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STABILITY OF SOLUTIONS OF 2,3-DIPHENYLCYCLOPROPENONE IN VARIOUS SOLVENTS. A NOVEL FORMULA – DIPHENYLCYCLOPROPENONE IN ISOPROPANOL MAY BE USEFUL IN TOPICAL THERAPY OF PATIENTS WITH ALOPECIA AREATA

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Abstract: The aim of this study was to evaluate the chemical stability of diphenylcyclopropenone (DCP) in various solvents. DCP is being used in topical therapy in patients with alopecia areata (AA). The solvents for DCP used so far were acetone, ethanol and propylene glycol. DCP is supposed to be an immune-modulating therapeutic agent, however the studies on it's pharmacokinetics including chemical stability are lacking. In a present study, DCP was dissolved in acetone (A), ethanol (E), propylene glycol (PG) and isopropanol (I). Solutions at two concentrations: 0.1 and 3.0% were prepared in each of these solvents. Then, the solutions were divided into two parts – one of which was stored at room temperature and the other in a refrigerator (at about 4˚C) without the access of light. In determined time intervals the solutions were analyzed and the content of DCP and it's main decomposition product DPA was assessed. The stability of solutions of DCP with all the solvents kept in a refrigerator (at about 4˚C) without light was maintained, the decomposition rate after 60 days was negligible. In contrary, DCP solutions kept at room temperature after 60 days decomposed visibly and in different rates according to a solvent (PG > I > E > A). The most surprising finding was that DCP solutions in acetone, which was supposed to be a good solvent for the purpose of AA treatment, decomposed completely (100%) after just 45 days at room temperature. The most stable solutions at room temperature turned out to be the ones in propylene glycol and isopropanol. Results suggest: 1. the preferable storage condition for all DCP solutions is at a temperature of about 4˚C without the access of light; 2. there is a limited benefit from using acetone as a DCP solvent; 3. the novel solvent for DCP - isopropanol, showed good stability in both temperatures and has favorable cosmetic qualities. In conclusion, authors suggest to make further investigations on DCP in isopropanol solutions in clinical studies regarding treatment of AA.

Keywords: alopecia areata, 2,3-diphenylcyclopropenone

Abbreviations: A - acetone, AA - alopecia areata, DCP - diphenylcyclopropenone, DPA - diphenylacetylene, E – ethanol, I – isopropanol, PG – propylene glycol, PUVA - psoralen plus ultraviolet A therapy

Alopecia areata (AA) is an autoimmune disease involving hair follicles characterized by hair loss on the scalp and/or body. Frequency of AA ranges from 0.7% to 3.8% of patients attending dermatology clinics (1). Etiopathogenesis of AA is unknown but evidence exists to support genetic, immune and environmental factors (2-5). Treatment modalities include corticosteroids, photochemotherapy (PUVA), biological drugs and contact immunotherapy (6-12). The latter include topical sensitizers, from which the most commonly used in alopecia areata is 2,3-diphenylcyclopropenone (DCP, DPCP; synonyms: 2,3-diphenylcycloprop-2-en-1-one; diphenylcyclopropenone monohydrate, diphencyprone).

Topical immunotherapy with DCP is considered to be an effective treatment of AA with success

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rates ranging from 6% (13) to 85% (14, 15). Most authors reported a cosmetically acceptable hair regrowth rate of 50-70% (16-18). The molecular effects of DCP in human skin are unknown. Although the mechanism of action of DCP has not been clearly defined, DCP is a hapten that induces DTH (delayed-type hypersensitivity) reactions involving a cytokine response and local infiltration of T-cell subpopulations, resulting in contact dermatitis. DCP induces change the perifollicular CD41/CD81 T-lymphocyte ratio (18), apoptosis of perifollicular lymphocytes (19) and modulate proinflammatory cytokines (20).

