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Development of a perillyl alcohol topical cream formulation

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Abstract

Perillyl alcohol (POH) is a relatively non-toxic agent that has been shown to be a promising anticancer monoterpene in preclinical models. Studies have indicated that topical application of POH may prove to be effective as a skin cancer chemoprevention therapy. The main aim of this study was to determine the influence of several factors on the stability of POH in solution and develop a topical formulation of POH. During preformulation, the influence of pH, temperature, ionic strength, and organic solvents, on the stability of POH was evaluated at four different temperatures: 4, 25, 37, and 48 °C. POH was found to degrade under acidic conditions with degradation following apparent first-order kinetics. A hydrophilic topical cream formulation of POH was developed and prepared for toxicology and clinical studies. A reverse phase gradient HPLC method was developed to quantitate POH in the complex formulation. Stability studies of the formulation and a placebo were performed and the formulation was found to be physically and chemically stable over a period of 1 year at 4 and 25 °C.

Keywords: Perillyl alcohol; Stability; Topical cream formulation; Reverse phase HPLC assay

1. Introduction

Perillyl alcohol (POH) (Fig. 1), is a cyclic monoterpene (*p*-metha, 1, 7-diene-6-ol or 4-isoprophenylcylcohexenecarbinol) and is found in the essential oils of lavendin, peppermint, spearmint, sage, cherries, lemongrass, caraway, and celery seeds. Perillyl alcohol has a calculated octanol/water partition coefficient of 2.38 and is an oil at room temperature. The intrinsic solubility of POH in water is about 115 μg/ml. POH is a relatively non-toxic agent that has been shown in preclinical trials to have therapeutic and chemopreventive activity against a wide variety of cancers. In animal studies it has been shown to regress pancreatic, mammary, and liver tumors, and as a chemopreventive agent for colon, skin, and lung cancers (Wattenberg, 1983; Elson et al., 1988; Haag et al., 1992; Crowell and Gould, 1994; Mills et al., 1995). It has also been shown to inhibit photocarcinogenesis in a non-melanoma model of mouse skin carcinogenesis and UVB-induced skin carcinogenesis. Barthelman et al. (1998), have shown that POH causes a reduction in UVB-induced non-melanoma tumors. Prevatt et al. (2002), found that topically applied POH is effective as a chemopreventive agent in melanoma. As a result, the topical application of POH may prove to be a safe and effective skin cancer chemoprevention therapy.

Because of the increasing incidence of skin cancer, effective chemoprevention strategies need to be developed. There is a need to develop oral and topical agents that will complement primary skin cancer prevention. Despite the promise of POH as a therapeutic agent for many applications, there is little data available concerning the stability of POH under

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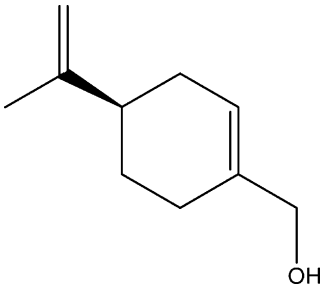

Fig. 1. Perillyl alcohol structure.

various conditions of pH and in various solvents that would potentially be suitable for the development of an appropriate topical preparation for use in clinical trials. In order to find suitable solvents to formulate POH, the stability of the compound was determined in various aqueous and non-aqueous solutions. Upon consideration of physiochemical properties and stability, a hydrophilic cream formulation of POH was developed and evaluated for long term stability.

2. Materials and methods

2.1. Materials

R-(+)-Perillyl alcohol, *S*-(−)-perillyl aldehyde, *S*-(−)-perillic acid, 4-isopropylbenzaldehyde and 2,6-di-*tert*-butyl-4-methylphenol and glycerin, USP, were obtained from Aldrich Chemical Company (Milwaukee, WI, USA). White petrolatum, USP and mineral oil (light), NF, were obtained from Penreco (Dickinson, TX, USA). Lanolin alcohol, NF, and polyoxypropylene-2-myristyl ether propionate were obtained from Croda Inc. (Parsipanny, NJ, USA). Stearic acid, USP, and isopropyl palmitate were obtained from Uniqema. Paragon III [phenoxyethanol (48%), DMDM hydantoin (30%), methyl paraben (11%), propyl paraben (3%) and water (8%)] was obtained from McIntyre Group Ltd. (University Park, IL, USA). Sterile water for irrigation, USP, was obtained from Baxter Healthcare Corporation (Deerfield, IL, USA). Triethanolamine, USP, was obtained from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals were analytical or high performance liquid chromatographic assay (HPLC) grade.

