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 in clinical studies.
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 α,α'-dibromodibenzylketone. A cyclopropenone compound has phenyl substituents at the 2- and 3-positions. It’s molecular formula is C15H10O 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>
<thead>
<tr>
<th>Solution</th>
<th>Content of DCP</th>
<th>Mean</th>
<th>Content of DPA</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>E 0.1</td>
<td>98.06</td>
<td>98.07</td>
<td>1.94</td>
<td>1.94</td>
</tr>
<tr>
<td>A 0.1</td>
<td>97.92</td>
<td>97.92</td>
<td>2.08</td>
<td>2.09</td>
</tr>
<tr>
<td>I 0.1</td>
<td>98.15</td>
<td>98.16</td>
<td>1.85</td>
<td>1.85</td>
</tr>
<tr>
<td>GP 0.1</td>
<td>97.54</td>
<td>97.51</td>
<td>2.46</td>
<td>2.50</td>
</tr>
<tr>
<td>E 3</td>
<td>98.32</td>
<td>98.33</td>
<td>1.68</td>
<td>1.68</td>
</tr>
<tr>
<td>A 3</td>
<td>98.29</td>
<td>98.29</td>
<td>1.71</td>
<td>1.71</td>
</tr>
<tr>
<td>I 3</td>
<td>98.39</td>
<td>98.39</td>
<td>1.61</td>
<td>1.62</td>
</tr>
<tr>
<td>GP</td>
<td>96.43</td>
<td>96.88</td>
<td>3.57</td>
<td>3.12</td>
</tr>
</tbody>
</table>
Stability of solutions of 2,3-diphenylcyclopropenone in various solvents. A novel...

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 attempts 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>
<thead>
<tr>
<th>No.</th>
<th>Peak area of DCP in 3% solution</th>
<th>Peak area of DCP in 0.1% solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>504297</td>
<td>16285</td>
</tr>
<tr>
<td>2</td>
<td>520271</td>
<td>16699</td>
</tr>
<tr>
<td>3</td>
<td>516110</td>
<td>16617</td>
</tr>
<tr>
<td>4</td>
<td>512061</td>
<td>16389</td>
</tr>
<tr>
<td>5</td>
<td>493218</td>
<td>16454</td>
</tr>
<tr>
<td>Mean</td>
<td>509191</td>
<td>16489</td>
</tr>
<tr>
<td>SD</td>
<td>10700</td>
<td>168</td>
</tr>
<tr>
<td>RSD [%]</td>
<td>2.10</td>
<td>1.02</td>
</tr>
</tbody>
</table>
Stability of solutions of 2,3-diphenylcyclopropenone in various solvents. A novel...

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 \textit{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 stability 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.

Acknowledgments

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REFERENCES


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