

## **Diffusion of preservatives from topical dosage forms: A comparative study**

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### **Synopsis**

A study of the diffusion of parabens from topical formulations is presented here. In particular, four different topical formulations, namely, a water-in-oil emulsion, an oil-in-water emulsion, and two hydrophilic gels (Pemulen gel and Carbopol gel) were produced, containing a mixture of three common parabens, namely, methylparaben (MP), ethylparaben (EP), and propylparaben (PP). An analytical method based on liquid extraction, followed by reversed-phase HPLC for the quantitative determination of MP, EP, and PP, was developed. The method allowed good separation of paraben mixtures and high percentages of recovery (> than 97%). The diffusion kinetics of parabens from the produced formulations was determined by an *in vitro* system based on a Franz cell assembled with a synthetic membrane, followed by a reversed-phase HPLC analytical method. The comparative study demonstrated that, in the case of emulsions, diffusion coefficients are a function of the substituent of preservatives: the higher the solubility, the higher the diffusion of parabens. On the contrary, in the case of the hydrophilic gels, the higher the parabens solubility, the lower the diffusion coefficients. The method described here could represent a means of controlling the extent of diffusion of parabens from topical formulations in order to minimize percutaneous absorption and to control the availability of microbes.

### **INTRODUCTION**

The main deterioration factors in pharmaceuticals or cosmetics can be divided into chemi-physical changes and microbial contamination. Among these, the importance of microbial contamination is relevant for sanitary problems in dermal usage. Two strategies should be adopted in order to prepare microbiologically acceptable pharmaceutical formulations or cosmetic products: the minimization of the risks of contamination from sources and the addition of preservatives to the formulation. The use of preservatives is

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preferable in order to guarantee the long-term absence of contamination from formulations (1).

Among the preservatives used in the pharmaceutical and cosmetic fields, the methyl, ethyl, propyl, and butyl esters and their sodium salts (parabens, USP) are probably the most widely used molecules (2,3), being active against molds, yeasts and, to a lesser extent, bacteria (4). In addition, parabens exhibit antimicrobial activity over a wide pH range (between 4 and 8). The activity of the parabens increases with the increasing chain length of the alkyl moiety and could be potentiated by the use of combinations of parabens, since an additive effect occurs. In order to preserve topical preparations, parabens are normally used in the concentration range of 0.02–0.3% (5,6).

Besides the desirable requisites for a preservative to be suitable for use in a topical formulation (i.e., a wide spectrum of activity, bactericidal rather than bacteriostatic activity, a wide pH range, and temperature and water solubility), an essential feature should be the absence of toxic, irritant, or sensitizing activity (6,7).

Parabens are nonmutagenic, nonteratogenic, and noncarcinogenic (7). Nevertheless, parabens, analogously to most other preservatives, may be harmful to consumers because of their tendency to induce allergic contact dermatitis, especially when they are included in topical formulations (8,9).

The history of contact dermatitis from parabens falls into two phases, corresponding to their use first as medicaments and later as preservatives. As medicaments, Bonnevie (10) first reported a case of contact dermatitis from ethyl paraben used as an antifungal agent in a concentration of 5%. As preservatives, Sarkany first reported a case of sensitization from parabens (11). Since these reports, various investigators have found so many instances of sensitization to parabens in various topical therapeutic agents that most pharmaceutical manufacturers have removed parabens from topical therapeutic agents and have replaced them with other preservatives (12–14).

In 1973 Fisher (15) reported several puzzling aspects, the so-called “paraben paradox,” which is that parabens in topical therapeutic agents occasionally sensitize, whereas parabens in cosmetics are “safe.” The explanation of this seeming paradox is that cosmetics are usually applied to normal skin, whereas therapeutic agents are applied to inflamed, eczematous, excoriated, or otherwise damaged skin. However, a case of a patient with hypersensitivity to parabens in several cosmetic creams and several cases of immediate-type hypersensitivity to parabens have been reported (16–18). With regard to the sensitizing effects related to the topical administration of parabens, it is fundamental that an *in vitro* method should be able to determine the extent of parabens diffusion from topical formulations.