2.2. HPLC method

The HPLC system consisted of a Waters 2690 separation module (Waters, Milford, MA, USA) coupled with a Waters 996 Photodiode array (PDA) detector. Sample analysis was performed by a reverse phase HPLC assay, using a 150 mm × 2.1 mm, Altima C18 5 μ column, maintained at 30 °C. Ultraviolet detection was done at 210 nm. For the formulation samples the total run time of the method was 45 min. The gradient HPLC method utilized acetonitrile:water (40:60) as the initial isocratic run for 10 min at a flow rate of 0.35 ml/min. This was followed by a linear gradient for 2 min, at the end of which the mobile phase composition was 100% isopropyl alcohol at a flow rate of 0.15 ml/min. In order to elute the non-polar ingredients of the formulation, the isocratic run with 100% isopropyl alcohol was continued for another 18 min. At the end of 30 min, a linear ramp was used to resume the original mobile phase conditions. At the end of 45 min, the final mobile phase composition was acetonitrile:water (40:60, 0.35 ml/min). The injection volume was 5 μl and the retention time of POH was 7.8 min. For the aqueous buffer and organic stability samples the total run time of the method was 10 min and only the initial isocratic portion of the method with acetonitrile:water (40:60) at 0.35 ml/min was used. Quantitation was done based on peak area, using a standard curve prepared daily.

2.2.1. Selectivity of the RP-HPLC assay for perillyl alcohol

The specificity of the method for POH was confirmed by library spectra matching and mass spectrometry. A library spectra match was performed on each of the POH peaks using a Waters 996 Photo Diode Array detector, in order to check for peak purity. In addition, aliquots of the POH samples were collected corresponding to the elution time of the POH peak. The eluates were then combined and analyzed using GC-MS (electron ionization) in the positive ion mode (Varian Saturn 2000 GC/MS, Varian Inc., Palo Alto, CA, USA). Compounds structurally related to POH and potential degradation products, including perillyl aldehyde, perillic acid, 4-isopropylbenzaldehyde, and 2,6-di-*tert*-butyl-4-methylphenol were also evaluated for their possible interference with the POH peak under the initial isocratic mobile phase solvent conditions.

2.3. Preformulation

2.3.1. Stability studies

The influence of pH on the stability of POH was studied using a citrate buffer (0.1 M) in the pH range of 2.0–5.0, a phosphate buffer (0.1 M) in the pH range of 6.0–8.0, and a borate buffer (0.1 M) in the pH range of 9.0–11.0. All pH values were adjusted to a constant ionic strength of 0.2 M. The influence of ionic strength on the stability of POH was tested by using citrate buffer (pH 4.0, 0.1 M) and adjusting the ionic strength to 0.2, 0.3, and 0.5 M using NaCl. The influence of organic solvents on the stability of POH was studied by preparing samples of POH in acetonitrile, ethanol, and isopropyl alcohol. In addition, studies were performed in 3% hydrogen peroxide (pH 5.2) to check the susceptibility of POH for oxidation. Stability testing was conducted for all samples at four different temperatures (4, 25, 37, and 48 °C). The target concentration of POH in all samples was 100 μg/ml. All samples were prepared in duplicate and each sample was assayed in duplicate. Stability samples were analyzed using an isocratic method (previously described) with acetonitrile:water (40:60) at 0.35 ml/min for 10 min. The retention time of POH was 7.8 min.

2.3.2. Method of preparation

The POH topical formulation (cream) is an oil in water emulsion. The ingredients of the cream formulation along with the quantities (%, w/w) are listed in Table 1. The procedure to make the POH cream formulation is as follows.

Table 1
Perillyl alcohol topical formulation ingredients

Perillyl alcohol formulation	
Ingredients	Quantity (%, w/w)
R-(+)-Perillyl alcohol	0.3
White petrolatum, USP	0.8
Lanolin alcohol, NF	0.8
Polyoxypropylene-2-myristyl ether propionate	4.2
Mineral oil (light), NF	8.3
Triethanolamine, USP	1.0
Glycerin, USP	3.3
Stearic acid, USP	16.7
Isopropyl palmitate	12.5
Distilled water, USP	51.49

White petrolatum, lanolin alcohol, stearic acid, and mineral oil (light) were melted in beaker I, by heating the beaker to 55 °C using a Corning hot plate stirrer. After the ingredients were melted, the contents of the beaker were stirred at 200 rpm using a Lightnin Labmaster Mixer (Model DS 1010). Polyoxypropylene-2 myristyl ether propionate and isopropyl palmitate were heated in a separate beaker II to 55 °C, using a Corning hot plate stirrer. Once the desired temperature was reached the contents from beaker II were added to the beaker I and then were heated at 55 °C for 30 min while maintaining stirring at 500 rpm. A weighed amount of POH was then added to the beaker I and stirred for another 5 min at 500 rpm. Triethanolamine, glycerin, paragon III, and about 65% of the water were preheated in a separate beaker III to 55 °C. The contents of beaker III were then added to beaker I. The above mixture was maintained at 55 °C, while stirring the contents at 1300 rpm for 20 min. The remaining quantity of water was then added to the beaker I and heating was discontinued. The above mixture was stirred at 1300 rpm for 30 min. The resultant smooth cream formulation was kept in the refrigerator (approximately 4 °C) for 24 h and subsequently analyzed for POH content. The resultant smooth cream had a pH of 5.9, when analyzed by Ross combination flat surface pH electrode (Model #8135BN, ThermoOrion, Beverly, MA, USA) using a pH meter (Model 225, Denver instrument co., Arvada, CO, USA).

