Suitability of Microemulsions as Vehicle System for Dermal Protein Application

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Introduction

Formulating a protein for dermal application (e.g. intra-dermal or vaccination effects) is challenging due to the protein’s molecular weight, hydrophilicity and sensitivity to external conditions, which mostly result in a short product shelf-life [1]. Here pre-microemulsion concentrates are promising topical formulations. Due to their spontaneous formation upon addition of water they facilitate a reduced contact time between the API and aqueous phase. Lipoid S LPC 80 was chosen as phospholipid-based surfactant with high self-emuilsifying power, besides its enhanced skin penetration effects and good tolerability [2, 3]. A successful penetration of the protein-loaded microemulsion will be supported by a microneedle system, stimulating a local imitation. Therefore, an ideal formulation should balance between good skin tolerability and high drug stability.

Aim of the Study

• Detection of structural changes of a model protein in a “natural”-based phospholipid microemulsion compared to a system including PEGylated surfactants
• Focus on time-dependent changes in structure and activity

Results

Enzymatic Activity Assay

Values were calculated compared to PC (100 %). Between 1 and 24 hr at RT values vary around a level of 57 %.

NanoDSF

Time dependent changes of catalase stability in two microemulsion systems. Catalase in phospholipid-based formulations shows a shift to lower values of thermal unfolding transition midpoint (Tm), but no loss in absorption maxima (λ). Stronger shifts of Tm along with an obvious decrease of signal intensities.

Size Exclusion Chromatography (SEC)

SEC profiles of catalase after different contact times with microemulsion systems. (A) Comparison of unchanged protein (PC) and after formulation preparation. Ingredients like Lipid S LPC 80 and Pluronic CC 407 were detected as small signals. (B) The longer the storage time of catalase the higher intermingling signals of emulsifying and lipophilic components. After 24 hr a small shoulder is separating from main peak, which underlines structural changes. (C) Catalase profiles after first contact with PEG microemulsion (F) Shift of protein retention time results in an overlay of catalase and water signal.

Materials and Methods

Testformulations

<table>
<thead>
<tr>
<th>Phospholipid-based Formulation (PA)</th>
<th>PEGylated Formulation (PA)</th>
<th>Positive Control (PC)</th>
<th>Negative Control (NC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipophilic Phase</td>
<td>Hydrophilic Phase</td>
<td>Surfactant/Co-surfactant</td>
<td></td>
</tr>
<tr>
<td>Lipid S LPC 80</td>
<td>Phosphate buffered saline (PBS)</td>
<td>Lipid S LPC 80/ Isopropanol</td>
<td></td>
</tr>
<tr>
<td>Pluronic Pluronic CC 407</td>
<td>pH 7.2</td>
<td>Cremophor EL</td>
<td>Polysorbate 80 Isopropanol</td>
</tr>
</tbody>
</table>

Catalase activity

Method: Spectroscopic measurements modified according to Beers & Sizer [4]

Description: $2H_2O_2 + 2H_2O + O_2$ NanoDSF

Size Exclusion Chromatography (SEC)

Schematic representation of NanoDSF setup. It is an advanced Differential Scanning Fluorimetry technology based on the detection of changes in chemical and thermal stability of proteins modified to Promethius IT-40 product information.

Summary and Conclusion

• Tested microemulsion systems have a different influence on protein stability
• Cremophor EL and Polysorbate 80 as PEGylated surfactants interfere catalase structure and reduce activity of less than a half
• Low stability of catalase in PEG formulation did not facilitate an appropriate application period
• Lipoid S LPC 80-based microemulsions provides best protein stability in an application period of at least 1 hr
• They are suitable vehicle systems for enzymes like catalase

Next to protein stability and activity measurements, final tolerability studies are ongoing as important investigations for the acceptance as dermal application system.

References


Acknowledgements

The authors would like to thank NanoTemper Technologies GmbH, Lipoid GmbH and Gattefosse for their support. This project is financed by the Phospholipid Research Center.

DPhG Annual Meeting 2016
Munich, 4th – 7th October