This paper describes (a) the production of four different topical formulations, namely, a water-in-oil emulsion, an oil-in-water emulsion, and two hydrophilic gels (Pemulen gel and Carbopol gel), containing a mixture of three common parabens; (b) an analytical method based on liquid extraction and reversed-phase HPLC for the quantitative determination of MP, EP, and PP in semisolid formulations; and (c) the use of an *in vitro* system based on a Franz cell to study the diffusion of the different parabens from the formulations.

## EXPERIMENTAL

### MATERIALS

Methylparaben (MP), ethylparaben (EP), and propylparaben (PP) were purchased from Fluka Chemie AG (Buchs, Switzerland). Pemulen<sup>®</sup> TR2 (acrylates/C10-30 alkyl acrylate crosspolymer) and Carbopol<sup>®</sup> 940 (Carbomer 940) were a generous gift of Biochim (Milan, Italy). All other materials and solvents of high purity grade were from Sigma Chemical Co. (St. Louis, MO).

### PRODUCTION OF TOPICAL DOSAGE FORMS

Four different topical formulations were produced, namely a water-in-oil emulsion, an oil-in-water emulsion, and two hydrophilic gels (Pemulen gel and Carbopol gel), whose compositions are reported in Table I. After production, all the dosage forms here described were stored at 4°C until use, to minimize possible degradations.

*W/O emulsions.* Briefly, for the preparation of the water phase, MP, EP, and PP were solubilized in boiling water. The oil-soluble components of the formulation were fused and heated to about 70°C. Production of the W/O emulsion was performed by slow addition of the aqueous phase to the oil phase under vigorous stirring by a turbine mixer. The emulsion obtained was then cooled down at room temperature.

*O/W emulsions.* As for the preparation of the W/O emulsion, MP, EP, and PP were solubilized in boiling water. The oil-soluble components of the formulation were fused and heated to about 70°C. Production of the O/W emulsion was performed, slowly adding the oil phase to the aqueous phase under vigorous stirring by a turbine mixer. The emulsion was then cooled at room temperature.

*Hydrophilic gels.* The production procedure was the same for both gels. MP, EP, and PP were solubilized in boiling water. For the preparation of the hydrophilic gel, the acrylates/C10-30 alkyl acrylate crosspolymer (Pemulen<sup>®</sup> TR2) or the carboxyvinyl polymer carbomer (Carbopol<sup>®</sup> 940) was added to the solution obtained and left to swell at room temperature to obtain a homogenous and liquified mixture. After an overnight incubation, triethanolamine was added to neutralize the solution.

### QUANTITATIVE DETERMINATION OF PRESERVATIVES

*Preservatives extraction from formulations.* A liquid procedure was performed in order to extract parabens from formulations. Briefly, 20 ml of a mixture constituted of tetrahydrofuran (THF)/water 90:10 v/v was added to 1 g of topical formulation and stirred for 30 min at room temperature. The solution obtained was vortexed and sonicated at 25°C for 2 min in a bath-type sonicator, Branson 2200 (Branson Ultrasonics Co., Danbury, CT) and centrifuged (Centrifuge Hareus Sepatech GmbH, Germany) at 6000 rpm for 10 min. Extracted samples (5 µl) were injected onto an HPLC column for the quantitative analysis.

*HPLC analysis.* A high-performance liquid chromatographic method was employed for the quali-quantitative analysis of preservatives. The analyses were performed with a Bruker apparatus (Bremer, FRG) consisting of three plungers, an alternative pump, a