2.3.3. Partitioning study

A partitioning study was performed in order to determine the apparent partition coefficient (*P*) of POH between the oil (lipid) phase and the aqueous phase of the cream formulation. This was done in order to determine the phase in which POH would be predominantly present. The oil (lipid) phase components that were used for the partition study were isopropyl palmitate, light mineral oil, PPG-2-myristyl ether propionate, triethanolamine, and glycerin. These components along with water were used approximately in the same ratio, as they were present in the original formulation. The oil (lipid) phase components were mixed with water and a known amount of POH (0.035%, w/w) was added to this two-phase system. Six vials containing this mixture were then kept on a shaker for 5 days in order for the system to equilibrate. This two-phase system was then centrifuged in order to obtain the oil

(lipid) layer and the aqueous layer. Both these phases were analyzed separately by the reverse phase HPLC assay mentioned earlier, in order to determine the POH concentration.

2.3.4. Sample preparation for topical formulation stability

The formulation was stored at two different temperatures: 4 and 25 °C. Prior to analysis, a weighed amount of the formulation was diluted with a known volume of IPA and sonicated for 20 min (Bransonic-1510R, Bransonic Ultrasonics Corp., Danbury, CT, USA), until the contents were in solution. The samples were inspected with a laser tyndall beam in order to make sure that all the components in the vials were in solution. Five microliters of the aliquots of these formulation samples were injected onto the HPLC analytical column for analysis.

3. Results and discussions

3.1. Chromatography

The initial isocratic conditions for the RP-HPLC assay were chosen such that it offered separation of POH from any potential degradation products in the aqueous buffer solutions. Since none of the degradation products coeluted with POH, the same initial isocratic conditions were chosen to analyze the formulation. Under these conditions POH was observed to be well resolved from other formulation ingredients and any potential degradation products. The subsequent gradient with isopropyl alcohol was conducted to elute the nonpolar formulation ingredients. The specificity of the RP-HPLC method for POH was confirmed by library spectra match and mass spectrometry. The peak purity data confirmed the absence of any impurities coeluting with POH. In addition, aliquots of POH samples were collected corresponding to the elution time of the POH peak. GC-MS analysis of POH eluates confirmed the identity and uniqueness of the POH peak. The same initial isocratic mobile phase solvent conditions were used to analyze compounds that were potential degradation products or structurally related to POH. Perillyl aldehyde, perillic acid, 4-isopropylbenzaldehyde, and 2,6-di-*tert*-butyl-4-methylphenol gave retention times of 14, 5.4, 10.3, and 24.5 min, respectively.

Fig. 2(a) and (b) show the degradation of POH in an aqueous buffer solution (pH 2.0) after 1 day (a) and after 7 days (b). Surprisingly, the degradation profiles of the samples did not indicate the presence of degradants in 200–400 nm wavelength range. This may be due to the formation of degradation products without a significant chromophore or because they were eluting with the mobile phase solvent front. The GC-MS analysis of the degraded samples also did not give a clear indication about the degradation products,

