through thick eschar was significantly lower than thin samples [19], as is expected by Fick's law for passive permeation that indicate direct relationship between the flux and concentration gradient and hence flux chang by membrane thickness. Such a correlation was not found for HSP values in the present investigation, as HSP is a polarity parameter and is not expected to be affected by thickness, as far as the structure and composition of the membrane remains the same. The present data might show that eschar polarity does not depend on its thickness when a full-thickness eschar is used.

4.4. *Correlation of solvents uptakes and individual Hansen solubility parameters*

Multiple linear regression analysis was performed to evaluate the correlation between solvents HSP parameters and their uptakes by normally and fully-hydrated eschar samples at 25 and 32 °C (Tables 6 and 7).

Pearson correlation analysis indicated that there are significant correlations between all three parameters of solvents (δ_D, δ_P and δ_H) and their uptakes for both hydration levels at both temperatures ($P < 0.05$) (Tables 6 and 7). Pearson coefficient (r) for normally-hydrated eschar samples showed descending order of $\delta_P < \delta_D < \delta_H$, at 25 and 32 °C (Table 6). It shows that the δ_P parameter is the most important parameter that influences solvent uptakes by normally-hydrated eschar with greater uptakes for solvents with higher δ_P ($P < 0.05$).

Comparison of solvent uptakes and their HSP parameters for fully-hydrated eschar also shows that the descending order of the Pearson coefficient is $\delta_P < \delta_H < \delta_D$ at 25 and 32 °C (Table 7). These data show that δ_P is still the main parameter that influences solvent uptake in fully-hydrated eschar, but it seems that δ_H plays a more important role than δ_D for solvent uptakes by fully-hydrated eschar.

This analysis suggests that polar drug molecules and polar solvents used as drug carriers are more likely to partition into

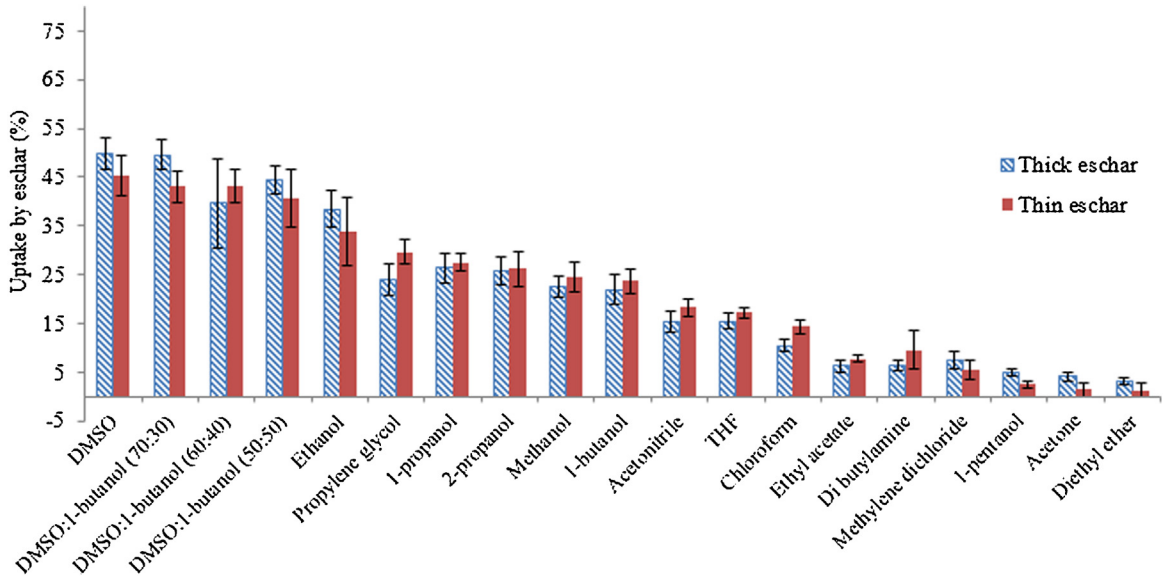

Fig. 4 – Sample graph showing comparison of solvents uptakes (%) by thin and thick normally-hydrated eschar samples at 25 °C.

the eschar and that the polarities of drugs and excipients should be taken into consideration in eschar drug development.