**Table I**  
Composition of Utilized Topical Formulations

Component	% (w/w)	Component	% (w/w)
<i>A. W/O emulsion</i>			
Oil phase		Water phase	
Cetearyl alcohol	10.0	Glycerine	3.00
White petrolatum	7.50	Methylparaben	0.05
Mineral oil	4.00	Ethylparaben	0.05
Ceteth-20	2.00	Propylparaben	0.05
		Water	q.s. to 100
<i>B. O/W emulsion</i>			
Oil phase		Water phase	
Cetyl alcohol	2.50	Glycerine	4.00
Cetearyl glucoside	2.00	Methylparaben	0.05
Joboba oil	3.00	Ethylparaben	0.05
Tocopherol	0.05	Propylparaben	0.05
Lecithin	6.00	Citric acid	0.05
Ascorbyl palmitate	5.00	Water	q.s. to 100
Capric/caprylic triglyceride	2.00		
<i>C. Pemulen gel</i>			
Acrylates/C10-30 alkyl acrylate			
Crosspolymer	1.00		
Methylparaben	0.05		
Ethylparaben	0.05		
Propylparaben	0.05		
Triethanolamine	1.00		
Water	q.s. to 100		
<i>D. Carbopol gel</i>			
Carbomer 940	0.80		
Isopropyl alcohol	4.50		
Mineral oil	2.50		
Methylparaben	0.05		
Ethylparaben	0.05		
Propylparaben	0.05		
Triethanolamine	0.80		
Tetrasodium EDTA	0.10		
Water	q.s. to 100		

variable-wavelength UV detector, a Rheodine Inc. injection valve, and a Shimadzu integrator.

For the analysis of parabens, a Hypersil C18 stainless steel column (25 × 0.46 cm) packed with 5- $\mu$ m particles and equipped with a precolumn was eluted with an isocratic mobile phase consisting of acetonitrile/water (40:60 v/v), the flow rate being 1.0 ml/min, at room temperature, with detection at 260 nm. Extracted samples were quantitated by a calibration curve constructed from standard preservative solutions.

Quantitative analysis of preservatives was, in addition, performed by UV spectroscopic analysis. The analyses were performed by an NIR Lambda 19 (Perkin-Elmer) spectrophotometer equipped with a double ray and a double monochromator UV-VIS-NIR.

#### DIFFUSION EXPERIMENTS

The experiments were carried out using a standard glass Franz diffusion cell (12,13) with

a 1-cm-diameter orifice ( $0.78 \text{ cm}^2$  area), assembled with a system composed of two different overlapped synthetic membranes: (a) a polydimethylsiloxane-based membrane, 250  $\mu\text{m}$  in thickness (Perthese<sup>®</sup>, Dow Corning Corporation, Midland, MI), in contact with the donor phase, and (b) a nylon-based membrane, 150  $\mu\text{m}$  in thickness, 0.22- $\mu\text{m}$  pore size (Alltech Associates Inc., Deerfield, IL), in contact with the receptor phase.

As receptor phase, an isotonic solution of 60 mM phosphate buffer, pH 7.4, was used. This solution was always degassed before use and poured in the cell body to overflowing, in order to avoid air bubble formation. To study preservative diffusion, 1 ml of paraben solution or 1 g of the formulation to be analyzed was placed into the donor cell compartment and tamped down on the membranes, previously moistened with the receptor phase. The upper part of the chamber was sealed to avoid evaporation. The receptor phase was stirred by means of a constantly spinning bar magnet and thermostated at 37°C. At predetermined time intervals between 1 and 8 hours, samples (0.15 ml) of receptor phase solution were withdrawn and the preservative concentration in the receptor phase was measured using HPLC. Each removed sample was replaced with an equal volume of simple receptor phase. The calculated preservative concentrations were plotted as a function of time, and the permeability coefficients were computed from the linear portion of the accumulation curve and expressed both as experimentally observed fluxes ( $J_o$ ) and as normalized fluxes  $J_n$  ( $J_n = J_o/C$ , where C is the preservative concentration in the analyzed form, expressed in mg/ml). All the obtained permeation rates were determined six times in independent experiments, and the mean values  $\pm$  standard deviations were calculated.