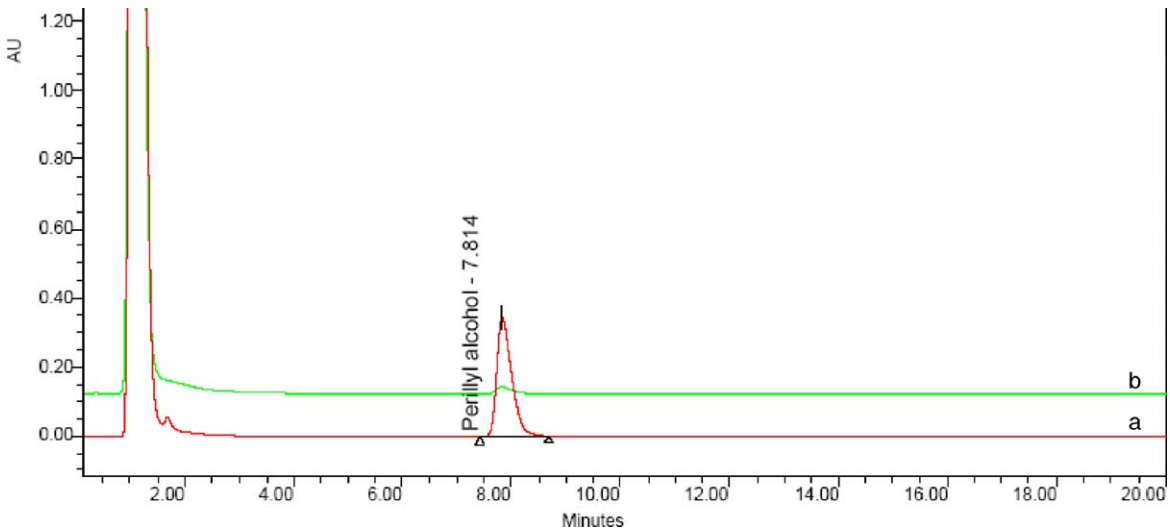

Fig. 2. Chromatogram overlay for the degradation of POH at pH 2.0 at 48 °C: (a) after 1 day and (b) after 7 days.

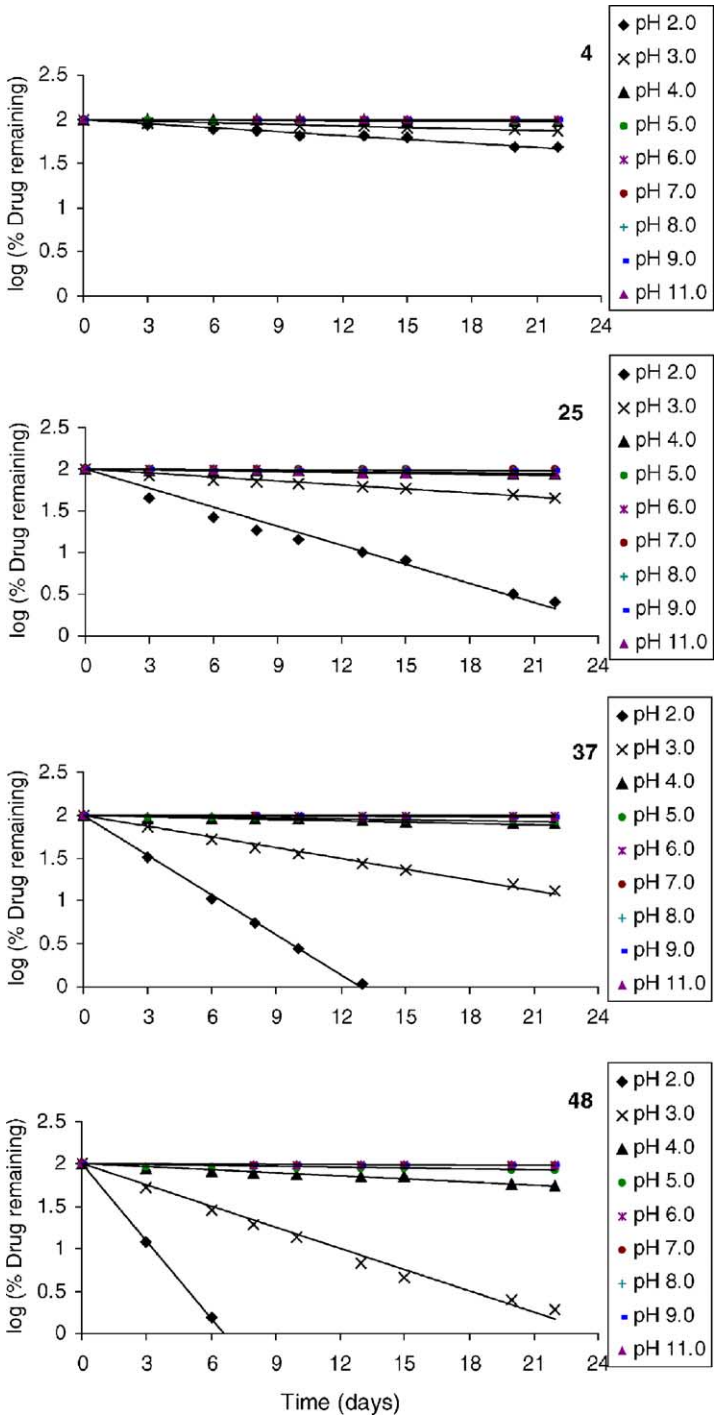

Fig. 3. pH degradation profile (log of the percentage of perillyl alcohol remaining vs. time) of perillyl alcohol at four different temperatures (4, 25, 37, and 48 °C).

precluding any identifiable degradation mechanism. Related monoterpenes have been shown to degrade by multiple pathways. These include epoxidation or oxidative cleavage of the double bond, oxidation of allylic C–H bonds and OH-initiated oxidation, leading to the formation of cyclic β-hydroxyperoxy radicals as intermediates (Ziegler et al., 1991; Lempers and Sheldon, 1996; Sakaguchi et al., 1996; Ferreira et al., 2002; Boyd et al., 2003; Schoflied et al., 2002; Santos et al., 2003; Silva et al., 2003). The intermediates thus formed (e.g. allylic hyperoxides) have been shown to deteriorate to several products. Degradation of monoterpenes (especially by oxidation) in these cases has been shown to be enhanced by acidic conditions and high temperature.