There are not much data available on the role of drug polarities on eschar drug delivery. However, Murdan et al. [21] observed similar results for human nail drug delivery after nail HSP calculation. Ghaffari et al. [6] also investigated permeation of clindamycin phosphate (a hydrophilic drug) and diazepam (a lipophilic drug) through human third-degree burn eschar in the presence and absence of ethanol. Permeability coefficient (K_p) values of clindamycin phosphate and diazepam were calculated to be 13.1×10^{-3} and 17.4×10^{-3} (cm/h) respectively [6]. Close permeability coefficients of clindamycin phosphate and diazepam, despite higher molecular weight of clindamycin that restricts the diffusion, might indicate higher uptake of clindamycin phosphate by eschar. It seems that permeation restrictions due to increased size are compensated by increased permeation through higher uptake.

4.5. *Comparison of HSP values of eschar, intact skin and the stratum corneum*

Table 8 shows the HSP parameters of normally-hydrated eschar at two temperatures, compared to the parameters obtained in previous studies for human intact skin [12,17], the psoriatic scale [15] and a similar study for human stratum corneum performed by our group [18].

Data show that the main difference among the eschar parameters and those of human skin and the stratum corneum is in the δ_H parameter which is related to hydrogen bonds (Table 8). Data also show that δ_D of psoriatic scales is very

Table 6 – Pearson correlation between solvent uptakes by normally-hydrated eschar samples at 25 and 32 °C and HSP parameters of solvents (δ_D: dispersion, δ_P: permanent molecular bipolar forces and δ_H: hydrogen bonding).

Parameter	25 °C			32 °C		
	δ_D	δ_P	δ_H	δ_D	δ_P	δ_H
Pearson correlation (r)	0.534	0.600	0.473	0.546	0.595	0.444
P-value	0.019	0.007	0.041	0.016	0.007	0.057

Table 7 – Pearson correlation between solvent uptakes by fully-hydrated eschar samples at 25 and 32 °C and HSP parameters of solvents (δ_D: dispersion, δ_P: permanent molecular bipolar forces and δ_H: hydrogen bonding).

Parameter	25 °C			32 °C		
	δ_D	δ_P	δ_H	δ_D	δ_P	δ_H
Pearson correlation (r)	0.498	0.603	0.567	0.476	0.648	0.570
P-value	0.030	0.006	0.011	0.039	0.003	0.011

Table 8 – Comparison of HSP values of eschar with human skin, stratum corneum, and psoriatic scale.

Type of skin	HSPs ($MPa^{1/2}$)			Reference
	δ_D	δ_P	δ_H	
Human skin[a]	17.6	12.5	11.0	[12]
Human skin[b]	17	8	8	[17]
Psoriatic scale[c]	24.6	11.9	12.9	[15]
Stratum corneum 32 °C	16.5	12	7.7	[18]
Eschar 25 °C	17.0	12.5	14.6	Present study
Eschar 32 °C	16.8	12.4	14.4	Present study

[a] Obtained from the human epidermis using permeation as a measurement method [12].
[b] Estimated for human skin [17].
[c] Obtained from psoriatic scales using swelling as a measurement method [15].

different from δ_D of the other skin types including eschar (Table 8) [12,15]. Previous studies have shown that lipids, proteins and water content of eschar are lower than those of intact skin, but the weight ratios of these components in eschar are similar to those of intact skin [22]. Please note that according to the classification of burned skin based on the depth of burn, third-degree burn eschar does not contain stratum corneum [2].

4.6. RED and its correlation with the partition coefficient (logP)

Partition coefficient (usually represented as logP) and HSP are both considered as polarity scales and are used to show the affinity of different systems/solvents to each other. In this section, these two scales are compared in terms of correlation to solvent uptake by the eschar.

LogP is usually expressed as the logarithm of octanol/water partition coefficient, considering octanol as a lipophilic model for biological barriers [23,24]. In the HSP concept, the Relative Energy Difference (RED) parameter, that is the ratio of R_a (distance) and R_0 (radius of interaction sphere) in Hansen space (Eq. (9)), is used to shows the affinity of a compound to a solvent/medium [12]. A RED number of 0 represents no energy difference, RED numbers less than 1 indicate high affinity, RED equal to or close to 1 are boundary conditions and higher RED numbers indicate lower affinity between two solvents [12].

$$\text{RED } \hbox{\tf="P48722B"\{[125TDDIFF]\}} = R_a/R_0 \quad (9)$$

Since logP is usually reported at 25 °C, we compared the RED calculated from present data using normally and fully-hydrated eschar data at 25 °C and logP from the literature [25] (Table 9). Please note that mixtures of DMSO and 1-butanol were not included in this comparison due to the inaccessibility of their logP.

