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BRIEF REPORT

Solubility and stability of melatonin in propylene glycol, glycofurol, and dimethyl sulfoxide [version 1; peer review: 2 approved with reservations]

Dennis Zetner, Jacob Rosenberg

Center for Perioperative Optimization, Department of Surgery, Herlev Hospital, Herlev, 2730, Denmark

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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

Open Peer Review

Approval Status ? ?

	1	2
version 1 05 Feb 2020	? view	? view

1. **Marcel Henrique Marcondes Sari**, Universidade Federal de Santa Maria, Santa Maria, Brazil

2. **Aroonsri Priprem**, Khon Kaen University, 123 Mittraphap Road, Amphur Muang, Thailand

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Dennis Zetner (Dennis.zetner@gmail.com)

Author roles: Zetner D: Conceptualization, Methodology, Project Administration, Writing – Original Draft Preparation, Writing – Review & Editing; **Rosenberg J**: Conceptualization, Methodology, Project Administration, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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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:

a) 20% w/w glycofurol in MilliQ water containing 10 mg/g melatonin (glycofurol, 1.5 g; MilliQ water, 6 g; melatonin, 75 mg).

b) 20% w/w glycofurol and 40% w/w DMSO in MilliQ water containing 10 mg/g melatonin (Glycofurol, 1.5 g; DMSO, 3 g; MilliQ water, 3 g; melatonin, 75 mg).

c) 50% DMSO in MilliQ water containing 1 mg/g melatonin (DMSO, 3.75 g; MilliQ water, 3.75 g; melatonin, 7.5 mg).

All formulations were prepared by dissolving the relevant amount of melatonin in the organic solvents. Subsequently, the organic solution was added to the relevant volume of MilliQ water. Each formulation was portioned into 12 Eppendorf tubes. These were stored in a heating cabinet at 25°C for up to 45 days. Three Eppendorf tubes from each formulation were taken for analysis at Day 10, 17, 31 and 45. The amount of melatonin in each formulation was determined immediately after preparation (Day 0). Prior to HPLC analysis, the samples were diluted 40 times in acetonitrile. Settings for the HPLC are listed available as *Extended data* (Zetner, 2020).

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

Table 1. Solubility of melatonin in prepared formulations.

Solvent	Melatonin concentration (mg/ml)
Propylene glycol 20%	3.8 3.6
Glycofurol 20%	11.1 10.5

Table 2. Measured concentrations of melatonin in prepared formulations over 45 days.

Formulation	20% glycofurol			20% glycofurol/ 40% DMSO			50% DMSO		
Nominal concentration	10 mg/ml			10 mg/ml			1 mg/ml		
Measured concentration (mg/ml)									
Day 0	10.5	10.6	-*	11.4	11.3	-*	1.2	1.2	-*
Day 10	10.7	10.8	11.3	11.3	11.4	11.4	1.3	1.2	1.3
Day 17	10.4	10.5	10.8	11.3	11.1	11.3	1.2	1.2	1.2
Day 31	11.4	11.1	11.0	11.8	12.4	12.9	1.2	1.3	1.3
Day 45	10.3	10.0	9.6	10.6	11.3	11.1	1.1	1.1	1.1

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

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 (Cavallo & 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.