3.2. Kinetic profile

The pH of a solution is known to affect the degradation of many drugs. This can be attributed to the fact that degradation reactions can be catalyzed by hydrogen ions and hydroxide ions. The log of the percentage of POH remaining versus time, at various pH conditions, is shown in Fig. 3. Stability testing was conducted for nine pH conditions at four different temperatures (4, 25, 37, and 48 °C). On the basis of the data in Fig. 3, it is evident that the degradation of POH follows apparent first-order kinetics under all conditions. The degradation rate constants were calculated from the linear relationship between the logarithm of the remaining drug concentration and time. These were then used to estimate the half-life ($t_{50\%}$) and shelf life ($t_{90\%}$) at each pH value. A summary of these data is presented in Table 2. The duplicate injections had a maximum relative standard deviation (R.S.D.) of 3.5. Table 2 also indicates the difference between the rate of reaction for a 3% hydrogen peroxide solution (pH 5.2) and at pH 5.0 (in the absence of any oxidizing agent). From Table 2, it is evident that the rate of reaction at the same pH is higher in the presence of an oxidizing agent, indicating that POH may be susceptible to peroxide catalyzed oxidation.

3.3. pH rate profile

Fig. 4 shows the pH rate profile of POH at the four different temperatures: 4, 25, 37, and 48 °C. The stability of POH is pH-dependent, with significant degradation at pH values less than 4.0. From Fig. 4, it is evident that at all of the test temperatures, POH is most stable at pH values higher than 5.0. At lower pH values, a negative slope is observed in the pH rate profile. The shape of the pH rate profile suggests that the degradation of POH increases with the increase in temperature.

3.4. Effect of temperature: Arrhenius expression

The degradation rate constants are related to the temperature by the Arrhenius expression as:

$$\ln k = \frac{\ln A - E_a}{RT},$$

Table 2
Half-life ($t_{50\%}$) and shelf life ($t_{90\%}$) for degradation of perillyl alcohol in different pHs

pH	Temperature (°C)											
	4			25			37			48		
	k (per day)	$t_{50\%}$ (days)	$t_{90\%}$ (days)	k (per day)	$t_{50\%}$ (days)	$t_{90\%}$ (days)	k (per day)	$t_{50\%}$ (days)	$t_{90\%}$ (days)	k (per day)	$t_{50\%}$ (days)	$t_{90\%}$ (days)
2	0.012	57.75	8.75	0.069	10.04	1.52	0.151	4.59	0.70	0.3116	2.22	0.34
3	0.0027	256.67	38.88	0.012	57.75	8.75	0.0396	17.50	2.65	0.0753	9.20	1.39
4	0.0005	1386.00	210.00	0.0023	301.30	45.65	0.0052	133.27	20.19	0.0099	70.00	10.61
5	0.0003	2310.00	350.00	0.0005	1386.00	210.00	0.0013	533.08	80.77	0.0024	288.75	43.75
6	0.00025	2772.00	420.00	0.00035	1980.00	300.00	0.0005	1386.00	210.00	0.00055	1260.00	190.91
7	0.00026	2665.38	403.84	0.00043	1611.62	244.19	0.0006	1155.00	175.00	0.0006	1155.00	175.00
8	0.00027	2566.67	388.88	0.0005	1386.00	210.00	0.0006	1155.00	175.00	0.0008	866.25	131.25
9	0.0003	2310.00	350.00	0.0006	1155.00	175.00	0.0008	866.25	131.25	0.0007	990.00	150.00
11	0.0003	2310.00	350.00	0.0011	630.00	95.45	0.0011	630.00	95.45	0.0007	990.00	150.00
3% H_2O_2 (pH 5.2)	0.073	9.55	1.45	0.183	3.79	0.57	0.454	1.53	0.23	0.751	0.92	0.14

