BRIEF REPORT

Solubility and stability of melatonin in propylene glycol, glycofurol, and dimethyl sulfoxide [version 1; peer review: awaiting peer review]

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Abstract

Introduction: Local administration of melatonin might prove useful in future clinical studies. Melatonin possesses poor solubility and stability in aqueous solutions. The aim of this study was to investigate the solubility and stability of melatonin when dissolved in glycofurol, propylene glycol, and dimethyl sulfoxide (DMSO).

Methods: Two experiments were performed: solubility and stability. In the solubility experiment, we dissolved melatonin in 20% propylene glycol and 20% glycofurol solutions, respectively. For the stability experiment, we prepared three different formulations: melatonin and glycofurol (20% w/w, 10 mg/g); melatonin, glycofurol, and DMSO (20%, 40% w/w, 10 mg/g); and melatonin and DMSO (50% w/w, 1 mg/g). All three solutions were stored at 25°C for 45 days. Concentrations of melatonin in all solutions were measured through high-performance liquid chromatography.

Results: Melatonin demonstrated poor solubility in propylene glycol (3.6–3.8 mg/g) and better solubility in glycofurol (10.5–11.1 mg/g). All three formulations of the stability experiment showed no degradation of melatonin over 45 days.

Discussion: Glycofurol and DMSO provide better solubility and stability than aqueous solutions. The formulations used in this experiment have adequate stability to be used in clinical trials.

Keywords
Melatonin, stability, solubility, dimethyl sulfoxide, DMSO, glycofurol, propylene glycol
Introduction
Oral melatonin has poor oral bioavailability (DeMuro et al., 2000; Di et al., 1997; Harpsøe et al., 2015; Lane & Moss, 1985). So, if high local doses of melatonin are wanted, other routes of administration might be advantageous, e.g. intravesical, vaginal, rectal, and pulmonal. A liquid solution of melatonin is required for these routes of administration. Since melatonin has poor solubility and stability in aqueous solutions (Hamed et al., 1991), we wanted to investigate alternative solvents. Possible solvents include dimethyl sulfoxide (DMSO), glycofurol, and propylene glycol. DMSO is used as a solvent for intravesical administration of drugs used in the treatment of inflammatory diseases of the bladder (Petrou et al., 2009; Shirley et al., 1978). Glycofurol is considered non-toxic and is used as a solvent in various intravenous formulations (Crowther et al., 1997). Propylene glycol is used extensively in cosmetic products and has been considered safe in this application (Fiume et al., 2012).

The aim of this study was to investigate the solubility of melatonin in glycofurol and propylene glycol formulations, as well as the stability of melatonin in glycofurol and DMSO formulations.

Methods
Two experiments were performed: a solubility and a stability experiment.

Solubility
Two formulations were prepared, one containing 20% w/w glycofurol in type 1 purified (MilliQ) water and the other 20% w/w propylene glycol in purified water. From each formulation, 2 x 1 ml was transferred to separate Eppendorf tubes (1.5 ml). Melatonin was added to each Eppendorf tube in larger quantity than the anticipated aqueous solubility. The Eppendorf tubes were agitated by means of end-over-end rotation overnight. Prior to high-performance liquid chromatography (HPLC) analysis, each sample was filtered (0.45 µm Q-Max RR syringe filters).

Stability
For the stability experiment, the following formulations were prepared:

<table>
<thead>
<tr>
<th>Formulation</th>
<th>20% glycofurol</th>
<th>20% glycofurol/40% DMSO</th>
<th>50% DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nominal concentration</td>
<td>10 mg/ml</td>
<td>10 mg/ml</td>
<td>1 mg/ml</td>
</tr>
<tr>
<td>Measured concentration (mg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>10.5</td>
<td>10.6</td>
<td>-</td>
</tr>
<tr>
<td>Day 10</td>
<td>10.7</td>
<td>10.8</td>
<td>11.3</td>
</tr>
<tr>
<td>Day 17</td>
<td>10.4</td>
<td>10.5</td>
<td>10.8</td>
</tr>
<tr>
<td>Day 31</td>
<td>11.4</td>
<td>11.1</td>
<td>11.0</td>
</tr>
<tr>
<td>Day 45</td>
<td>10.3</td>
<td>10.0</td>
<td>9.6</td>
</tr>
</tbody>
</table>

DMSO, Dimethyl sulfoxide, *Only two samples were tested on Day 0.