2,3-Diphenylcyclopropenone was first synthesized in 1959 (21, 22). DCP is synthesized by cyclization of a,a'-dibromodibenzylketone. A cyclopropenone compound has phenyl substituents at the 2- and 3-positions. It's molecular formula is $C_{15}H_{10}O$ and it's molecular weight is 206.2393 g/mol. DCP is sensitive to ultraviolet light degradation (23). Unlike dinitrochlorobenzene it is nonmutagenic in the Ames assay (24). Studies on the chemical stability of 2,3-diphenylcyclopropenone in solutions are generally lacking. Hence the aim of this study was to evaluate the chemical stability of 2,3-diphenylcyclopropenone in different solvents and in different temperature and light conditions.

MATERIALS AND METHODS

The study was performed for standard of 2,3-diphenylcyclopropenone dissolved in acetone (A), ethanol (E), propylene glycol (PG) and isopropanol (isopropyl alcohol, 2-propanol) (I). Solutions at two concentration levels: 0.1 and 3.0% were prepared for each of these solvents. Then, the solutions were divided into two parts – one of which was stored at room temperature (about 25°C) and the other in a refrigerator (at about 4°C) without light. In determined time intervals (after 7, 14, 30, 45 and 60 days) the solutions were analyzed and the content of DCP and DPA (diphenylacetylene – the main decomposition product of DCP) was assessed. The analyses were performed with the gas chromatography technique with flame ionization detector (GC-FID) using internal normalization method. Because DCP decomposes to DPA, the quantification in the stability studies was based on the relative content of DCP and DPA (the relative percentage of DCP to DPA).

Apparatus and equipment

Gas chromatograph Agilent Technologies 6890 Plus with COC (cool on-column) injector and flame ionization detector (FID); column: HP-1, (30 m × 0.53 mm; film 3 μm), Agilent Technologies.

Table 1. Analysis of DCP solutions on the "time zero".

Solution	Content of DCP	Mean	Content of DPA	Mean
E 0.1	98.06	98.07	1.94	1.94
	98.07		1.93	
A 0.1	97.92	97.92	2.08	2.09
	97.92		2.09	
I 0.1	98.15	98.16	1.85	1.85
	98.17		1.84	
GP 0.1	97.54	97.51	2.46	2.50
	97.48		2.53	
E 3	98.32	98.33	1.68	1.68
	98.33		1.67	
A 3	98.29	98.29	1.71	1.71
	98.29		1.71	
I 3	98.39	98.39	1.61	1.62
	98.39		1.62	
GP	96.43	96.88	3.57	3.12
	97.32		2.68	

Table 2. Analysis of DCP solutions stored at room temperature.

Solution	Content assessed [%]											
	at 1st day		after 7 days		after 14 days		after 30 days		after 45 days		after 60 days	
	DCP	DPA	DCP	DPA	DCP	DPA	DCP	DPA	DCP	DPA	DCP	DPA
E 0.1(25)	98.07	1.94	91.88	8.13	64.04	35.97	36.31	63.70	22.93	77.07	5.07	94.93
A 0.1(25)	97.92	2.09	85.41	14.59	37.44	62.57	8.36	91.65	0	100	0	100
I 0.1(25)	98.16	1.85	91.52	8.49	67.94	32.07	51.03	48.97	33.76	66.24	13.01	87.00
GP 0.1(25)	97.51	2.50	90.65	9.35	73.63	26.38	61.82	38.18	48.10	51.91	29.51	70.50
E 3(25)	98.33	1.68	97.68	2.33	95.92	4.09	92.45	7.55	90.02	9.99	83.38	16.63
A 3(25)	98.29	1.71	96.95	3.06	94.35	5.65	90.20	9.80	86.34	13.67	77.41	22.60
I 3(25)	98.39	1.62	97.29	2.71	95.47	4.53	93.42	6.59	91.50	8.51	84.90	15.10
iGP 3(25)	96.88	3.13	96.53	3.48	94.32	5.69	92.92	7.09	90.92	9.09	89.12	10.88

Table 3. Analysis of DCP solutions stored in refrigerator with no exposure to the light.