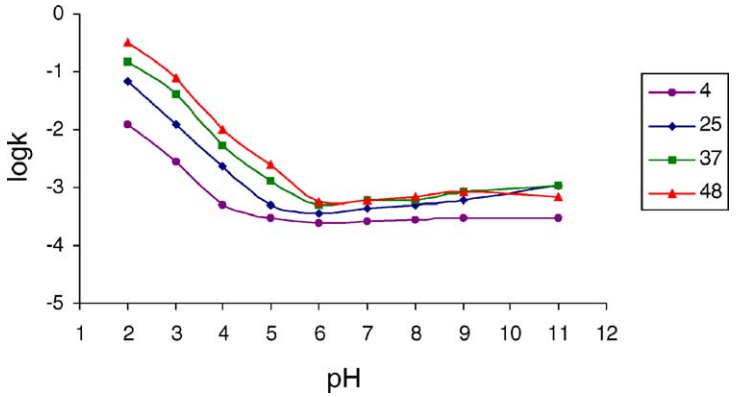

Fig. 4. pH-rate (log k vs. pH) profile of perillyl alcohol at four different temperatures (4, 25, 37, and 48 °C).

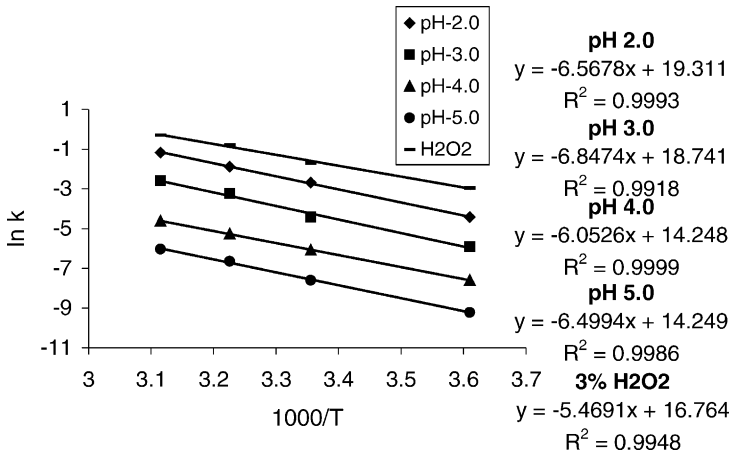

Fig. 5. Arrhenius plot: temperature dependence of the degradation rate constants at five pH values (2.0, 3.0, 4.0, 5.0, and 5.2 (3% hydrogen peroxide)).

where 'k' is the degradation constant, 'A' is the collision factor, 'E_a' is the energy of activation, 'R' is the gas constant, and 'T' is the temperature in Kelvin. The various buffered samples of POH along with the aqueous and non-aqueous samples were analyzed at four different temperatures: 4, 25, 37, and 48 °C. The natural logarithms of the degradation constants were plotted against the inverse of temperature for the four pH conditions (2.0, 3.0, 4.0, and 5.0) and for 3% hydrogen peroxide solution (pH 5.2) to obtain the Arrhenius parameters (Connors et al., 1986; Martin, 1993; Carstensen, 1995). Five linear relationships are shown in Fig. 5. The energies of activation, calculated from the slopes, are listed in Table 3. The calculated energies of activation are within the usual range of 50–100 kJ/mol (or 12–24 kcal/mol) commonly reported for degradation reactions of other drugs (Connors et al., 1986).

Table 3
Energy of activation for the solutions at pH 2.0, 3.0, 4.0, 5.0, and 3% hydrogen peroxide solution (pH 5.2)

Energy of activation for the five solutions		
Sample	Slope	E_a (kJ/mol)
2.0	−6.56	54.60
3.0	−6.84	56.92
4.0	−6.05	50.32
5.0	−6.50	54.03
3% H_2O_2 (pH 5.2)	−5.47	45.47

3.5. Effect of ionic strength

The effect of ionic strength on the stability of POH (at pH 4.0) is shown in Fig. 6. From Fig. 6 it is evident that ionic strength has no significant effect on the degradation of POH at low temperatures (4 and 25 °C). The effect of ionic strength becomes significant at 48 °C, where the degradation of POH is increased as the ionic strength is decreased.

