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Influence of Urea, Isopropanol, and Propylene Glycol on Rutin In Vitro Release from Cosmetic Semisolid Systems Estimated by Factorial Design

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Rutin, one of the major flavonoids found in an assortment of plants, was reported to act as a sun protection factor booster with high anti-UVA defense, antioxidant, antiaging, and anticellulite, by improvement of the cutaneous microcirculation. This research work aimed at evaluating the rutin in vitro release from semisolid systems, in vertical diffusion cells, containing urea, isopropanol and propylene glycol, associated or not, according to the factorial design with two levels with center point. Urea (alone and in association with isopropanol and propylene glycol) and isopropanol (alone and in association with propylene glycol) influenced significant and negatively rutin liberation in diverse parameters: flux (μg/cm^2.h); apparent permeability coefficient (cm/h); rutin amount released (μg/cm^2); and liberation enhancement factor. In accordance with the results, the presence of propylene glycol 5.0% (wt/wt) presented statistically favorable to promote rutin release from this semisolid system with flux = 105.12 ± 8.59 μg/cm^2.h; apparent permeability coefficient = 7.01 ± 0.572 cm/h; rutin amount released = 648.80 ± 53.01 μg/cm^2; and liberation enhancement factor = 1.21 ± 0.07.

Keywords rutin; propylene glycol; urea; in vitro release; isopropanol; factorial design

INTRODUCTION

Flavonoids exert a range of physiological activities, including antioxidant, anti-inflammatory, antibacterial, immune-stimulating, and antiviral effects (Gao, Xu, Chen, & Chen, 2003; Harborne & Williams, 2000; Walle, 2004). Reactive oxygen species have relationship with oxidative stress and studies demonstrated that the overproducing of these reactive species can aggravate oxidative stress and the result is the development of many diseases, such as heart injury, diabetes, cancer, and aging. Therefore, the use of antioxidants has been used to prevent the occurrence of such diseases (Wu et al., 2008). Attention has been drawn to the utilization of health care products based on natural active substances due to the presumable safe utilization, ecological orientation and preservation, and reduced ambient impact (Rolim et al., 2006).

Rutin (quercetin-3-rutinoside) is one of the major flavonoids found in an assortment of plants. The attention regarding on the application of this flavonol-type is due to its well-recognized health benefits. This natural active substance can be used as natural pigment, antioxidant, stabilizer, food preservative, UV absorber in food, animal feed, pharmaceutical, cosmetic, and chemical industries (Calabrò et al., 2005; Kim et al., 2005). As a topical active compound, rutin was reported to act as a sun protection factor (SPF) booster with high anti-UVA defense (Velasco et al., 2008), antioxidant, antiaging, and anticellulite, by improvement of the cutaneous microcirculation (Baby et al., 2007; Nishikawa et al., 2007).

Active substances topically applied must be able to be released from topical preparations and reach the action site, or interact with, in adequate concentration to exert their physiological and biological activities. Therefore, rutin, when incorporated in cosmetic products or pharmaceutical semisolid dosage forms must be able to be released from the formulations to the epidermis.

In vitro diffusion techniques are widely used in the assessment of release and percutaneous absorption of topically applied active compounds and this research work aimed at evaluating the rutin in vitro release from cosmetic semisolid systems containing urea, isopropanol, and propylene glycol, as penetration enhancers, associated or not and in distinct proportions, according to a design of experiments by factorial design with two levels (DOE 2^k) with center point.

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EXPERIMENTAL PROCEDURE

Semisolid Systems and Design of Experiments

The development of the cosmetic formulations involved the elaboration of semisolid systems (O/W emulsions) containing rutin 5.0% (wt/wt) and urea, isopropanol and propylene glycol, as penetration enhancers for cosmetic purpose, either in association or not and in different proportions, according to factorial design with two levels (DOE 2^k) with center point, employing three concentration values: minimum (without addition of the penetration enhancer), median (2.5%, wt/wt), and maximum (5.0%, wt/wt). Tables 1 and 2 describe the design of experiments and Table 3, the qualitative/quantitative composition of the cosmetic semisolid systems. The enhancers were selected according to their compatibility with topical formulations, biocompatibility with the skin, and their concentrations were in accordance with the safety use in semisolid systems intended for cosmetic purposes. Experiments were performed in genuine replicates of three and in random order to eliminate systematical errors (Gabrielsson, Lindberg, & Lundstedt, 2002).

TABLE 1
Factorial Design with Two Levels (DOE 2^k) with Center Point[a]

Semisolid Systems	Urea	Isopropanol	Propylene Glycol
E-1	−1	−1	−1
E-2	+1	−1	−1
E-3	−1	+1	−1
E-4	+1	+1	−1
E-5	−1	−1	+1
E-6	+1	−1	+1
E-7	−1	+1	+1
E-8	+1	+1	+1
E-9	0	0	0

[a]Factors: 3. Runs: 36. Blocks: 1. Base design: 3;8. Genuine replicates: 3; Center points: 12.

