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Solubility and Stability Studies of Benzoyl Peroxide in Non-Polar, Non-Comedogenic Solvents for Use in Topical Pharmaceutical Formulation Developments

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SUMMARY. **Non-irritant, non-comedogenic and non-polar emollients were pre-selected for determinations of relative dielectric permittivity and solubility of benzoyl peroxide (BP). Those solvents capable of solubilizing BP in concentrations commonly utilised in topical formulations (between 1 and 10 %) were taken into account for stability studies. The developed pre-formulations were also studied for acute irritation both clinically and instrumentally. Even though the solubility of BP in the solvents studied had relatively low values; in some cases, such as with caprylic/capric triglyceride (CapCap) and dicaprylyl carbonate (DicCar) it has been possible to obtain acceptable concentrations of BP from a therapeutic viewpoint (19.9 and 19.5 mg/mL, respectively). Two BP pre-formulations (PBCapCap and PBDicCar) with enhanced stability and with the capability to decrease adverse application site reaction by maintaining moisture in the *stratum corneum* were developed with potential application in topical formulations of BP with solvents of low relative dielectric permittivity (CapCap and DicCar, respectively).**

INTRODUCTION

Benzoyl peroxide (BP) has a proven track record of safety and efficacy for the treatment of acne [1,2]. It is a first-line topical treatment in acne vulgaris and rosacea for its antimicrobial, anti-inflammatory, comedolytic and keratolytic properties [3-6]. *Propionibacterium acne* is the primary micro-organism associated in the development of inflammatory as well as non-inflammatory acne [7]. BP antimicrobial activity is nonspecific and it is based on the generation of highly reactive oxygen radicals, a physicochemical effect causing a reduction of populations of *Propionibacterium acnes* [7]. In this sense, the use of BP has advantages in comparison to the use of antibiotics because potential bacterial resistance is avoided, and it is also preferred over other keratolytic agents due to its bactericidal effect [7].

Prescriptions and over the counter products contain anywhere from 1.0 to 10 % BP in a wide variety of preparations, including creams, lotions, pads, cleansers and gel [3]. The majority of topical BP formulations are gels with BP dispersion formulated at 2.5, 5.0 or 10 % [8]. Although efficacy does not appear to increase with concentrations higher than 2.5 % of BP, side effects including the most common one –irritation– are largely dose-dependent and tend to be less favourable at increasing concentrations [4,8-11]. The degree of irritation is believed to be related to the amount of BP present in the product [12]. Another concern from a pharmaceutical perspective is that BP is extremely reactive in terms of stability. BP is destroyed by thermal degradation in solution due to the instability of peroxide bond (O-O bond) present in the molecule, and the degradation pathway through a free radical mechanism is widely studied and explained in detail elsewhere [13-18]. Further, BP is poorly soluble in many pharmaceutical solvents and practically insoluble in water [4]. In addition, some solvents frequently used in the formulation of commercially available gels, such as ethanol [19] and polyethylene glycol (PEG) 400 [13], are known to produce an adverse effect on stability with time during the storage of BP [4]. Thus, two mayor

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concerns surround the topical formulation of BP: skin irritation and BP chemical instability on storage when it is solubilised in a vehicle.

In view of these concerns, the purpose of the present investigation was to a) determine the saturation solubility of BP in various non-polar, non comedogenic emollients commonly used in topical pharmaceutical and cosmetic formulations, b) study the rates of degradation of saturated solutions of BP during a stability test, and c) investigate the potential of selected saturated solutions of BP to cause irritation in human skin as preliminary steps to design an elegant and stable BP topical formulation with enhanced stability, which could also be able to minimize BP adverse application site reactions so as to be considered a real benefit for topical acne treatment.

MATERIALS AND METHODS

Materials

Benzoyl peroxide (75 % w/w) and methanol were obtained from Merck (Buenos Aires, Argentina). Octyldodecanol (Eutanol G), propylheptyl caprylate (Cetiol Sensoft), dicaprylyl carbonate (Cetiol CC), decyl oleate (Cetiol V), cetearyl isononate (Cetiol SN) and caprylic/capric triglyceride (Myrtiol 318) were a gift from Cognis (Buenos Aires, Argentina). Polyethylene glycol 400 and polydimethylsiloxane were supplied from Dow Corning. All other chemicals and solvents were of analytical grade from Fluka.