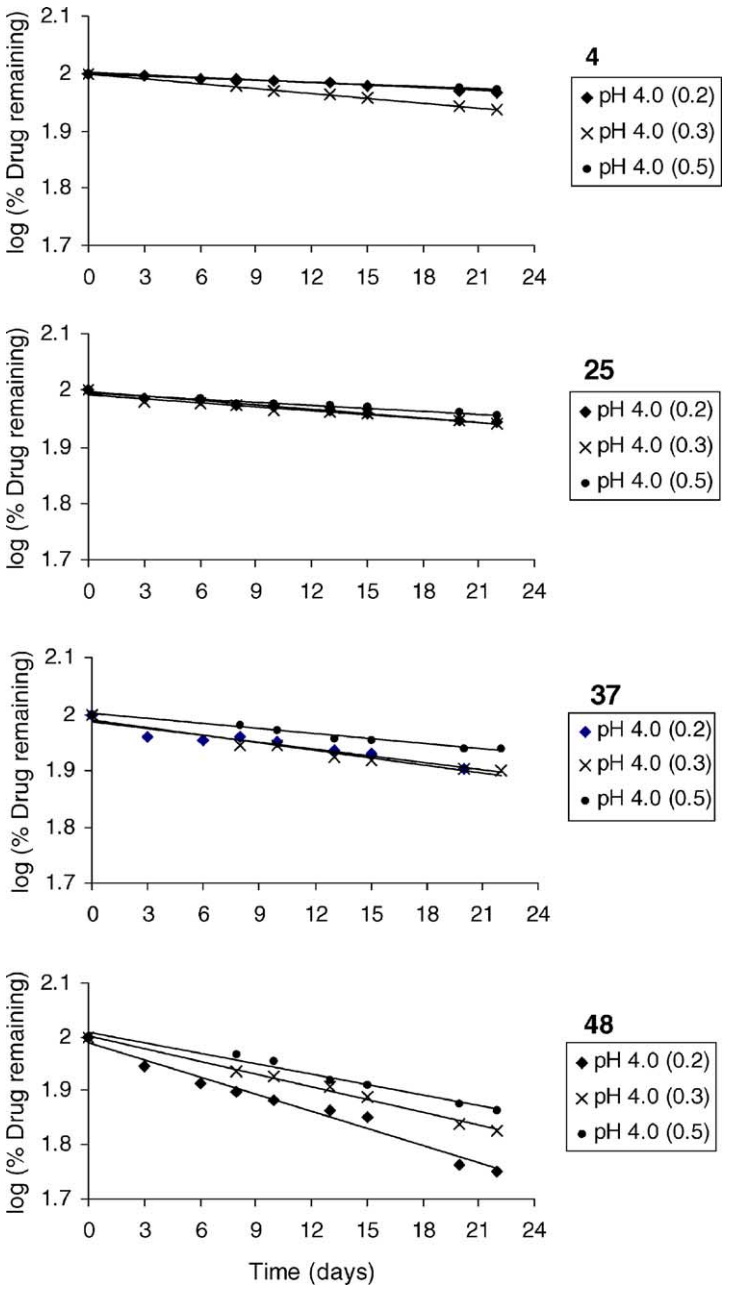

Fig. 6. Effect of ionic strength on pH degradation of perillyl alcohol at four different temperatures (4, 25, 37, and 48 °C).