TABLE 2
Codification of the Factorial Design of the Semisolid Systems According to the Proportions of the Enhancers in Three Concentration Values

Codification	Urea (%, wt/wt)	Isopropanol (%, wt/wt)	Propylene Glycol (%, wt/wt)
−1	0	0	0
0	2.5	2.5	2.5
+1	5.0	5.0	5.0

In Vitro Release Experiments

Prior to experiment performing, cellulose acetate membranes with an average pore size of 0.45 μm (GE® Cellulose Acetate Membranes, 25.0 mm, GE® Osmonics, Minnetonka, MN, USA) were treated to achieve standardization. Membranes were adequately cut and washed in distilled water. Following, they were immersed in distilled water at 100°C for 60 s (Haigh & Smith, 1994; Valenta, Nowack, & Bernkop-Schnürch, 1999). This treatment was performed three times and, at each time, water volume was discarded and a new volume at same temperature was used. The standardization process was performed to remove additives that might be present on the membranes, such as plasticizers and preservatives, among others, that could affect rutin in vitro release and quantitative determination.

All release experiments were performed using an infinite dosing technique. Vertical diffusion cells (Hanson Research®, Chatsworth, CA, USA) consisted of a donor and acceptor chambers between which the membranes were positioned. Diffusion area was 1.77 cm^2 with 15.0-mm orifice and acceptor chamber volume was 7.0 mL. The acceptor medium consisted of a 1:1 (vol/vol) water–ethanol 99.5% (LabSynth, São Paulo, Brazil) solution, to ensure sink conditions (Reichling, Landvatter, Wagner, Kostka, & Schaefer, 2006; Saija et al., 1998), and it was constantly mixed with a magnetic stirring bar (300 rpm), except during the period of the sample collecting. Diffusion experiments were conducted at 37.0 ± 0.5°C (Li & Birt, 1996; Ngawirunpat, Opanasopit, & Prakongpan, 2004). Amounts of 300.0 mg of semisolid systems were applied to the donor compartment. Samples of 1.0 mL were collected over 6 h, in predetermined intervals, and immediately replaced with same volume of fresh solution. Samples were analyzed by spectrophotometry determination at 410.0 nm. In vitro release experiments were performed in replicates of three.

Quantitative Determination of Rutin Released in the Acceptor Medium

For quantitative determination of rutin released in the acceptor fluid, the spectrophotometric method (Beckman DU-640 UV-visible Spectrophotometer with a 1.0 cm quartz cuvette; Beckman, Fullerton, CA, USA) at 410.0 nm was used, previously validated for linearity, specificity, lacking of interferents, limits of detection and quantification, recovery, precision and accuracy. To the 1.0 mL collected sample from acceptor chamber was added 1.5 mL of the mixture of spectrophotometric solvent and blank consisted of distilled water–ethanol 99.5%–sodium hydroxide 0.25 M (1:1:0.025) (LabSynth). The presence of sodium hydroxide was required to provoke a bathocromic shift of the rutin (Valenta et al., 1999), intensifying its absorbance obtained at 410.0 nm.

TABLE 3
Qualitative/Quantitative Composition of the Semisolid Systems

Components		Composition (%, wt/wt)
Cetearyl alcohol (and) dicetyl phosphate (and) ceteth-10 phosphate	Crodafos® CES (Croda)	2.50
Diisopropyl adipate	Ceraphyl® 230 (ISP)	1.00
Isopropyl myristate	Crodamol® IPM (Croda)	2.00
BHT	—	0.10
Disodium EDTA	Uniquelan® NA2S (Chemyunion)	0.10
Xanthan gum	Rhodicare® S (Rhodia)	1.00
2-Bromo-2-nitropropane-1,3-diol	Protectol® BN (Basf)	0.01
Aqua (q.s.)	—	100.00
Urea	LabSynth	0, 2.50, and/or 5.00
Isopropanol	LabSynth	
Propylene glycol	LabSynth	
PEG-8	Carbowax® 400 (Dow)	5.00
Rutin	Rutina (Henrifarma)	5.00

Analysis of the In Vitro Release

Rutin release profiles were determined experimentally by the diffusion studies and involved the calculation of the following parameters:

A. Rutin amount released (μg/cm^2)
B. Liberation model kinetic : linear regression coefficient (R^2) (Kalia & Guy, 2001; Larrucea, Arellano, Santoyo, & Ygartua, 2001; Reichling et al., 2006; Shah, Kaka, Tenjarla, Lau, & Chow, 1994).
C. Flux (J, μg/cm^2.h) (Shah, 1993),

$$J = \frac{DK_{V/M}C_R}{E} = CA \quad (1)$$

where D is the diffusion coefficient, $K_{V/M}$ is the vehicle/membrane partition coefficient, C_R is the rutin concentration, E is the membrane thickness, and CA is the slope from the regression line of rutin amount released in function of time.

D. Lag time (L_T, h) (Parks, Cleek, & Bunge, 1997; Shah, 1993; Shah et al., 1994)

$$L_T = \frac{E^2}{6D} \text{ (intersection with the } x\text{-axis)} \quad (2)$$

E. Apparent permeability coefficient (P, cm/h) (Reichling et al., 2006; Shah, 1993; Shah et al., 1994).

$$P = \frac{K_{V/M}D}{E} = \frac{J}{C_R} \quad (3)$$

F. Diffusion coefficient (D, cm^2/h) (Shah, 1993; et al., 1994).

$$D = \frac{E^2}{6L_T} \quad (4)$$

G. Semisolid system/membrane partition coefficient ($K_{V/M}$) (Shah, 1993).

$$K_{V/M} = \frac{JE}{DC_R} \quad (5)$$

H. Liberation enhancement factor (LEF): quotient of rutin flux in presence of the penetration enhancers by the rutin flux without enhancers (Bonina & Montenegro, 1994).