## RESULTS AND DISCUSSION

### PRODUCTION OF TOPICAL FORMULATIONS

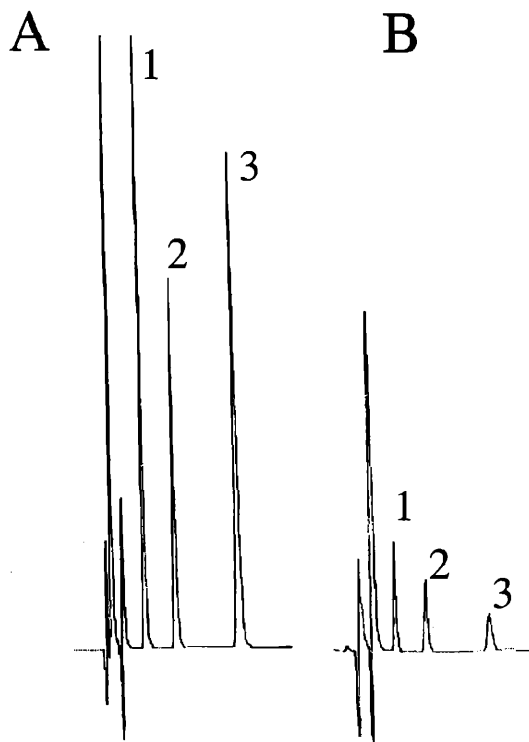
Four different topical formulations were produced, namely a W/O emulsion, an O/W emulsion, and two hydrophilic gels based on the use of acrylates/C10-30 alkyl acrylate crosspolymer or carboxyvinyl polymer resins. In all formulations a mixture of parabens consisting of MP, EP, and PP was included. In all cases paraben concentrations were 0.05%, w/w (0.5 mg/ml). In Table I are reported the compositions of the paraben-containing formulations.

### QUANTITATIVE ANALYSIS OF PRESERVATIVES

Parabens were extracted by a liquid procedure followed by reversed-phase HPLC analysis as reported in the Experimental section. The method allowed to obtain good separation of the MP, EP, and PP mixture is shown in the chromatograms in Figure 1. Table II reports percentages of recovery of parabens included in the different formulations. In all cases the high percentages of recovery obtained (> than 97%) could suggest the suitability of the method for quality control studies. Data obtained by UV spectroscopic analysis confirmed the HPLC analysis results (data not shown).

### DIFFUSION EXPERIMENTS

The experiments described here were performed utilizing an "*in vitro*" test based on a



**Figure 1.** Chromatograms of a standard mixture (A) of methyl-, ethyl- and propylparabens aqueous solution or (B) extracted from an oil-in-water emulsion (see Experimental section).

**Table II**  
Percentages of Recovery of Parabens

Preservative	Recovery average $\pm$ RSD <sup>1</sup> (%) <sup>2</sup>			
	A	B	C	D
Methylparaben	97.8 $\pm$ 1.3	97.1 $\pm$ 1.9	97.5 $\pm$ 1.4	99.2 $\pm$ 1.8
Ethylparaben	98.2 $\pm$ 1.5	99.1 $\pm$ 2.1	98.2 $\pm$ 1.2	98.8 $\pm$ 1.6
Propylparaben	97.2 $\pm$ 1.7	97.3 $\pm$ 1.6	97.4 $\pm$ 2.2	97.4 $\pm$ 2.0

<sup>1</sup> Relative standard deviations.

<sup>2</sup> Percentage w/w with respect to total amount of preservative used in the formulation.

A: W/O emulsion. B: O/W emulsion. C: Pemulen gel. D: Carbopol gel.

percutaneous absorption glass cell (Franz diffusion cell) (19,20), assembled with single or multiple synthetic membranes (see Experimental section). For the calculation of the permeability coefficients, in all cases the following procedure was used: the amount of paraben penetrated through the membrane(s) per unit area was plotted against time, and the slopes, which represent the steady-state fluxes, were calculated by linear regression. The calculated regression coefficients were never less than 0.97. The slopes were then substituted into the following equation for the determination of  $J_n$  (permeability coefficient):  $J_n = J_o/C$ .