Results
The solubility of melatonin in the prepared 20% w/w propylene glycol and 20% w/w glycofurol formulations is shown in Table 1. The solubility of melatonin in propylene glycol was only 3.6–3.8 mg/ml, while it was 10.5–11.1 mg/ml in glycofurol.

The results of the melatonin measurements from Day 0 to 45 are shown in Table 2. Melatonin was stable at 25°C for
45 days in all three formulations. None of the melatonin concentrations in the formulations varied considerably from the original concentration. However, when inspecting the HPLC chromatograms of all three products, two peaks were identified in the 20% glycofurol w/w solution at 7.9 and 8.3 minutes. These two peaks were not present in the chromatograms of either solution containing DMSO. All output results are available as Underlying data (Zetner, 2020).

Discussion
The solubility of melatonin was nearly three times higher in the glycofurol formulation than in the propylene glycol formulation. A concentration of 10 mg/ml was achieved in the glycofurol formulation. Melatonin concentrations were stable for 45 days in all three formulations of the stability experiment; however, two unidentified peaks were present in the glycofurol solution (Figure 1).

Previous studies of melatonin stability in aqueous solutions have documented varying results. One study demonstrated stable melatonin concentrations of 100–113 µg/ml in a solution consisting of 5% ethanol and 95% isotonic saline for at least 6 months. The solution was created in sterile conditions and kept in sterile vacuum tubes, protected from light, at room temperature, 4°C, and -70°C (Cavalllo & Hassan, 1995). Interestingly, another study investigated the stability of melatonin at 50 µg/ml dissolved in a phosphate buffer at pH 1.2, 2, 4, 7.4, 7, 10, and 12. This showed that up to 30% of the melatonin degraded over 21 days at all pH ranges. These samples were kept at 20°C and 37°C (Daya et al., 2001). This makes it difficult to draw conclusions about whether melatonin is stable in aqueous solutions, but it seems that melatonin dissolved in aqueous solutions is unreliable to use for clinical trials. Furthermore, the concentrations in these studies might be too small to be relevant in a clinical setting compared with the 10 mg/g achieved in the glycofurol formulation in the present study.

To our knowledge, this is the first trial investigating the solubility and stability of melatonin dissolved in DMSO, glycofurol, and propylene glycol. Study limitations were present since we only had data for 45 days, and solely at 25°C. Also, we did not make a comparison to melatonin in an aqueous solution.

![Figure 1. High-performance liquid chromatography elution profiles. Profiles shown are of melatonin 10 mg/mL in 20% (w/w) glycofurol, 10 mg/mL in 20% w/w glycofurol and 40% w/w dimethyl sulfoxide (DMSO), and 1 mg/g 50% DMSO stored for 45 days at 25°C.](image-url)
Since our experiments were performed, propylene glycol has received the dubious honor of being named the American Contact Dermatitis Society’s ‘Allergen of the Year 2018’ (Jacob et al., 2018). Adding this to the low solubility of melatonin in propylene glycol makes it hard to recommend using propylene glycol as a solvent for melatonin in clinical settings.

Both formulations containing DMSO demonstrated sufficient stability. The solution containing only glycofurol showed two unidentified peaks in the chromatogram at 45 days. Therefore, it can be speculated that these two peaks represent degradation products of melatonin. However, further studies aimed at identifying these two peaks are needed before they can be named as degradation products of melatonin. Both glycofurol and DMSO provide practical and relatively cheap ways of storing melatonin in a liquid solution. The stability of the DMSO formulations is good enough for them to be used for pharmacokinetic and safety trials in humans. If the formulations are to be used commercially in the long term, a longer stability experiment will have to be performed to determine a clinically relevant shelf life and requirements for storage temperatures.

Conclusion
The solubility of melatonin in propylene glycol was low, but melatonin was easily soluble in glycofurol. Glycofurol alone demonstrated sufficient stability, but also showed two unidentified peaks in the chromatogram. Both glycofurol/DMSO, and DMSO alone demonstrated a sufficient stability for melatonin solutions over 45 days at room temperature.

Data availability
Underlying data

This project contains the following underlying data:
- Data.xlsx (All results of HPLC analysis).
- Chromatograms.xlsx (Chromatograms for Day 0–45 measurements).

Extended data

This project contains the following extended data:
- Appendix 1 (Settings used for the HPLC-analysis).

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

References


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