Data were statistically treated to achieve the effects or interactions of the urea, isopropanol and propylene glycol on the in vitro release of rutin and one way ANOVA followed by Tukey Multiple Comparison Test was also used (Graphpad Prism® 5.00, San Diego, CA, USA).

RESULTS AND DISCUSSION

In vitro release experiments through synthetic model membranes allow the determination of maximum liberation of an active compound from semisolid dosage forms, which permit to characterize and differentiate formulations, to evaluate the production quality and batch-to-batch uniformity, and to compare the performance of innovative products with those available to the consumers. However, it is indispensable to attend prerequisites during the experiments, like: infinite dosing, no

rate limitation by the model membrane separating the donor and receptor compartments, and the receptor phase must act as a perfect sink to the active substance (Guy & Hadgraft, 1990; Reichling et al., 2006). The cosmetic semisolid systems were developed with pH values ranging from 6.5 to 6.9 and apparent viscosity, from 3,243 to 4,546 cP.

The rutin in vitro release profiles (kinetic liberation) from the semisolid systems, containing urea, isopropanol, and propylene glycol, associated or not and in distinct proportions, according to factorial design with two levels (DOE 2^k) with center point, and employing cellulose acetate as a model membrane, are represented in Figures 1 and 2, as rutin amount released (μg/cm^2) in function of time (h) and in function of square root of time (√h). Results obtained of rutin release experiments from semisolid dosage forms were treated mathematically and statistically to determine the liberation kinetic model employing linear regression.

All semisolid systems (E-1 to E-12) presented zero-order kinetic model for rutin liberation in agreement with the linear correlation coefficient (R^2) higher than .9923 ± .0019 to the relationship between rutin amount released (μg/cm^2) in function of time (h), in Table 4. In accordance with Shah et al. (1994) and Larrucea et al. (2001), it is considered an adequate value of R^2 to determine the kinetic model values equals or superiors than .997.

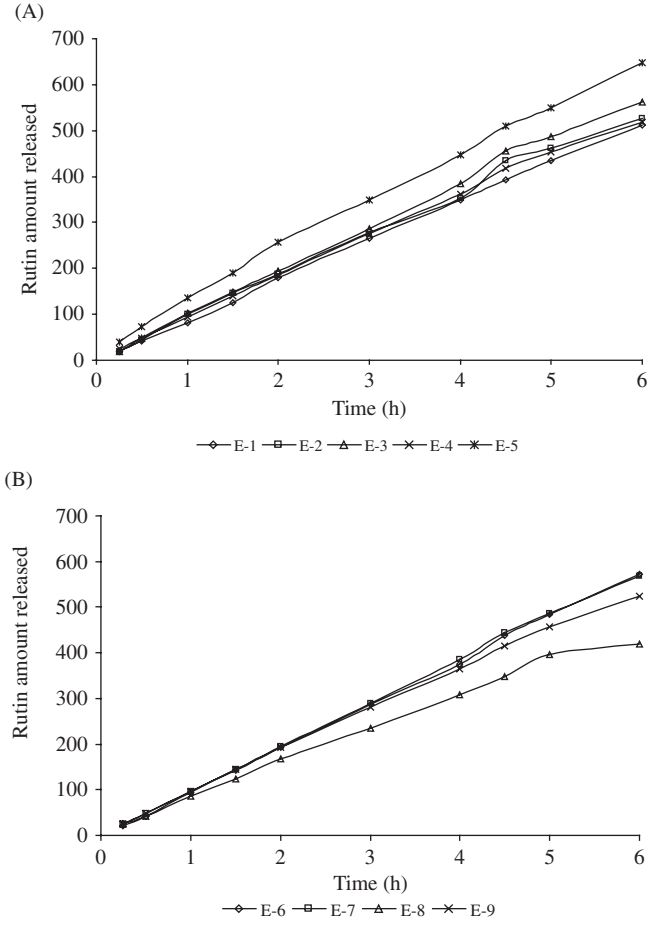

FIGURE 1. Rutin amount released (μg/cm^2) in function of time (h). Semisolid systems E-1 to E-5 (A) and E-6 to E-9 (B).