*Diffusion of parabens from topical formulations.* Figure 2 reports the diffusion kinetics of parabens from the aqueous solution and from the produced topical formulations. The

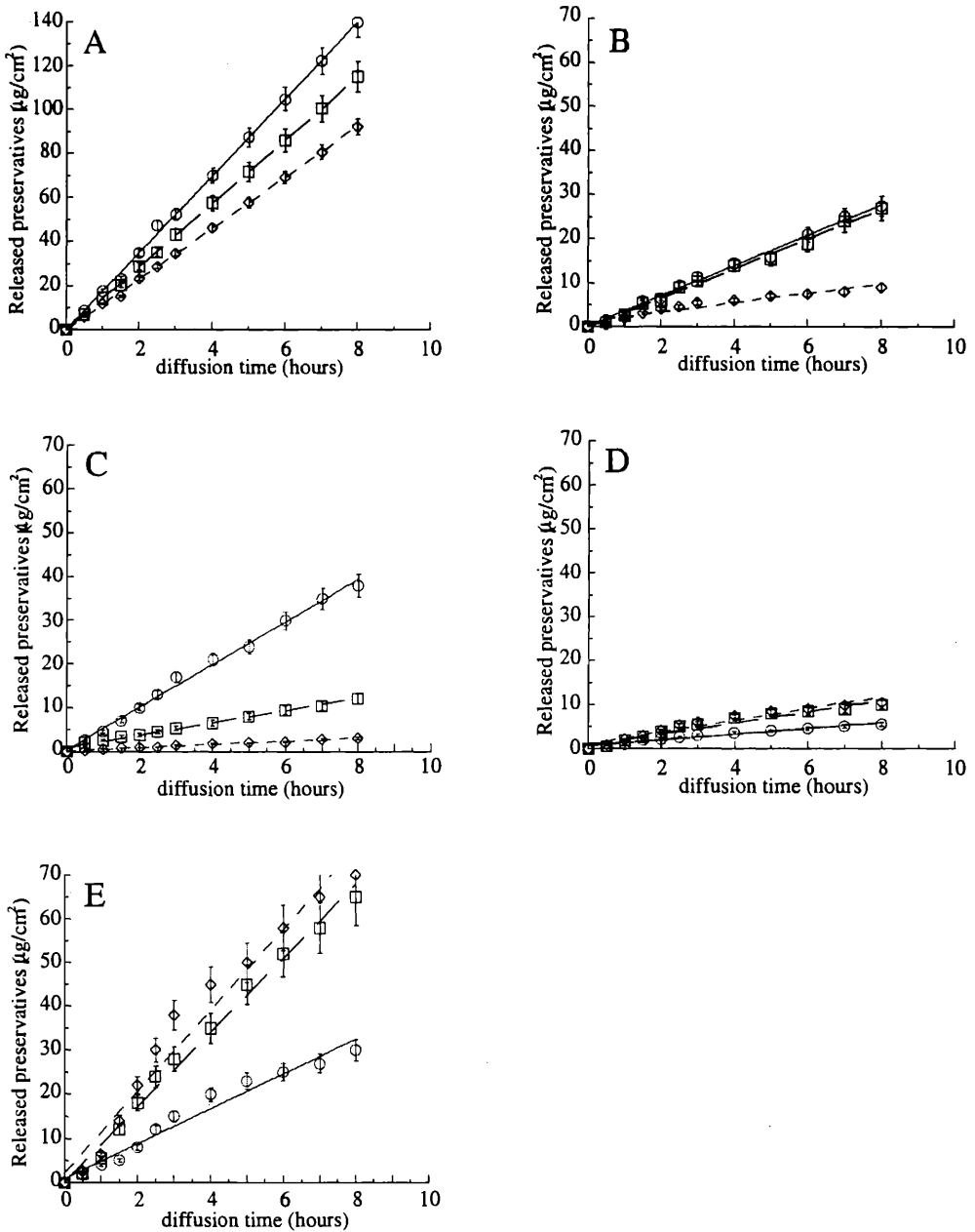


Figure 2. "In vitro" diffusion kinetics of methyl- (○), ethyl- (□) and propyl- (◇)-parabens incorporated in the reported topical dosage forms. A bimembrane system was employed: a silicone-based membrane (250-µm thickness) in contact with the donor phase, and a nylon-based membrane (150-µm thickness), 0.22-µm pore size) in contact with the receptor phase. The results reported represent the mean values ± SD of six independent experiments. A: Aqueous solution. B: Water-in-oil emulsion. C: Oil-in-water emulsion. D: Pemulen gel. E: Carbopol gel.

calculated diffusion coefficients for parabens incorporated into the different topical forms are reported in Table III. Figure 3 shows a comparison between the diffusion coefficients of the different parabens from topical formulations.