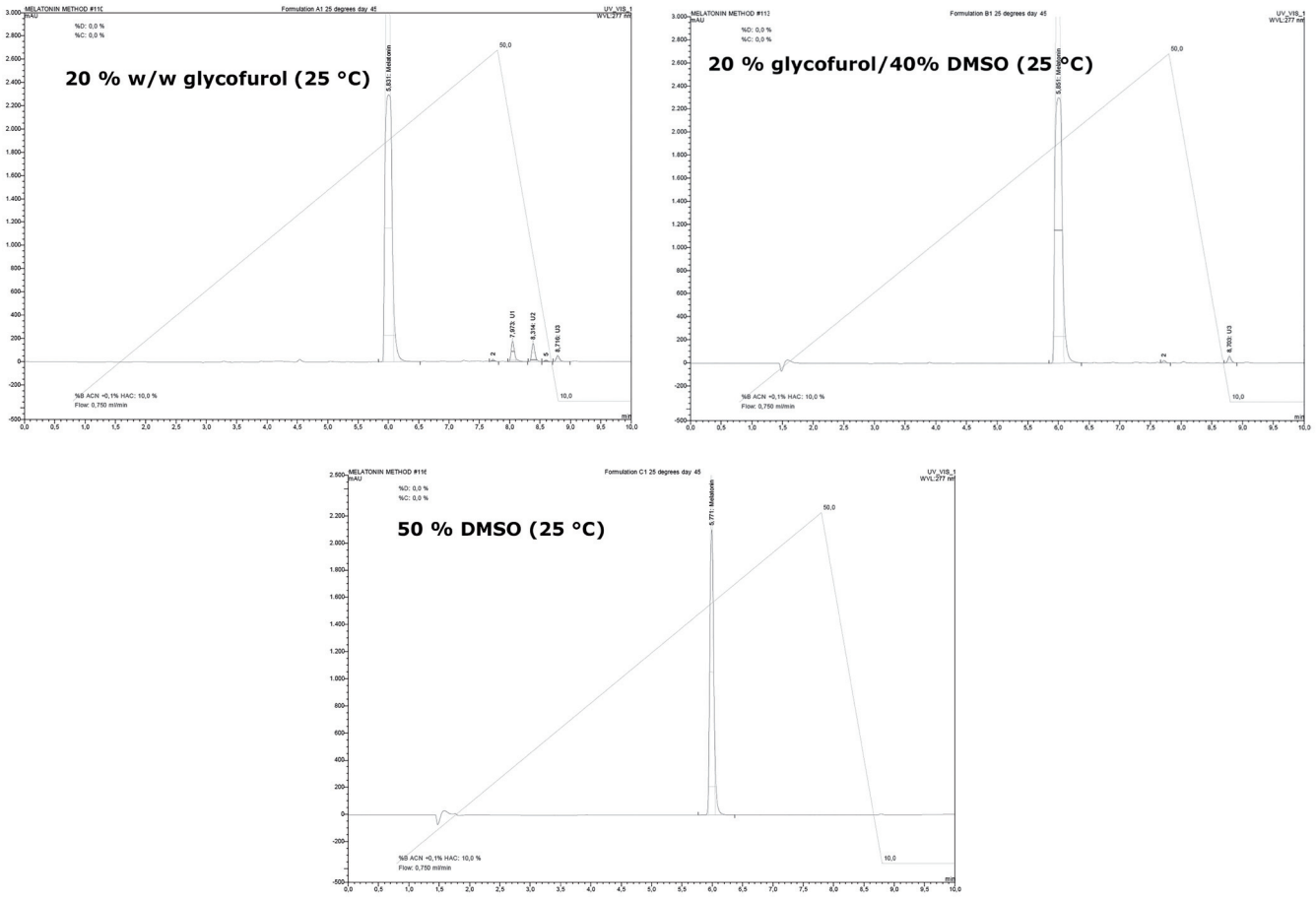

Figure 1. High-performance liquid chromatography elution profiles. Profiles shown are of melatonin 10 mg/mL in 20% (w/w) glycofurol, 10 mg/mL in 20% w/w glycofurol and 40% w/w dimethyl sulfoxide (DMSO), and 1 mg/g 50% DMSO stored for 45 days at 25°C.

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

Open Science Framework: Solubility and stability of melatonin in propylene glycol, glycofurol, and dimethyl sulfoxide. https://doi.org/10.17605/OSF.IO/N9Y7V (Zetner, 2020).

This project contains the following underlying data:

- Data.xlsx (All results of HPLC analysis).
- Chromatograms.xlsx (Chromatograms for Day 0–45 measurements).

Extended data

Open Science Framework: Solubility and stability of melatonin in propylene glycol, glycofurol, and dimethyl sulfoxide. https://doi.org/10.17605/OSF.IO/N9Y7V (Zetner, 2020).

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).

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http://www.doi.org/10.17605/OSF.IO/N9Y7V

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Aroonsri Priprem
Melatonin Research Group, Khon Kaen University, 123 Mittraphap Road, Amphur Muang, Khon Kaen, 40002, Thailand

Overall
If the formulations used in this study is to be used in clinical trials, as mentioned, it should provide concrete evidence on safety of the formulation and stability of melatonin (including potential degraded contaminant), as exemplified in a study: http://dx.doi.org/10.1016/j.jpha.2017.04.001.

Specific comments

1. The rationale of local administration of melatonin in a solution dosage form and the solvents or solvent mixes which were selected for studies are not fully addressed. Additional citations are essential to support the rationale.

2. Validations of all tests, particularly HPLC, are not provided. Controls and standards are not adequately provided. Without validated results, controls and standards, it is not certain if the results are reproducible.

3. There is no statistical analysis in all tests. Interpretation of stability studies can only be conclusive after statistical analysis of the data.

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Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?
No

If applicable, is the statistical analysis and its interpretation appropriate?
No

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: pharmaceutical technology, physicochemical characteristics, melatonin

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 18 March 2021

https://doi.org/10.5256/f1000research.24252.r80743

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Marcel Henrique Marcondes Sari
Laboratório de Tecnologia Farmacêutica, Centro de Ciências da Saúde, Departamento de Farmácia Industrial, Universidade Federal de Santa Maria, Santa Maria, Brazil

The present study provides important data regarding the solubility of melatonin and its stability in different solvents. The document is well-written and well-organized, which facilitates comprehension. During my reading, I identified a few issues that could be considered in the revision process.

1. The title is not informative. It should contain the main findings of the study.

2. In the introduction section, the authors could mention the clinical applications of melatonin and reinforce the importance of obtaining a suitable solvent for solubilizing this substance.

3. Methods – All the materials and reagents used in this study as well as the source of them must be informed.

4. Water solubility of melatonin for comparative purposes should be included to demonstrate if the proposed solvents improved the stability of the active substance.

5. Melatonin solubility in DMSO was not provided. Please, include it.

6. The authors could attach both chromatograms obtained in the stability evaluation (day 0 and 45) to better demonstrate the profiles. It would improve comprehension and readability.

7. Statistical evaluation of the data is missing and must be included in order to reinforce the conclusions made. More details regarding data expression and treatment must be informed.

8. The figures' resolution is not optimal. Besides, it is difficult to read the "melatonin" identification at the peak. The authors should improve figures' quality and also increase the font size.

9. Toxic issues must be considered in selecting an adequate solvent. Besides, the biocompatibility of substances is another critical concern. The authors should include a brief paragraph regarding such subjects in the discussion section.

10. Any idea regarding the superior stability of melatonin in DMSO solvent? There are some studies that demonstrated the potential antioxidant property of DMSO as a protective effect against melatonin oxidation (DOI 10.1002/nau.23204[1]). The authors could improve the discussion based on such scientific evidence.

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Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

No

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Nanotechnology; pharmaceutical formula development; biochemistry and toxicology; pharmaceutical technology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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