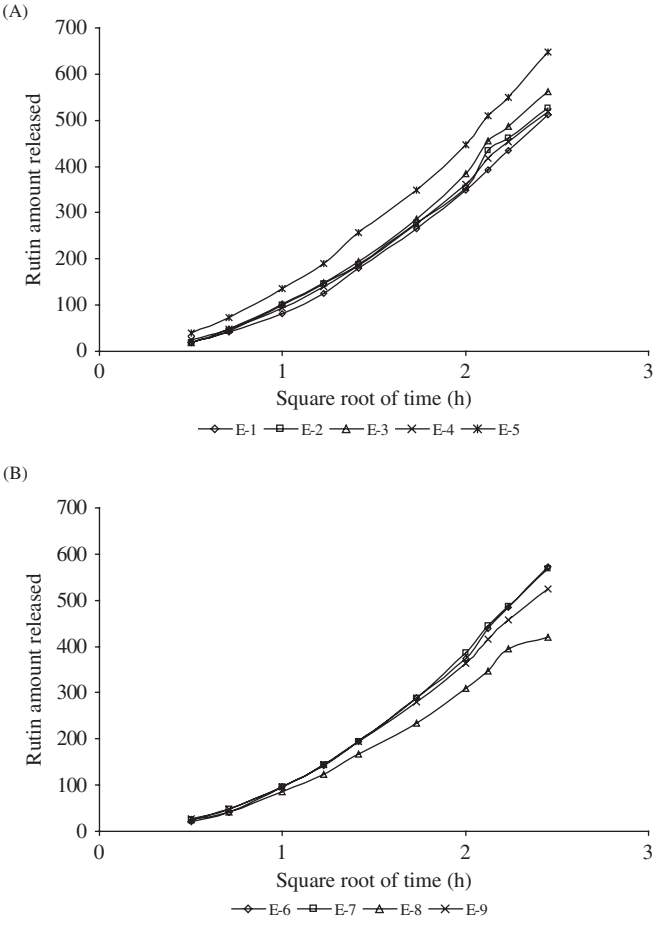

FIGURE 2. Rutin amount released (μg/cm^2) in function of square root of time (√h). Semisolid systems E-1 to E-5 (A) and E-6 to E-9 (B).

Even though it was observed R^2 values inferior than .997 to semisolid system E-2 (.9948 ± .0026) and E-8 (.9923 ± .0019), the relationship of rutin liberation in function of square root of time resulted in R^2 values not exceeding .9808 ± .0051, suggesting that Higuchi kinetic model was not applied to these diffusion experiments. Exposed so, it was verified that rutin transport across the acetate membrane had the active compound dissolution as a limitation factor to the diffusion process (Kalia & Guy, 2001; Larrucea et al., 2001; Shah et al., 1994) which corroborated with the low permeability of this flavonol.

Rutin in vitro release profiles were complemented with the determination of the parameters: flux (J, μg/cm^2.h), lag time (L_T, h), apparent permeability coefficient (P, cm/h), diffusion coefficient (D, cm^2/h), semisolid system/membrane partition coefficient ($K_{V/M}$), and LEF, described in Table 4.

TABLE 4
Parameters of Rutin In Vitro Release: Rutin Amount Released (μg/cm^2), Liberation Kinetic Model, Flux (J, μg/cm^2.h), Lag Time (L_T, h), Apparent Permeability Coefficient (P, cm/h), Diffusion Coefficient (D, cm^2/h), Semisolid System/Membrane Partition Coefficient ($K_{E/M}$), and Liberation Enhancement Factor (LEF)

Parameters	E-1	E-2	E-3	E-4	E-5	E-6	E-7	E-8	E-9
J (μg/cm^2.h)	86.82 ± 4.71	89.97 ± 4.53	96.09 ± 8.34	88.16 ± 1.03	105.12 ± 8.59	96.58 ± 10.76	96.18 ± 7.42	72.36 ± 3.66	88.26 ± 8.93
L_T (× 10^{-3} h)	8.45 ± 1.50	−63.55 ± 39.58	−21.09 ± 6.81	−85.87 ± 8.16	−269.17 ± 46.74	25.34 ± 67.88	−13.91 ± 1.06	−197.09 ± 12.82	−108.23 ± 137.50
P (× 10^{-3} cm/h)	5.79 ± 0.314	6.00 ± 0.302	6.41 ± 0.556	5.88 ± 0.069	7.01 ± 0.572	6.44 ± 0.717	6.41 ± 0.495	4.82 ± 0.244	5.88 ± 0.596
D (× 10^{-6} cm^2/h)	1.27 ± 0.225	−9.53 ± 5.94	−3.16 ± 1.02	−12.88 ± 1.22	−40.38 ± 7.01	3.8 ± 0.102	−2.09 ± 0.159	−29.56 ± 1.92	−16.23 ± 20.62
$K_{V/M}$	140.40 ± 30.30	−31.65 ± 30.89	−65.07 ± 20.19	−13.77 ± 1.37	−5.29 ± 0.86	−68.75 ± 87.83	−92.92 ± 14.07	−4.91 ± 0.41	−32.48 ± 31.94
Zero-order kinetic	0.9991 ± 0.0003	0.9948 ± 0.0026	0.9971 ± 0.0010	−0.9979 ± 0.0002	0.9973 ± 0.0022	0.9989 ± 0.0001	0.9991 ± 0.0003	0.9923 ± 0.0019	0.9965 ± 0.0035
Higuchi kinetic	0.9742 ± 0.0017	0.9754 ± 0.0078	0.9744 ± 0.0023	0.9769 ± 0.0008	0.9808 ± 0.0051	0.9716 ± 0.0077	0.9717 ± 0.0004	0.9791 ± 0.0023	0.9770 ± 0.0045
LEF	—	1.04 ± 0.02	1.11 ± 0.05	1.07 ± 0.05	1.21 ± 0.07	1.11 ± 0.08	1.11 ± 0.05	0.83 ± 0.03	1.02 ± 0.10
Rutin (μg/cm^2)	512.17 ± 26.24	527.47 ± 24.62	561.34 ± 52.32	518.67 ± 6.59	648.80 ± 53.01	571.61 ± 60.49	567.67 ± 41.31	419.76 ± 17.98	523.58 ± 80.05

E-1 to E9, semisolid systems.

Flux values were on the interval between 72.36 ± 3.66 and 105.12 ± 8.59 μg/cm^2.h, respectively, for rutin from semisolid systems E-8 and E-5. E-8 flux was statistically different from E-3, E-5, E-6, and E-7 ($p < .0011$). Calculated flux values for all other systems were homogeneous.

Calculated lag time values were near to zero (–269.17 ± 46.79 × 10^{-3} to 25.34 ± 67.88 × 10^{-3} h) to all formulations and apparent permeability coefficient presented values were between 4.82 ± 0.244 × 10^{-3} and 7.01 ± 0.572 × 10^{-3} cm/h to E-8 and E-5, respectively.