High pressure liquid chromatography quantification of benzoyl peroxide

The quantitative determination of benzoyl peroxide (BP) was carried out using a reversed phase isocratic high pressure liquid chromatography (Shimadzu HPLC 10) equipped with a UV detector. A 25-cm C-18 reversed phase column (particle size of 5 µm, internal diameter of 4 mm; Licrhrospher 100) was used for separation. A mixture of methanol/distilled water (75:25) was utilised as mobile phase. The filtered mobile phase was pumped at a flow rate of 2 mL/min and the eluent was monitored using the UV detector set at 254 nm. Different concentration of BP in methanol (50, 10, and 1 µg/mL) were used as standard solutions in order to adequate the system. Studies were carried out to estimate precision and accuracy of this HPLC method for analysis of BP. A standard curve was used to estimate the concentration of BP in the different samples studied. Each determination was calculated in triplicate, and the mean of concentrations were reported.

Criteria used for selecting solvents

The solvents were emollients non-miscible with water, and they were selected based on their non-toxic, non-irritant, non-comedogenic and acceptable organoleptic characteristics. Once a preliminary list was obtained, only those solvents capable of solubilizing BP (at concentrations of saturation) within the range of concentrations commonly utilised in topical formulations (between 1 and 10 mg/mL) were considered for stability studies.

Relative dielectric permittivity measurements

Relative dielectric permittivity (RDP) measurements were carried out to samples of selected solvents at several temperature (28, 35, 45, 55, 65 and 75 °C) and extrapolated to 25 °C with a RCL-meter model 5100 made by Topward (Taiwan) and a sample cell for liquids with platinum electrodes made by Parsec (Argentina). The calibration of the cell was verified with ciclohexane. The temperature of the sample was controlled within ± 0.1 °C with a thermostat made by Lauda (Germany). For each measured temperature, RDP was determined at frequencies of 1, 5, 10, and 15.7 kHz and the mean value was calculated. The accuracy of results was better than 0.5 % at all the measured temperatures.

Solubility determinations

The solubility of BP was determined in different solvents (octyldodecanol, caprylic/capric triglyceride, propylheptyl caprylate, dicaprylyl carbonate, decyl oleate, cetearyl isononanoate, octyldodecanol, polydimethylsiloxane) in triplicate at 25 ± 2 °C and protected from light. polyethyleneglycol 400 (PEG400) and mineral oil were used as references. A volume of 10 mL of appropriate solvent was added to 15 mL glass vial containing an excess of BP (1.0 g). The vials were stirred at room temperature for 24 h to ensure equilibrium solubilization. Samples of saturated solutions were then centrifuged (5 min, 5000 rpm) and filtered through polysulfonato membranes (pore size 0.45 µm). Aliquots of 1 mL were withdrawn from the filtrates and suitably diluted to 50 mL with methanol for HPLC measurements according to the method outlined before.

Stability studies

This step was performed to examine the degradation process as a consequence of BP solvent interaction over time. To this end, only

those solvents capable of solubilizing BP (at concentrations of saturation) within the range of concentrations commonly utilised in topical formulations (between 1 and 10 %) were considered for stability studies. Formulations were transferred to amber glass vials and kept at 25 ± 2 °C in the dark. The working temperature was determined based on an earlier work showing that at temperatures higher than 25 °C, BP undergoes thermal decomposition [13]. Every 15 days, for a total period of 90 days, samples of 1 mL were taken from each formulation series and dissolved in 50 mL of methanol and these were analysed using HPLC according to the method outlined in section.