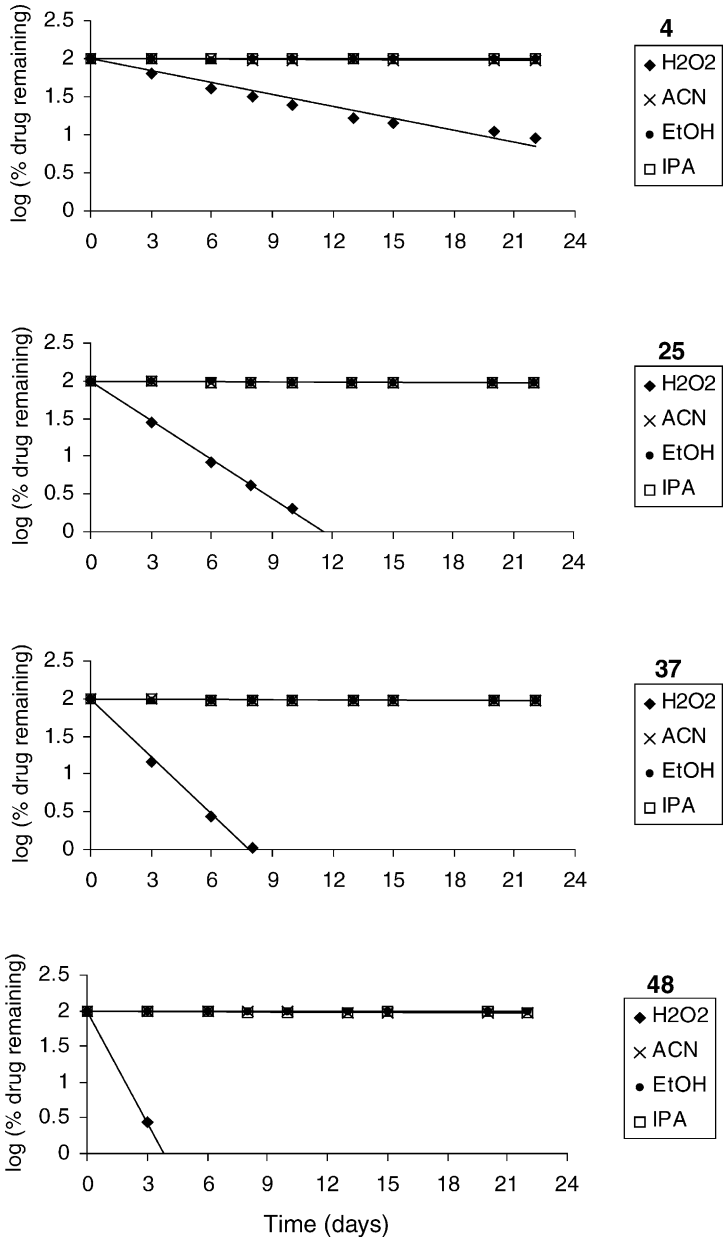

Fig. 7. Degradation (log of the percentage remaining vs. time) of perillyl alcohol in four different solvents.

3.6. Effect of organic and aqueous solvents

Fig. 7 shows the effect of organic solvents (ethanol, acetonitrile, and isopropyl alcohol) and 3% hydrogen peroxide, on the stability of POH at four different temperatures (4, 25, 37, and 48 °C). It is evident from Fig. 7 that POH is stable in the three organic solvents, in the temperature range studied. On the other hand, significant degradation of POH was seen in 3% hydrogen peroxide (pH 5.2), which increased dramatically with the increase in temperature. This is indicative of the fact that

POH is susceptible to peroxide catalyzed oxidation.

Although the mechanism of degradation of POH is not known, from the results it is evident that POH is stable at the pH range of the topical cream formulation (5.9–6.0). The $t_{90\%}$ of POH at pH 6.0 is about 14 months when stored at 4 °C and about 10 months when stored at room temperature (25 °C). The ionic strength does not have a significant effect on the degradation of POH at these temperatures (4 and 25 °C). POH is also stable in the solvents used during the HPLC analysis (water, acetonitrile, and isopropyl alcohol). POH is susceptible to oxidation when strong oxidizing agents (e.g. hydrogen peroxide) are used. Temperature also increases degradation.

3.7. Partition study

The topical formulation of POH is a hydrophilic oil/water emulsion. The stability of POH in the formulation will be governed by the phase in which POH is predominantly present. The concentration of POH in the oil (lipid) phase of the two-phase mixture was determined to be 403 times higher than in the aqueous phase, from an average of six determinations. Hence, the $\log P$ (apparent oil/water partition coefficient) was approximately 2.61. This value of $\log P$ (apparent oil/water partition coefficient) is in agreement with the octanol/water partition coefficient ($\log P_{\text{oil/water}} = 2.3$) of POH. This indicates that POH in the formulation predominantly partitions in the oil (lipid) phase as compared to the aqueous phase.

3.8. Topical formulation stability

The physical and chemical stability of the POH topical formulation was observed for 1 year. The formulation was physically stable, with no mold growth or phase separation during the entire duration of the study. The chemical stability of the topical formulation was analyzed by the reverse phase HPLC method mentioned earlier.

Table 4 shows the percent theoretical recovery of POH in the formulation for a period of 1 year. The results are reported for two different temperatures: 4 and 25 °C, along with the standard deviations. From the results, it is evident that POH topical formulation is physically and chemically stable for a period of 1 year.

The $t_{90\%}$ of POH at the pH of the aqueous phase of the topical formulation was determined to be approximately 14 months at 4 °C and approximately 10 months at 25 °C. The formulation stability indicates that POH is chemically stable at both these temperatures for at least 1 year. The increased formulation stability can be explained by the fact that most of the POH partitions into the oil (lipid) phase as indicated by the apparent oil/water partition coefficient ($\log P$) of 2.61.

Table 4
Perillyl alcohol topical formulation stability (as a percentage of initial recovered) at 4 and at 25 °C

Time (months)	Temperature			
	4		25	
	Percentage of initial	S.D. ($n = 3$)	Percentage of initial	S.D. ($n = 3$)
1	103.37	1.63	106.61	4.6
2	101.66	2.11	99.04	1.08
4	97.38	7.13	103.92	0.34
5	105.92	2.51	92.74	6.28
7	100.06	0.63	95.83	5.19
8	105.66	2.31	99.12	1.25
9	104.29	7.06	100.01	1.01
12	103.18	4.7	99.17	1.45
Mean	102.69		99.56	
S.D.	2.89		4.31	
C.V. (%)	2.82		4.33	

4. Conclusions

This study shows that the degradation of monoterpene perillyl alcohol follows apparent first-order kinetics. A topical cream formulation of perillyl alcohol has been developed. A stability indicating reverse phase HPLC method has been developed to analyze the formulation. Perillyl alcohol was found to be stable in the topical formulation for 1 year. Thus, the formulation has physical and chemical stability that can be used clinically to evaluate chemoprevention of UV radiation induced skin carcinogenesis.

Acknowledgements

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