The semisolid system/membrane partition coefficients were in the interval –92.92 ± 14.07 (E-7) and 140.40 ± 30.30 (E-1). E-5 presented the highest enhancement factor for rutin in vitro release (1.21 ± 0.07) and E-8 (semisolid system containing urea 5.0%, isopropanol 5.0%, and propylene glycol 5.0%, wt/wt) generated the inferior LEF value, equal to 0.83 ± 0.03 ($p < .0001$).

Rutin amount released (μg/cm^2) in function of time (h), after 6 h of experiment, was statically more expressive to E-5, analyzed by one-way ANOVA ($p < .0002$) followed by Tukey Multiple Comparison Test, possessing value equal to 648.80 ± 53.01 μg/cm^2. Therefore, the presence of propylene glycol 5.0% (wt/wt) presented a tendency to be more suitable to promote the rutin release from the semisolid system, while, unexpectedly, the formulation containing the association of urea, isopropanol and propylene glycol at maximum concentration (E-8) resulted in release of inferior magnitude, equal to 419.76 ± 17.98 μg/cm^2. It may be suggested that the inhibitor effect of rutin release by E-8 could have involved the increase of the active compound affinity to the semisolid system. Topical penetration enhancers of hydrophilic type, such as dimethyl sulfoxide (DMSO), pyrrolidones, dimethylacetamide, dimethylformamide, urea, propylene glycol, and other glycols, increase the extent of hydration of the membranes used in topical diffusion experiments, including the stratum corneum. Urea, one of the naturally occurring moisturizing factors (NMF), is known to possess water-binding capacity and this behavior could be associated with the increase affinity of rutin into the emulsions containing urea (Lippold & Hackemüller, 1990) and its associations.

The statistical treatment, established on the design of experiments by factorial design with two levels (DOE 2^k) with center point (Box, Hunter, & Hunter, 2005), evaluated the effect intensity of the urea, isopropanol, and propylene glycol, associated or not and in distinct proportions, on rutin in vitro release parameters. Statistical experimental design methodologies are powerful, efficient, and systematic tools in the design of pharmaceutical and cosmetic forms, allowing a rational study of the influence of formulation excipients on the selected responses with a shortening of the experiment time and an improvement in the research and development work. The objective of the experimental design is to plan and conduct experiments to extract the maximum amount of information from the collected data in the smallest number of experimental runs. Statistical analysis of data generated from the experiment clearly establishes the relationship between the measured parameters of interest (responses) and the process parameters (factors) being studied. Factors may have individual, simple effects on the response or may have effects that are interdependent, referred to as interaction effects. For statistical validation and estimative assay variability, the center point is of utmost relevance, as adopted for this research work. The center point is obtained from a run in which all factors are set in the middle of their range and it is replicated several times (typically 3–10) to provide an estimate of the variance of the response (Furlanetto, Cirri, Maestrelli, Corti, & Mura, 2006; Gabrielsson et al., 2002; Lutz et al., 1996; Martinello, Kaneko, Velasco, Taquedo, & Consiglieri, 2006).

Table 5 describes the effects caused by the enhancers on rutin in vitro release from the semisolid systems and Figures 3–9 illustrate the effects through the contour plots. According to results obtained for flux by factorial design, it was verified that the presence of urea alone, isopropanol alone, urea + isopropanol, and isopropanol + propylene glycol influenced significant and negatively the rutin transport across the cellulose acetate membrane per unit of area and time, in different levels of effects or interactions. Exceptionally, the propylene glycol alone did not provoke a result statistically significant; consequently, it did not promote the flux value reduction. The more intense effect that reduced the active transport across the membrane per unit of area and time occurred with the association of isopropanol + propylene glycol. This phenomenon may be a result of the decrease of the thermodynamic activity of the rutin on the semisolid system due to the solvent effect provoked by the presence of isopropanol and propylene glycol. Theoretically, the thermodynamic activity of a diffusant will be maximum in a saturated medium and a maximum flux of penetration might be expected and, ideally, the penetration rate will be proportional to the degree of saturation (Dugard & Scott, 1986). According to Figure 3, there was an evidence for the flux maximization when the isopropanol has its concentration reduced.

Lag time was demonstrated for urea alone and isopropanol alone; and for the associations between urea + propylene glycol and isopropanol + propylene glycol to be homogenous, that is, these substances did not cause effects or interactions statistically significant on this parameter.

Propylene glycol alone, urea + isopropanol, and urea + isopropanol + propylene glycol offered significant results, generating decreased lag time values which proved that these substances influenced positively the time for rutin to initiate its release to the receptor phase linearly in function of time. However, all lag time values for rutin liberation were near to zero, as presented in Table 4. Urea + isopropanol + propylene glycol generated a decrease on the lag time value with more intensity when compared with propylene glycol alone.