In vivo acute skin irritation assay

Acute skin irritation potential of selected formulations was investigated by performing an *in vivo* patch test in 15 volunteers of both sexes, recruited from the academic community (Professors, Assistant Professors and postgraduates students) with normal healthy skin (24-40 years old) after informed consents were obtained. Volunteers were free of eczema and had no history of atopic dermatitis or respiratory atopy. Clinical skin irritation testing can be easily and ethically conducted in volunteer human subjects providing that the chemicals lack other toxicities (*e.g.* genotoxicity, sensitization, corrosivity, etc.) that would preclude testing in humans at the desired exposure levels [20-25]. A single application of 0.5 mL of each tested formulation was spread uniformLy over a sheet of non-woven polyethylene cloth (1.5 cm x 1.5 cm), which was then applied on the fore arm area of a volunteer and fixed with an adhesive dressing (Tegaderm, 3M, USA). The application was made progressively from 1, 2, 3 and 4 h to assure that no excessive reactions would occur [20]. After 4 h, the cloth was removed, and the treated skin area was swabbed with a cotton swab. After withdrawal of the formulations, the treated skin sites were observed for signs of irritation over the next 72 h. The first assessment was performed at 30 min after patch removal using a four-point scale (Table 1). For evaluation of delayed-acute reactions the test sites were inspected after 24, 48 and 72 h. Mineral oil was used as a negative control and sodium lauryl sulphate (SLS) (20 %) in mineral oil as a positive control in the experiment. In each individual, two different formulations were tested plus a positive and a negative control.

The degree of irritation was also evaluated, 30 min after the formulations were removed, by non-invasive measurement of transepidermal water loss (TEWL) using a Tewameter TM 210 (Courage + Khazaka, Koln, Germany). At least 15 min prior to TEWL measurements, the 15 volunteers were seated in an air-conditioned room at 22 °C and 40-50 % humidity 26,27 and were kept relaxed during the experiment. Measurement of TEWL is a suitable non-invasive technique for determination of skin barrier function [21].

Data analysis

Statistical evaluation of the data of *in vivo* acute skin irritation assay was performed using the Wilcoxon–Mann–Whitney test to compare TEWL values recorded from verum treated skin with the control site.

RESULTS

Solubility determinations

The solubility values of BP in the different solvents tested in this study appear in Table 2. Caprylic/capric triglyceride and dicaprylyl carbonate presented good BP solubilities , followed by propylheptyl caprylate, decyl oleate and cetraryl isononanoate in descending order (the sample types of BP solubilised in the different solvents were coded as shown in Table 2). In addition, BP was slightly soluble in silicone, mineral oil and octyldodecanol. Further, a mixture of octyldodecanol:ethanol (85:15) prepared to increase the solubility of BP in octyldodecanol was tested but the solubility value obtained was still insufficient for this vehicle to be used in formulations for the treatment of acne. Finally, the solubility of BP in PEG 400 was the

Grading	Description of response
0	No reaction
1+	Mild erythema possibly spreading beyond treatment site
2+	Distinct erythema possibly spreading beyond treatment site
3+	Strong, often spreading erythema with edema

Table 1. Typical skin reaction scoring used for the evaluation of a human patch test for the identification and classification of skin irritation potential.

Sample	Solvents	Solubility (mg/mL) at 25 °C
PBPEG400	Polyethylene glycol 400	36.0
PBCapCap	Caprylic/capric triglyceride	19.9
PBDicCar	Dicaprylyl carbonate	19.5
PBProCap	Propytheptyl Caprylate	16.4
PBDecOl	Decyl oleate	15.8
PBCetIso	Cetearyl Isononanoate	11.5
PBOctEt	Octyldodecanol:Ethanol	6.7
PBOct	Octyldodecanol	3.6
PBMin	Mineral oil	3.0
PBSil	Polydimethilsiloxane	1.7

Table 2. Solubility of benzoyl peroxide (mg/mL) in various solvents at 25 °C.

highest among the solvents studied in agreement with previous investigations [4,13,28]. Based on these results, caprylic/capric triglyceride, dicaprylyl carbonate and propylheptyl caprylate were considered good candidate solvents and selected for the following section studies.

Relative dielectric permittivity measurements

The relative dielectric permittivity (RDP) values at 25 °C and low frequencies (1-15,7 kHz) of the selected solvents appear in Table 3. The solubility of BP in each solvent at 25 °C was plotted against the relative dielectric permittivity of the tested solvents, resulting in a characteristic "solubility pattern" that might serve as the fingerprint to identify BP (Fig. 1).