**Table III**  
 "In vitro" Diffusion Coefficients of Preservatives Incorporated in Different Topical Forms Determined Utilizing Franz-Type Diffusion Cell

Preservative	$J_0 \cdot \mu\text{g}/\text{cm}^2\text{h}$	C (mg/ml)	$J_n \cdot \text{cm}/\text{h} \times 10^3$	$\log J_n$
<i>Aqueous solution</i>				
Methylparaben	17.48	0.5	34.96	1.54
Ethylparaben	14.39	0.5	28.77	1.45
Propylparaben	11.55	0.5	23.16	1.36
<i>W/O emulsion</i>				
Methylparaben	3.48	0.5	6.96	0.84
Ethylparaben	3.37	0.5	6.74	0.82
Propylparaben	1.08	0.5	2.16	0.33
<i>O/W emulsion</i>				
Methylparaben	4.87	0.5	9.74	0.98
Ethylparaben	1.40	0.5	2.80	0.44
Propylparaben	0.37	0.5	0.74	-0.13
<i>Pemulen gel</i>				
Methylparaben	0.67	0.5	1.34	0.12
Ethylparaben	1.27	0.5	2.54	0.40
Propylparaben	1.34	0.5	2.67	0.42
<i>Carbopol gel</i>				
Methylparaben	3.95	0.5	7.90	0.89
Ethylparaben	8.47	0.5	16.94	1.22
Propylparaben	9.22	0.5	18.44	1.26

The reported results represent the average of six independent experiments.

In the case of aqueous solutions, the diffusion coefficients of parabens ( $J_n$  values) are at least fivefold higher than in the case of viscous forms. In addition, the diffusion coefficients are a function of the substituent of preservatives: the higher the solubility, the higher the diffusion of parabens. The same behavior is reliable for the emulsions, in particular in the case of the O/W, where the normalized fluxes ( $J_n$ ) were 9.74, 2.8, 0.74  $\text{cm}/\text{h} \times 10^3$  for MP, EP, and PP, respectively. On the contrary, in the case of the hydrophilic gels, the higher the parabens solubility, the lower the diffusion coefficients. In particular, in the case of Carbopol gel,  $J_n$  values were 7.9, 16.94, and 18.44  $\text{cm}/\text{h} \times 10^3$  for MP, EP, and PP, respectively.

The different types of vehicles could account for the differences in the diffusion coefficients of the preservatives. MP and EP exhibit more affinity with the hydrophilic matrix of gels in comparison to PP, which, being more insoluble, is less retained, resulting in a higher diffusion coefficient. Moreover, in the case of Pemulen gel,  $J_n$  values are lower with respect to those exhibited by parabens incorporated in Carbopol gel. The trend can be attributed to the different lipophilicity of the resins; in fact, the acrylates/C10-30 alkyl acrylate crosspolymer, having C10-C30 chains, is able to dissolve parabens better than the carboxy vinyl polymer carbomer. This behavior is particularly evident for PP, which is much more retained by Pemulen gel in comparison to Carbopol gel ( $J_n$  of 2.67 versus 18.44  $\text{cm}/\text{h} \times 10^3$ ) due to the chemical affinity of PP to the acrylates/C10-30