The effect analysis of urea, isopropanol, and propylene glycol for the apparent permeability coefficient, demonstrated that urea alone, isopropanol alone, urea + isopropanol, and isopropanol + propylene glycol provoked a significant decrease on

TABLE 5
Analysis of the Effect of the Enhancers: Flux (μg/cm^2.h), Lag Time (h), Apparent Permeability Coefficient (cm/h), Diffusion Coefficient (cm^2/h), Semisolid System/Membrane Partition Coefficient ($K_{V/M}$), Rutin Amount Released (μg/cm^2), and Liberation Enhancement Factor (LEF)

Parameters	Enhancers	Effect	Coefficient	Standard Error	t	p
	Urea (1)	−9.28	−4.64		−2.50	.018*
	Isopropanol (2)	−6.42	−3.21		−1.73	.094*
	Propylene glycol (3)	2.30	1.15		0.61	.540
Flux (μg/cm^2.h)	1 and 2	−6.58	−3.29	1.88	−1.77	.086*
	1 and 3	−6.89	−3.44		−1.85	.073*
	2 and 3	−10.15	−5.07		−2.73	.010*
	1, 2, and 3	−1.05	−0.52		−0.28	.783
Lag time (h)	Urea (1)	−0.0063	−0.003		−0.15	.876
	Isopropanol (2)	−0.0047	−0.002		−0.11	.907
	Propylene glycol (3)	−0.0731	−0.036		−1.80	.081*
	1 and 2	−0.1176	−0.058	0.02	−2.89	.007*
	1 and 3	0.0620	0.031		1.52	.137
	2 and 3	0.0211	0.010		0.52	.605
	1, 2, and 3	−0.1212	−0.060		−3.54	.001*
Apparent permeability coefficient (cm/h)	Urea (1)	−0.00061	−0.0003	0.0001	−2.50	.018*
	Isopropanol (2)	−0.00042	−0.0002		−1.73	.093*
	Propylene glycol (3)	0.00015	0.0001		0.61	.540
	1 and 2	−0.00043	−0.0002		−1.77	.086*
	1 and 3	−0.00046	−0.0002		−1.85	.073*
	2 and 3	−0.00067	−0.0003		−2.73	.010*
	1, 2, and 3	−0.00007	−0.0000		−0.28	.783
Diffusion coefficient (cm^2/h)	Urea (1)	−0.000001	−0.000000		−0.15	.875
	Isopropanol (2)	−0.000001	−0.000000		−0.11	.905
	Propylene glycol (3)	−0.000011	−0.000005		−1.80	.081*
	1 and 2	−0.000018	−0.000009	0.000003	−2.89	.007*
	1 and 3	0.000009	0.000005		1.52	.136
	2 and 3	0.000003	0.000002		0.52	.605
	1, 2, and 3	−0.000018	−0.000009		−3.54	.001*
Semisolid system/membrane partition coefficient ($K_{V/M}$)	Urea (1)	−24.05	−12.02		−1.05	.298
	Isopropanol (2)	−52.84	−26.42		−2.32	.027*
	Propylene glycol (3)	−50.44	−25.22		−2.22	.034*
	1 and 2	93.70	46.85	11.35	4.12	.000*
	1 and 3	36.32	18.16		1.60	.120
	2 and 3	40.95	20.47		1.80	.081*
	1, 2, and 3	−17.97	−8.99		−0.78	.442
Rutin amount released (μg/cm^2)	Urea (1)	−63.11	−31.55		−1.05	.012*
	Isopropanol (2)	−48.14	−24.07		−2.32	.051*
	Propylene glycol (3)	22.04	11.02		−2.22	.360
	1 and 2	−32.17	−16.08	11.86	4.12	.185
	1 and 3	−49.42	−24.71		1.60	.046*
	2 and 3	−68.33	−34.16		1.80	.007*
	1, 2, and 3	−3.18	−1.59		−0.13	.897
LEF	Urea (1)	−0.106	−0.053		−2.93	.006*
	Isopropanol (2)	−0.073	−0.036		−2.02	.051*
	Propylene glycol (3)	0.025	0.012		0.71	.480
	1 and 2	−0.074	−0.037	0.018	−2.06	.047*
	1 and 3	−0.080	−0.040		−2.21	.034*
	2 and 3	−0.116	−0.058		−3.22	.003*
	1, 2, and 3	−0.012	−0.006		−0.33	.740

*Statistically different, $p < .1$.

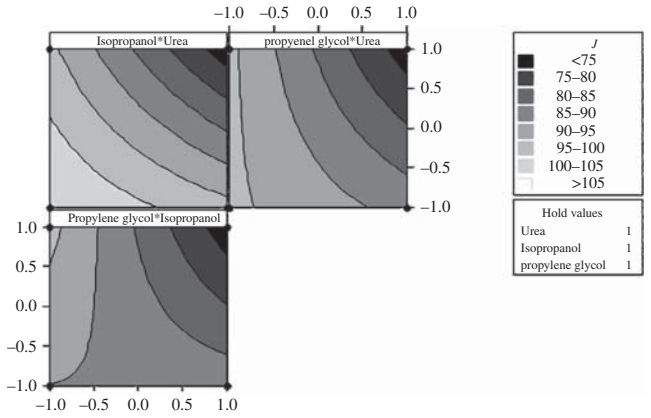

FIGURE 3. Contour plot for flux (μg/cm^2.h).

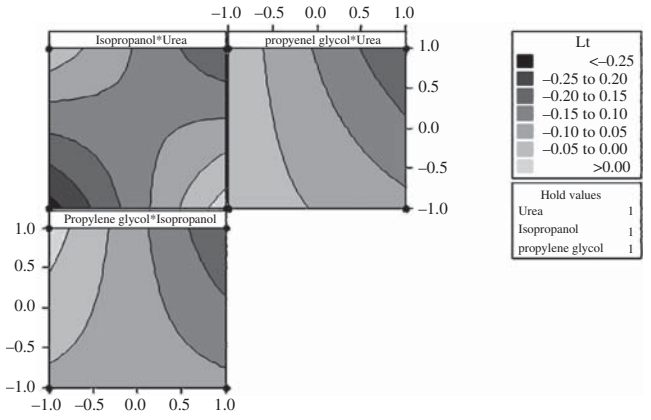

FIGURE 4. Contour plot for lag time (h).