Stability studies

A graph of the degradation of BP solubilised in the different solvents studied as a function of time is shown on a semi-log scale in Figure 2 and all decompositions were first-order processes. The constant rates of degradation of BP

Solvents	Relative dielectric permittivity (ε_r)*
Polyethylene glycol 400	14.40
Caprylic/capric triglyceride	3.83
Dicaprylyl carbonate	2.42
Propytheptyl Caprylate	3.33
Decyl oleate	3.14
Cetearyl Isononanoate	3.00
Octyldodecanol:Ethanol (85:15)	5.89
Octyldodecanol	2.70
Mineral oil	2.23
Polydimethilsiloxane	2.55

Table 3. Relative dielectric permittivity (ε_r) at 25°C and low frequencies (1-15,7 kHz) of solvents.

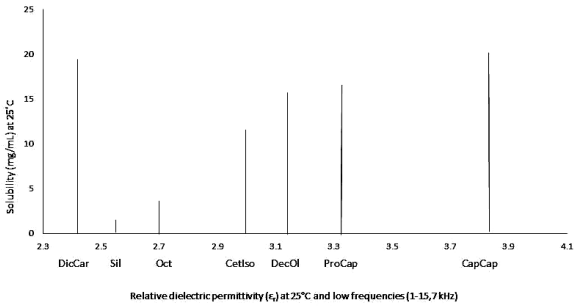

Figure 1. Solubility spectrum of Benzoyl Peroxide.

(kBP) for each of the solvents studied were calculated from the slopes of the degradation profiles of the different formulations (correlation coefficients higher than 0.9000 in all cases) and the results are shown in Table 4. The highest degradation rate was observed in BPPEG400 (kBP -0.02710), while the most stable formulations were PBCapCap (kBP -0.00092) and PBDicCar (kBP -0.00127).

In vivo acute skin irritation assay

The clinical scores (see Table 1) and TEWL data of the *in vivo* irritation assay for the formulations selected (PBCapCap and PBDicCar) are shown in Table 5. The number of volunteers with positive skin reactions are shown in relation to the total number of volunteers studied. No clinical reactions were observed in the volunteers exposed to the negative control, while the positive control caused an inflammatory reaction (sum of clinical scores 20) in 11 of 15 volunteers, which persisted over 48 h in most subjects. No skin irritation was observed when the subjects were exposed either to PBCapCap or to PBDicCar. No delayed-acute reactions at 24, 48 and 72 after treatment were observed in the subjects. The TEWL increased from 5.34 ± 0.78 g/m².h in non-treated skin to 8.05 ± 1.13 g/m².h 30 min after removal of the negative

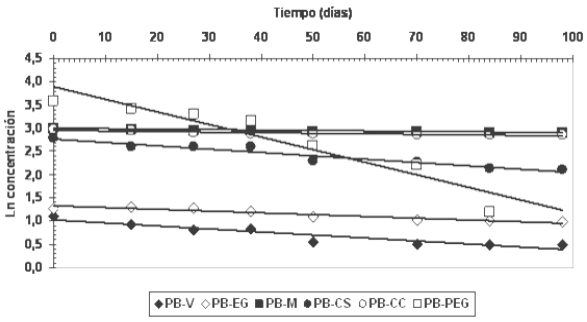

Figure 2. Stability of benzoyl peroxide (at saturated concentration) in pure solvents after storage at 20 °C ± 5 for 90 days.

Sample	Rate constant of degradation (k_{BP})	Correlation Coeficient (r)
PBPEG400	-0.02710	0.9479
BCapCap	-0.00092	0.9520
PBDicCar	-0.00127	0.9593
PBProCap	-0.00719	0.9656
PBOct	-0.00371	0.9524
PBMin	-0.00653	0.9458

Table 4. Stability of benzoyl peroxide (at saturated concentration) in pure solvents after storage at 25 ± 5 °C for 90 days.