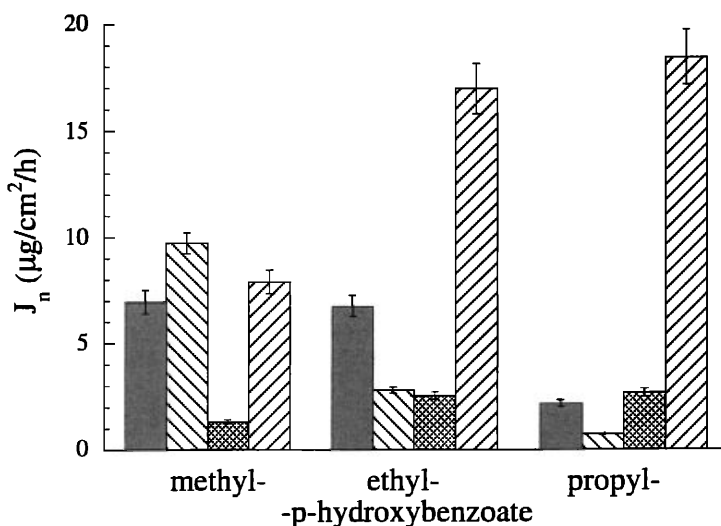


Figure 3. Comparative effect of different formulations on the *in vitro* diffusion of preservatives. Histograms represent the mean values ( $n = 6$ )  $\pm$  SD of  $\log J_n$ . Filled pattern: water-in-oil emulsion. Left diagonal lines pattern: oil-in-water emulsion. Criss-cross lines pattern: Pemulen gel. Right diagonal lines pattern: Carbopol gel.

alkyl acrylate (Pemulen) resin. The scarce attraction of Carbopol gel to parabens can suggest the use of acrylates/C10-30 alkyl acrylate crosspolymers (for instance carbomer 1342) instead of the carboxy vinyl polymer in order to produce well-preserved topical formulations, able to hold onto parabens.

Different considerations should be made in the case of the emulsions that, being two-phase systems, can dissolve parabens part in the oil and part in the aqueous phases. In this regard, the water phase concentrations of parabens in the different emulsions were determined by HPLC analysis. It was found that the aqueous phase concentrations of parabens in the W/O emulsion were 2.54, 1.12, and 0.6 mg/ml for MP, EP, and PP, respectively, while in the aqueous phase of the O/W emulsion, paraben concentrations were 2.6, 1.08, and 0.48 for MP, EP, and PP, respectively. The trend of paraben diffusion ( $J_n \text{ MP} > J_n \text{ EP} > J_n \text{ PP}$ , Table III) is in agreement with the water solubility of parabens, both in the W/O and O/W emulsions.

Considering diffusion of the different parabens, in the case of MP,  $J_n$  is higher in the O/W emulsion in comparison to the W/O emulsion (9.74 versus  $6.96 \text{ cm}/\text{h} \times 10^3$ ), probably because in the O/W emulsion, MP is mostly dissolved in the external aqueous phase and its diffusion in the receiving medium is faster. On the contrary, in the case of the more lipophilic EP and PP,  $J_n$  values are lower in the O/W emulsion (2.8 and  $0.74 \text{ cm}/\text{h} \times 10^3$  for EP and PP, respectively) in comparison to the W/O emulsion (6.74 and  $2.16 \text{ cm}/\text{h} \times 10^3$  for EP and PP, respectively). This trend can be related to the low solubilities in water of these parabens, mostly dissolved in the internal disperse phase of the O/W emulsion or in the continuous oil phase of the W/O emulsion. The different partition of parabens in the phases of the two emulsions can account for differences in  $J_n$  values.

In addition, one should consider the different compositions of the oil phases. In the O/W

emulsion, capric/caprylic triglyceride can dissolve EP and PP better than petrolatum present in the oil phase of the W/O emulsion. The EP and PP attraction to the oil phase of the O/W emulsion can justify their lower diffusion coefficients with respect to the W/O emulsion. These last considerations suggest that the lipophilic components of the matrix could represent a mean to hold on to parabens, promoting their preservative action in the formulation.