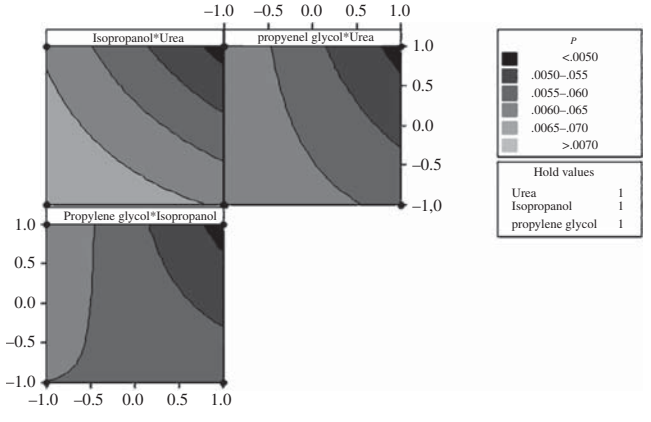

FIGURE 5. Contour plot for apparent permeability coefficient (cm/h).

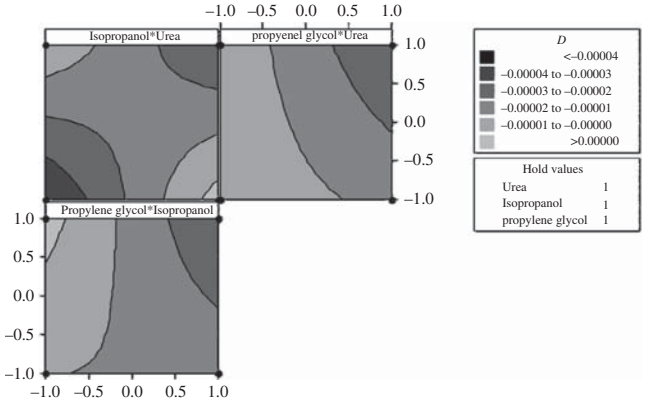

FIGURE 6. Contour plot for diffusion coefficient (cm^2/h).

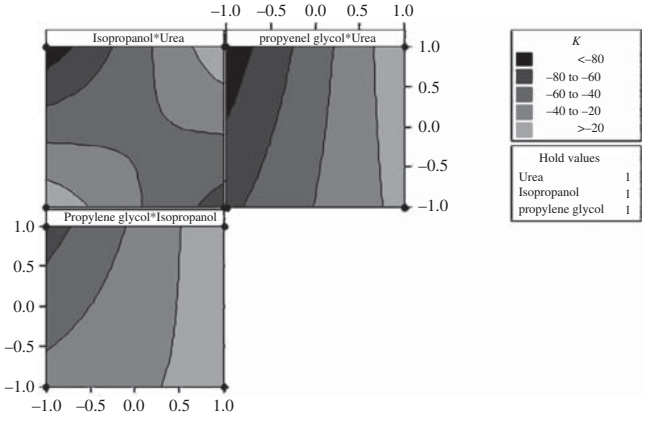

FIGURE 7. Contour plot for semisolid system/membrane partition coefficient ($K_{V/M}$).

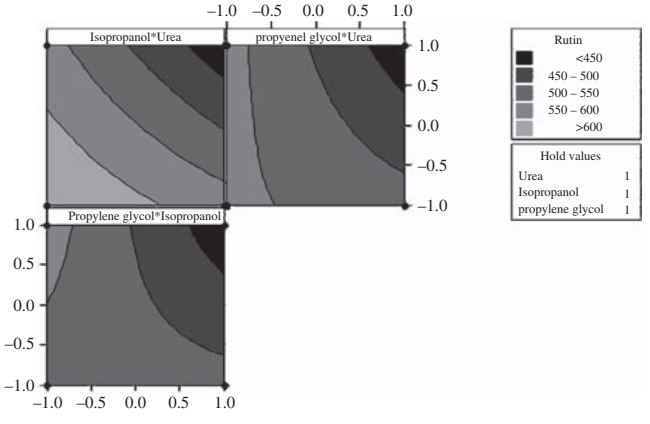

FIGURE 8. Contour plot for rutin amount released (μg/cm^2).

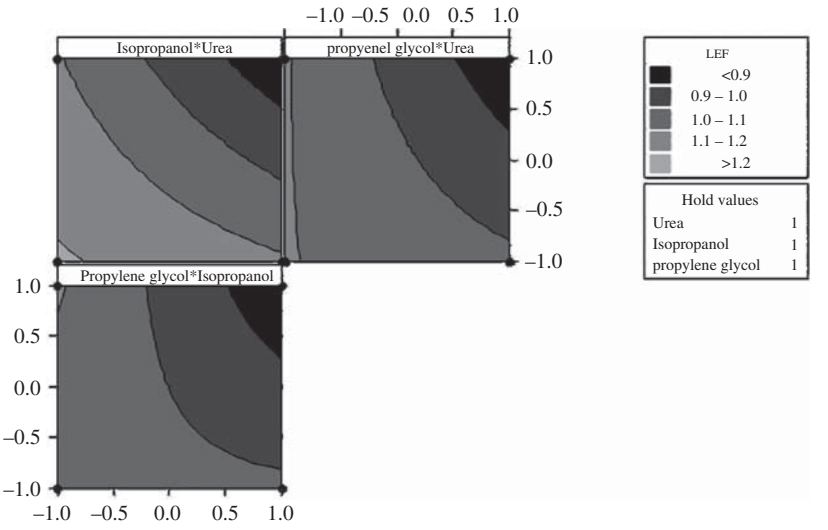

FIGURE 9. Contour plot for liberation enhancement factor (LEF).