Formulation	Clinical Reaction	Sum of clinical score	TEWL ($g/m^2.h$)
PBCapCap	0/15	0	9.11 ± 1.42
PBDicCar	0/15	0	7.57 ± 0.98
Mineral Oil	0/15	0	8.05 ± 1.13
SLS	11/15	20	33.45 ± 6.21*
None	0/15	0	5.34 ± 0.78

Table 5. Patch test results and measurements of the transepidermal water loss (TEWL) for the spin traps and controls in human skin *in vivo*. The number of subjects with positive skin reactions are shown in relation to the total number of subjects (15) studied. The clinical scores of the subjects with positive reactions were added and the total score of all reactions is shown. Mean values ± standard deviation are shown. * Significantly different: $p < 0.01$ from negative control (mineral oil).

control. This is interpreted as a transient damage to the skin barrier by the occlusive patch, which is a well-known phenomenon [29]. The positive control caused a large increase in TEWL to 33.45 ± 6.21 $g/m^2.h$, indicating grossly disturbed skin barrier function. PBCapCap and PBDicCar produced negligible changes in TEWL and did not significantly increase the TEWL when compared to the negative control (Table 5), suggesting a tolerance of the skin to the topically applied pre-formulations.

DISCUSSION

From a pharmaceutical perspective, two major concerns surround the topical formulation of BP for the treatment of acne: a) the skin irritation and b) BP chemical instability on storage when BP is solubilised in the vehicle. In this sense, the mayor finding of this study is that the incorporation of BP in a formulation can be done in a solubilised form, more stable than in other vehicles tested in previous publications, and without causing skin irritation. Chelquist & Gorman [13] were focused in hydric solvents, in particular polyethylene glycol 400 (PEG 400) water blends and cosolvents mixtures of PEG 400 and polyols. Due to poor stability of solution formulations of BP in PEG 400 solutions, the authors concluded that the vehicle should provide low BP solubility and they proposed the suspension of BP as the best topical formulation [13].

Based on our investigations, we speculate that the skin irritation could be associated with the non-diffused particles of BP which are kept in contact with the upper layer of the skin when BP is dispersed in the vehicle instead of solubilised. This speculation is in agreement with the fact that micronized BP causes less irritation that macro-sized BP in topical suspensions [11]. In a suspension, the highly insoluble particles of macro-sized BP are entrapped in the vehicle, creating an obstacle to effective delivery to the infected and inflamed follicle, delivering approximately 0.03 % to 1.0 % of the available BP to the follicle [30]. In addition, the non-diffused particles of BP are kept in contact intimacy with the upper layer of the skin for hours, which might cause skin irritation. In view of these difficulties, it is conceivable to 1) solubilise BP in a non-polar, non-comedogenic solvent with medium to low capability to solubilise BP, 2) maintain the concentration of BP in the vehicle as near to saturation as possible and 3) from a diffusion perspective, take advantage of the thermodynamic activity of BP in the formulation to warranty an effective drug delivery to the follicle.

In addition, it is worthwhile to speculate that a solution, or a formulation capable of encapsulating BP in a solubilised form, would be a more appropriate pharmaceutical form for the delivery of BP instead of an emulsion. Emulsifiers are basically detergents with the capability to emulsify not only the formulation ingredients but also the sebum within the follicular ostia and possibly the skin proteins around the ostia [31]. Solubility of BP in various non-polar solvents had already been studied extensively by Nielloud *et al.* in an attempt to develop stable formulations with the capability to increase the solubility of BP [4]. However, Nielloud *et al.* focused on the determination of PBO solubility in various non-polar solvents frequently used in the dermatological field with the aim to develop a submicron emulsion gel to solubilised BP [4]. According to the authors, caprylic/capric triglycerides was the most suitable solvent among the

ones studied, in concordance with the present investigation, but it was used to develop a submicron emulsion gel which was made with an ethylene oxide derivative of castor oil, glycerol, caprilyc/capric triglycerides, and water in the proportion of 20-20/35/25, respectively and 1.5 % BP. Although the information obtained from such a study could be very useful and even though the authors did not make an irritation test of the formulation reported, a formulation with a content of 20 % of emulsifier it is likely not the best alternative for an acne-prone skin [31].