Solution	Content assessed [%]											
	at 1st day		after 7 days		after 14 days		after 30 days		after 45 days		after 60 days	
	DCP	DPA	DCP	DPA	DCP	DPA	DCP	DPA	DCP	DPA	DCP	DPA
E 0.1(4)	98.07	1.94	97.89	2.11	97.72	2.31	97.87	2.13	97.88	2.13	98.23	1.77
A 0.1(4)	97.92	2.09	97.02	2.98	97.14	2.86	97.40	2.61	97.35	2.65	97.78	2.22
I 0.1(4)	98.16	1.85	97.74	2.29	97.62	2.39	97.76	2.24	97.75	2.26	98.04	1.97
GP 0.1(4)	97.51	2.50	97.40	2.60	96.76	3.24	96.66	3.34	96.60	3.40	97.87	2.13
E 3(4)	98.33	1.68	98.29	1.71	98.23	1.78	98.30	1.70	98.28	1.72	98.20	1.80
A 3(4)	98.29	1.71	98.26	1.74	98.22	1.79	98.28	1.73	98.24	1.77	98.14	1.86
I 3(4)	98.39	1.62	98.34	1.66	98.35	1.65	98.36	1.64	98.34	1.67	98.27	1.73
GP 3(4)	96.88	3.13	97.58	2.42	97.07	3.05	96.76	3.24	96.99	3.03	98.31	1.69

Chromatographic conditions

Variable temperature of injector: equal to column temperature + 3°C; column temperature programme: 60°C (3 min) – 10°C/min – 220°C (6 min); carrier gas: nitrogen 15 mL/min; detector temperature: 300°C; hydrogen flow: 35 mL/min; air flow: 350 mL/min, make-up gas: 20 mL/min; volume of sample injection: 1.0 μL. Under above conditions the retention times of the analytes are as follows: DPA ~ 17.90 min; DCP ~ 19.38 min.

Reagents

Diphenylocyclopropenone (DCP), 98.4%, Sigma Aldrich, batch BCBM5280V; diphenylacetylene (DPA), 98.1%, Sigma Aldrich, batch STBC7355V; acetone, 99.5%, ethanol anhydrous, 99.9%, 2-propanol, 99.9% (all POCh); propylene glycol, 99.8%, Sigma-Aldrich; nitrogen; hydrogen; synthetic air for FID.

The method precision was determined in five repetitions of 3% and 0.1% solution of DCP in isopropanol. The peak area of DCP was measured and the mean value as well as relative standard deviation were calculated. Details of these measurements are shown in Table 4.

RESULTS AND DISCUSSION

Analysis of DCP solutions in time zero

The analysis of DCP solutions on the 1st day are presented in Table 1. An example of chromatograms from analysis of 0.1% and of 3% solutions of DCP on the first day are shown in Figure 1.

Analysis of DCP solutions during storage at room temperature

The results are given as the mean value from two measurements. The analysis of DCP solutions stored at room temperature are shown in Table 2.

Analysis of DCP solutions during storage in the refrigerator with no light exposure

The results are also given as the mean value from two measurements. The analysis of DCP solutions stored in these conditions is shown in Table 3.

Figure 2 shows changes of DCP and DPA content during storage at room temperature in comparison with the storage in refrigerator. Figure 3 shows chosen chromatograms from final analyses of DCP solutions. The chromatograms were compared, with special attention paid to a storage period, solvent and storage conditions.

According to the results good chemical stability of all DCP solutions has been revealed at about 4°C without light. The chemical stability at about 25°C was significantly lower and it differed according to the solvent used. After 60 days, the amount of DCP was the highest in propylene glycol solutions, a bit lower in isopropanol, even lower in acetone and the lowest in acetone (PG > I > E > A). Moreover, 3% concentrations of DCP showed much better stability than that of 0.1% (Table 2). The most surprising finding was that DCP solutions in acetone which was supposed to be a good solvent for the purpose of AA treatment, decomposed completely (100%) after just 45 days at room temperature. Authors suggest to stop any further attemps to use acetone as a solvent for DCP.