this parameter values, indicating that the presence of these components and their associations on the semisolid systems containing rutin was not adequate due to the inhibition of the active compound transport to the receptor phase, reducing the flux. The effect caused by propylene glycol alone did not present significant response; hence, it did not contribute with the expressive reduction of the apparent permeability coefficient which is directly proportional to the flux.

In relation to diffusion coefficient, the presence of propylene glycol alone, urea + isopropanol, and urea + isopropanol + propylene glycol propitiated significant decrease on this parameter value. All other components, alone and in association, obtained homogeneity of the results. The effect of these penetration enhancers may have increased the structuring of water on the semisolid systems as was demonstrated by the decrease of the apparent diffusion coefficient (Lippold & Hackemüller, 1990). Although propylene glycol promoted better results on rutin release, the negative effect on this parameter suggested that this enhancer alone and in association with urea and isopropanol acted unfavorably as the known mode of action of penetration enhancers, as they decreased the rutin diffusivity, reflecting in a difficulty of this active compound to propagate through the membrane as, probably, a function of the molecular structure of the diffusant interaction with the presence of those enhancers and their association (Saija et al., 1998).

It was verified for semisolid system/membrane partition coefficient that isopropanol alone and propylene glycol alone presented significant effect on the value reduction while associations like urea + isopropanol and isopropanol + propylene glycol promoted an increase on this parameter value. Urea alone and in association with propylene glycol did not obtain effects statistically significant. The relative affinity of the rutin for the membrane and vehicle was evaluated by the determination of the semisolid system/membrane partition coefficient. Usually, high values of this parameter indicate that the vehicle possess poor affinity for the drug and a low value may be an evidence of high degree of mutual interaction, reflecting a tendency of the active to remain in the vehicle (Puglia et al., 2006).

Rutin in vitro release was negative and significant influenced, that is, inhibited, by the presence of urea alone, isopropanol alone, urea + propylene glycol, and isopropanol + propylene glycol. A factor for the elucidation of the decrease in rutin release could be the creation of hydrogen bond between the rutin molecules and these hydrophilic substances. The presence of such penetration enhancers probably originated intermolecular H-bond that resulted in the decrease in concentration of free, unbounded rutin molecules able to penetrate the membrane easier than the molecules involved in intermolecular interactions (Arct, Oborska, Mojski, Binkowska, & Swidzikowska, 2002).

The presence of propylene glycol alone and urea + isopropanol did not generate effect statistically significant for the rutin amount released ($\mu g/cm^2$), accordingly, they did not inhibit the rutin liberation from the semisolid systems.

LEF presented negatively influenced when formulations contained urea alone, urea + isopropanol, urea + propylene glycol, and isopropanol + propylene glycol. Only propylene glycol did not result in an effect statistically significant; therefore, it did not present tendency to reduce the enhancement factoring comparison with the semisolid system not added with urea, isopropanol, and propylene glycol. The rutin in vitro cutaneous permeation was tested (data not shown) with identical experiment conditions, substituting the synthetic membrane with an alternative biomembrane model constituted by pure stratum corneum obtained from *Crotalus durissus* shed skin (Baby et al., 2006a, b), for the semisolid system containing

propylene glycol 5.0% (wt/wt). Although propylene glycol promoted better rutin release through cellulose acetate membrane, the active substance presented low permeability and it was not able to permeate across the stratum corneum, being retained 0.931 ± 0.0391 μg/mg (μg rutin/mg alternative model biomembrane). Propylene glycol is considered as a solvent or a cosolvent that may improve the rutin solubility in the vehicle and it also can alter the stratum corneum structure, thereby modifying percutaneous permeation. It was reported for the propylene glycol its ability to permeate the skin, carrying the active molecules across, although, for this research, this enhancer was not able to promote rutin percutaneous permeation, possibly due to an inadequate concentration or to the flavonol interaction with the skin (Baby, 2007; Larrucea et al., 2001). According to these results, rutin applied topically is suggested to exert its biological activities only at the skin surface or at the upper layers of the epidermis (Velasco et al., 2008)

CONCLUSIONS

The rutin in vitro release from semisolid systems presented herein, employing cellulose acetate as synthetic model membrane, presented liberation kinetic of zero order. Urea (alone and in association with isopropanol and propylene glycol) and isopropanol (alone and in association with propylene glycol) influenced significant and negatively the rutin liberation from the semisolid systems in diverse parameters, like: flux (μg/cm^2.h); apparent permeability coefficient (*P*, cm/h); rutin amount released (μg/cm^2)—except for urea + isopropanol; and LEF. In accordance with the exposed and considering that the meaningful data obtained from a topical release experiment is usually the active substance diffused through the model membrane into the receptor phase (Shah, 1993), the presence of propylene glycol 5.0% (wt/wt) presented statistically favorable to promote rutin release from this semisolid system.

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