Pearson correlation analysis between solvent uptakes by normally and fully-hydrated eschar at 25 °C and logP or RED indicated that both logP and RED show significant correlations ($P < 0.05$) with solvents uptake by eschar (Table 10).

Table 10 – Pearson correlation between mean solvent uptakes by normally-hydrated (NH) and fully hydrated (FH) eschar samples at 25 °C and RED and logP of the solvents. Uptake data and RED data are from the present investigation and LogP data are from the literature (see Table 9).

Parameter	RED		LogP	
	NH	FH	NH	FH
Pearson coefficient (r)	−0.629	−0.744	−0.609	−0.706
P-value	0.009	0.001	0.012	0.002

Comparison of Pearson coefficient between solvents uptakes by normally and fully-hydrated eschar and logP or RED values showed that RED/solvent uptakes correlations and logP/solvent uptakes correlations are very close at 25 °C (Table 10). However, it seems that correlations for RED are stronger, especially for fully-hydrated eschar (Table 10). It seems that RED is a better factor for the prediction of solvent uptakes by eschar. Negative numbers of Pearson coefficient also indicate that solvent uptakes generally decrease with RED increase.

5. Conclusion

In this work, Hansen solubility parameters (HSP) of third-degree burned skin (eschar) were obtained for the first time at two temperatures of 25 °C and 32 °C for two eschar hydration levels of normally and fully-hydrated. The correlation between solvents uptakes by eschar and individual Hansen solubility parameters of solvents indicated that there are significant correlations between all three parameters of solvents (δ_D, δ_P and δ_H) and their uptakes for both hydration levels at both

Table 9 – Comparison of RED (calculated in the present investigation) and logP (from Ref. [25]) of solvents used for uptake by normally and fully-hydrated eschar samples at 25 °C.

Solvent	RED		LogP
	Normally-hydrated	Fully-hydrated	
Ethanol	0.99	0.99	−0.1
DMSO	0.99	0.99	−0.6
1-Propanol	1.01	1.05	0.3
2-Propanol	1.07	1.14	0.3
Propylene glycol	1.06	1.01	−0. 9
1-Butanol	1.08	1.16	0.9
Methanol	1.36	1.31	−0.5
Acetonitrile	1.62	1.69	−0.3
1-Pentanol	1.06	1.16	1.6
Acetone	1.28	1.41	−0.1
Methylene dichloride	1.38	1.51	1.5
THF	1.43	1.56	0.5
Ethyl acetate	1.59	1.74	0.7
Chloroform	1.97	2.09	2.3
Di butyl amine	2.19	2.34	2.8
Di ethyl ether	2.23	2.37	0.9

temperatures. However, data show that δ_P parameter is the most important parameter that influences solvent uptakes by normally-hydrated eschar with greater uptakes for solvents with higher δ_P.

HSP parameter comparison of two hydration levels indicate that all three parameters of eschars were increased after 24 h eschar hydration. Data show that hydrogen bonding parameter is most affected by eschar hydration at both temperatures. Results also indicated that by increasing the temperature from 25 °C to 32 °C, HSP parameters decreased for both hydration levels and that δ_H showed more reduction than the other two parameters for fully-hydrated eschar.

Comparison of HSP values of eschar and those of intact human skin and human stratum corneum showed that the main difference between the eschar parameters and those of human skin and the stratum corneum is in hydrogen bonding.

Practically, the HSP parameters and related information obtained here indicates the polarity of the membrane and can be used to measure the affinity of materials to the eschar and therefore are useful in designing drug carries and selecting materials and ingredients for drug delivery through burn eschar. Our data show that HSP might be a better parameter for predicting the affinity of solvents and materials to eschar than the partition coefficient.

Further investigations are in progress in our laboratories regarding HSP parameters for other skin conditions, differences between coagulation and stasis zones of burn wound and even skin models that can predict percutaneous absorption of drugs such as liquid crystalline models for the intercellular lamellar structure of the human stratum corneum [26].

Declaration of interest

The authors declare no conflict of interest.

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