2,3-Diphenylcyclopropenone (DCP) is used as an immune-modulating therapeutic factor. DCP is a topically administered drug intended for treating alopecia areata. DCP is applied at a high concentration of 2.0 or 3.0% usually once in order to obtain sensitization and then at lower concentrations (0.001-0.5%; usually about 0.1%) once weekly (1, 17, 18, 25, 26). Systemic immunological effects of DCP were observed in mice treated with 0.01% DCP or 0.1% DCP (28). For clinical

Table 4. Method precision determined for 3% and 0.1% DCP solutions in isopropanol.

No.	Peak area of DCP in 3% solution	Peak area of DCP in 0.1% solution
1	504297	16285
2	520271	16699
3	516110	16617
4	512061	16389
5	493218	16454
Mean	509191	16489
SD	10700	168
RSD [%]	2.10	1.02

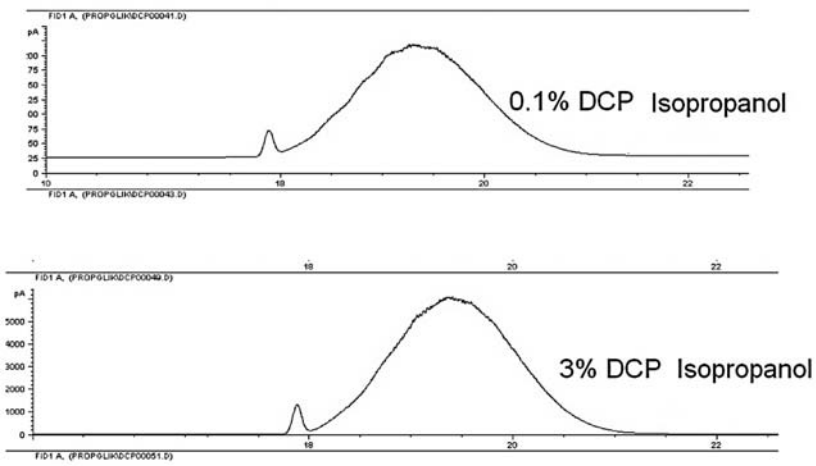

Figure 1. Chosen chromatograms from the beginning of the study. An analysis of 0.1% and 3% solutions of DCP in isopropanol at the "time zero". In each graph right peak indicates the amount of DCP and left it's main decomposition product – DPA (diphenylacetylene)

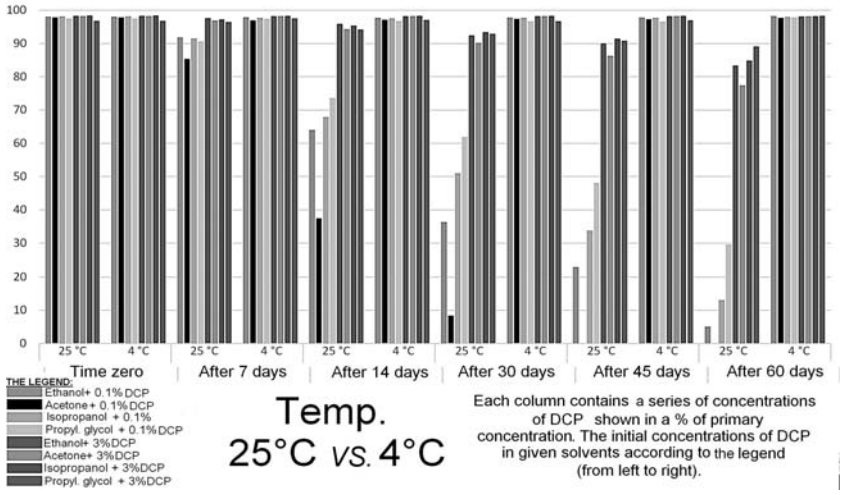

Figure 2. Comparison of stabilities of DCP solutions at room temperature *vs*. the temperature of 4ºC