As an appendix to Table III, Table IIIa reports the results of a statistical analysis based on a paired *t*-test performed to compare the  $J_n$  of parabens for the different formulations. The results obtained with different formulations were significantly different (being

**Table IIIa**  
Statistical Analysis (paired *t*-test) of the  $J_n$  Values of Parabens Incorporated in the Different Formulations Reported in Table III

	Aqueous solution	W/O emulsion	O/W emulsion	Carbopol gel	Pemulen gel
<b>Methylparaben</b>					
Aqueous solution	—	$t = 45.42$ $p < 0.0001$	$t = 43.92$ $p < 0.0001$	$t = 39.3$ $p < 0.0001$	$t = 54.58$ $p < 0.0001$
W/O emulsion	$t = 45.40$ $p < 0.0001$	—	$t = 9.55$ $p = 0.0002$	$t = 3.37$ $p = 0.0198$	$t = 22.50$ $p < 0.0001$
O/W emulsion	$t = 43.90$ $p < 0.0001$	$t = 9.55$ $p = 0.0002$	—	$t = 13.11$ $p < 0.0001$	$t = 73.55$ $p < 0.0001$
Carbopol gel	$t = 39.30$ $p < 0.0001$	$t = 3.37$ $p = 0.0198$	$t = 13.11$ $p < 0.0001$	—	$t = 60.16$ $p < 0.0001$
Pemulen gel	$t = 54.58$ $p < 0.0001$	$t = 22.5$ $p < 0.0001$	$t = 73.55$ $p < 0.0001$	$t = 60.16$ $p < 0.0001$	—
<b>Ethylparaben</b>					
Aqueous solution	—	$t = 2.46.57$ $p < 0.0001$	$t = 119.30$ $p < 0.0001$	$t = 271.68$ $p < 0.0001$	$t = 51.53$ $p < 0.0001$
W/O emulsion	$t = 246.57$ $p < 0.0001$	—	$t = 26.93$ $p < 0.0001$	$t = 297.87$ $p < 0.0001$	$t = 66.15$ $p = 0.0001$
O/W emulsion	$t = 119.30$ $p < 0.0001$	$t = 26.93$ $p < 0.0001$	—	$t = 1.78$ n.s.	$t = 142.91$ $p < 0.0001$
Carbopol gel	$t = 271.68$ $p < 0.0001$	$t = 297.87$ $p < 0.0001$	$t = 1.78$ n.s.	—	$t = 101.46$ $p < 0.0001$
Pemulen gel	$t = 51.53$ $p < 0.0001$	$t = 66.15$ $p = 0.0001$	$t = 142.91$ $p < 0.0001$	$t = 101.46$ $p < 0.0001$	—
<b>Propylparaben</b>					
Aqueous solution	—	$t = 8.81$ $p < 0.0001$	$t = 104.67$ $p < 0.0001$	$t = 13.24$ $p < 0.0001$	$t = 105.61$ $p < 0.0001$
W/O emulsion	$t = 8.81$ $p < 0.0001$	—	$t = 7.37$ $p < 0.0001$	$t = 47.27$ $p < 0.0001$	$t = 2.83$ $p = 0.0001$
O/W emulsion	$t = 104.67$ $p < 0.0001$	$t = 7.37$ $p < 0.0001$	—	$t = 50.76$ $p < 0.0001$	$t = 10.23$ $p < 0.0001$
Carbopol gel	$t = 13.24$ $p < 0.0001$	$t = 47.27$ $p < 0.0001$	$t = 50.76$ $p < 0.0001$	—	$t = 50.88$ $p < 0.0001$
Pemulen gel	$t = 105.61$ $p < 0.0001$	$t = 2.83$ $p = 0.0178$	$t = 10.23$ $p < 0.0001$	$t = 50.88$ $p < 0.0001$	—

mostly  $p < 0.0001$ ), apart from EP included in the Carbopol gel versus the O/W emulsion.

Parabens, analogously to most of other preservatives, can induce sensitizing effects such as allergic contact dermatitis, especially when they are included in particular topical formulations such as eyedrops, or contour eyes (8,9,16). In this respect, the method here described is proposed to control the extent of paraben diffusion from topical preparations, with the aim of minimizing percutaneous absorption.

## CONCLUSIONS

In conclusion, the results of this study show that the experimental methods presented here could be proposed (a) in preformulatory studies aiming to determine the extent of paraben diffusion from topical formulations, (b) to control the availability of parabens to microbes, (c) to perform premarketing quality controls for dermatological and cosmetic products (e.g. creams, gels, and ointments), and (d) to assure batch-to-batch uniformity.

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