Of the pre-formulations studied in the present investigation, the most stable ones were PBCapCap and PBDicCar (Table 4) with degradation rates 30 fold and 25 fold lower, respectively, than PBPEG400. Moreover, in these two cases it was possible to obtain acceptable saturated concentrations of BP (from a therapeutic point of view) which makes these pre-formulations strong candidates to be considered in future topical pharmaceutical formulations of BP. When the solubility values of BP in each solvent at 25 °C were plotted against the relative dielectric permittivity of the solvents (Table 3), the BP's characteristic "solubility pattern" was produced (Fig. 1). Nonetheless, there were no direct correlations between the relative dielectric permittivity of the solvents and BP's solubility values (Fig. 1). The implication is that the solubility was influenced by intermolecular interactions. In an attempt to study the influence of the polarity of solvents to the stability of the formulations, correlations between degradation rate and dielectric permittivity were tried to establish without success. In general, a free radical initiation reaction can be influenced by the polarity of the solvents [32]. Furthermore, changing the dielectric permittivity of an autoxidation medium often influences both the rate controlling propagation and termination reactions [32]. In BP, however, solvent polarity seems not to be a major factor and no correlation was found between dielectric permittivity and degradation rate. This finding is in agreement with previously published results [33,34]. Hongo *et al.* studied the rate of degradation of BP in alcoholic solvents and established that the stability of BP in methanol (ε_r = 32.6) was lower than in ethanol (ε_r =24) [35]. However, these results are not in opposition with the present investigations. Among polar solvents, aliphatic alcohols were reported to have a stimulatory role on the decomposition of BP [32]. BP is known to start its degradation process in primary alcohols with the abstraction of hydrogen from the alcohol [32]. The abstraction of hydrogen from the alcohol would be easier in methanol than in ethanol. This specific path for decomposition of BP in primary aliphatic alcohols can explain the correlation between stability of BP in alcoholic solvents such as methanol and ethanol and dielectric permittivity. In general situations, the decrease in the rate of BP degradation could be explained as the extinction of benzoyloxy radicals as result of interactions with solvent molecules without increasing the number of radicals [14,33]. In the particular case of a chain transfer to the solvent, the new free radicals generated would be comparable in activity to the old ones and, thus, the process would only affect the products and not the kinetics of the overall reaction [36].

Besides the chemical instability of BP in storage when it is solubilised in the vehicle, the skin tolerance is another concern for BP topical formulations. Skin irritation results in a disturbed barrier function and it can be analysed clinically (macroscopically) and instrumentally *in vivo*. In routine clinical patch testing for evaluation of the irritation potential of chemicals in human skin the exposure time is limited (4 h) [20]. Therefore the irritation potential of the selected saturated solution (PBCapCap and PBDicCar) with an application time of 4 h was tested in the skin of volunteers in comparison to the positive control. Sodium lauryl sulphate (SLS) is suitable for the purpose of irritant patch testing, because of its ability to influence the barrier function of the skin as well as to cause skin inflammation [29]. None of the 15 volunteers exposed to PBCapCap and PBDicCar reacted positively. Furthermore, the biophysical measurements of TEWL clearly showed no significant difference between PBCapCap and PBDicCar and negative control. The TEWL increased observed in PBCapCap, PBDicCar and negative control were interpreted as a transient damage to the skin barrier by the occlusive patch, which is a well-known phenomenon [20]. It is well established that the sensitivity of TEWL measurements as a screening technique for early and weak signs of irritancy is superior in comparison to visual scoring [29,37].

Considering all the results of this preliminary study, pre-formulations like PBCapCap and also PBDicCar may be used to design an elegant formulation of BP with enhanced stability and with the capability to decrease BP adverse application site reactions by maintaining moisture in the *stratum corneum*.

CONCLUSIONS

Two BP pre-formulations (PBCapCap and PBDicCar) with enhanced stability were carried out with solvents of low dielectric constant, caprylic/capric triglyceride and dicaprylyl respectively. Even though the solubility of BP in the solvents studied had relatively low values; in some cases (such as PBCapCap and PBDicCar) it was possible to obtain saturated concentrations of BP within the clinically relevant dose range. Furthermore, PBCapCap and PBDicCar pre-formulations had the capability to decrease BP adverse application site reactions. Therefore, we conclude that these pre-formulations, PBCapCap and PBDicCar, have potential application in topical pharmaceutical formulations of BP and they could be applied in the development of an elegant formulation of BP with enhanced stability and with the capability to minimize BP skin irritation so as to be considered a real benefit for topical acne treatment. Future efforts will be focused on the development of such formulations and the results will be discussed in future publications.

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