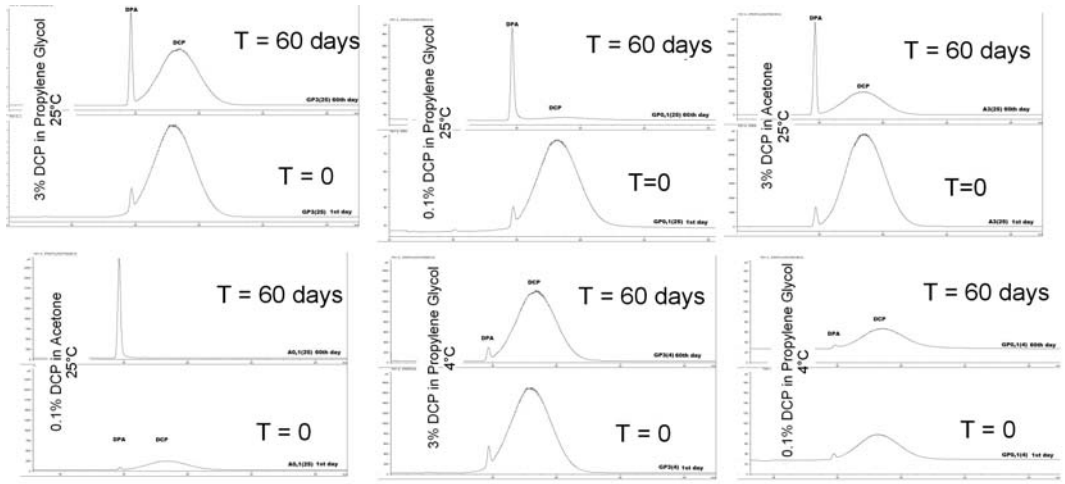

Figure. 3. Chosen chromatograms from the end of the study (compared with the initial ones, for better contrast). Comparison of DCP/DPA ratio in different solvents at given temperature and concentration. Please, note complete 0.1% DCP decomposition in acetone

use, DCP is dissolved in acetone (A), propylene glycol (PG) or ethanol (E) to the desired concentrations (18, 26, 27, 29), the latter very rarely. Technically, solutions in a propylene glycol rather than in acetone make the treatment easier. Acetone has an unpleasant scent and evaporates quickly disappearing even from apparently firmly closed bottles. Acetone itself causes skin irritation. This "irritation" is clinically only slightly different from the allergic reaction that investigators require to obtain as the effect of DCP. This is making the judgment of the allergic reaction difficult. Authors observed though some disadvantages in treatment with DCP in propylene glycol too. Propylene glycol makes the skin surface slimy, does not moisten the skin and eventually leaks from the top of the scalp to areas near neck and ears. This may cause unwanted irritation. In authors opinion, the physical properties of isopropanol make it a useful solvent for DCP. It moistens the skin easily, removes the layer of grease from it and disappears quickly, but not as quickly as acetone. According to available knowledge this is the first study on stability of DCP in isopropanol. The concept of using isopropanol as chemical solvent for DCP seems to be a promising one. Moreover, the results suggest that due to the faster decomposition rate of DCP at lower concentrations, these concentrations should be prepared *ex tempore* from primary 3% solution.

CONCLUSIONS

The preferable storage condition for DCP in a solution is at a temperature of about 4°C without light in concentration of 3%.
The novel solvent for DCP is isopropanol.
The stablility of DCP in solutions at room temperature (about 25°C) after 60 days is diminishing in the following order: PG > I > E > A.
Results suggest limited usefulness of acetone as a solvent for DCP.

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