Suitability of Microemulsions as Vehicle System for Dermal Protein Application

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Introduction

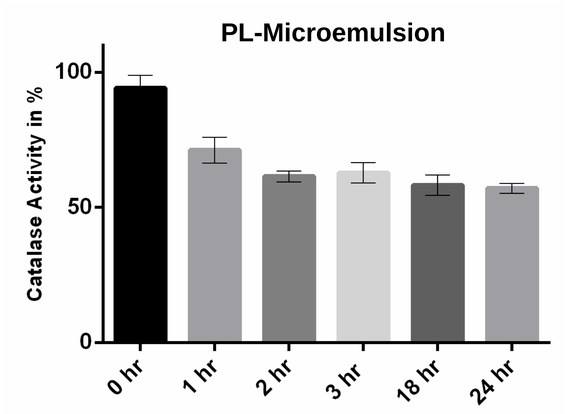
Formulating a protein for dermal application (e.g. intra-dermal or vaccination effects) is challenging due to the protein`s molecular weight, hydrophilicity and sensitivity to external conditions, which mostly results in a short product shelf-life [1]. Here pre-microemulsion concentrates are promising 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-emulsifying 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 irritation. Therefore, an ideal formulation should balance between good skin tolerability and high drug stability.

Aim of the Study

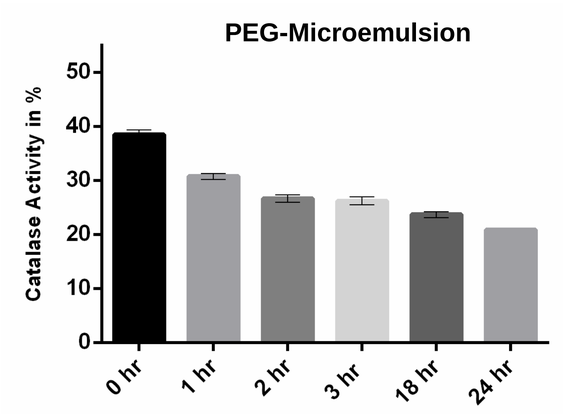
- **Detection of structural changes of a model protein in a "natural"–based phospholipid microemulsion compared to a system including PEGylated surfactants**
- **Focus on time-dependent changes in structure and activity**

Results

Enzymatic Activity Assay

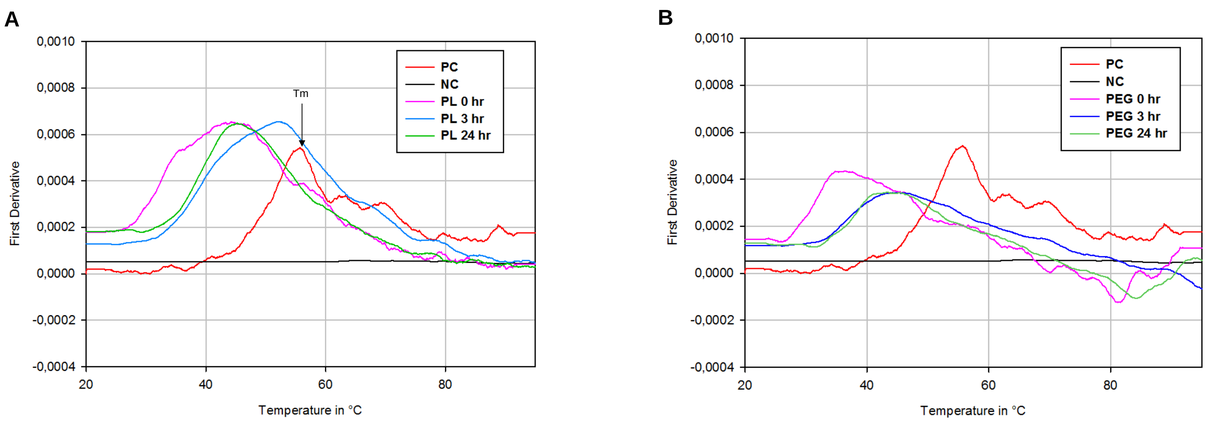


Values were calculated compared to PC (100 %). Between 1 and 24 hr at RT values vary around a level of 57 %.
(n = 4, error bars represent standard deviation)



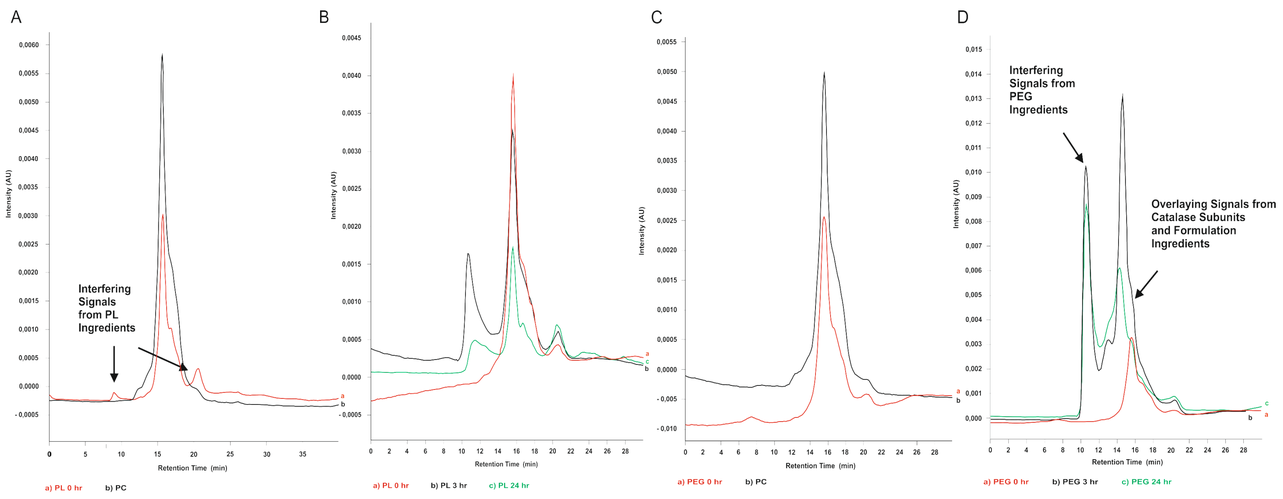
Values were calculated according to first graph. Directly after preparing final formulation, the protein activity level has been halved and evenly decreased to 21 %.
(n = 4, error bars represent standard deviation)

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 midpoints (Tm), but no loss in absorption intensity (B) 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 Lipoid S LPC 80 and Plurol Oleique CC 497 were detected as small signals. (D) The longer the storage time of catalase the higher intefering signals of emulsifying and lipophilic components. After 24 hr a small shoulder is separating from main peak, which underlines structural changes. (E) Catalase profiles after first contact with PEG microemulsion (F) Shift of protein retention time results in an overlay of catalase and ingredient signal.

Materials and Methods

Testformulations

PL formulation with a "natural"- based surfactant.

	Phospholipid -based Formulation (PL)	PEGylated Formulation	Positive Control (PC)	Negative Control (NC)
Lipophilic Phase	Plurol Oleique CC 497			
Hydrophilic Phase	Phosphate buffered saline (PBS) pH 7.0			PBS pH 3.5
Surfactant/ Cosolvent	Lipoid S LPC 80/ Isopropanol	Cremophor EL, Polysorbate 80/ Isopropanol		
Model Protein	0.1 % Catalase		0.1 % Catalase	

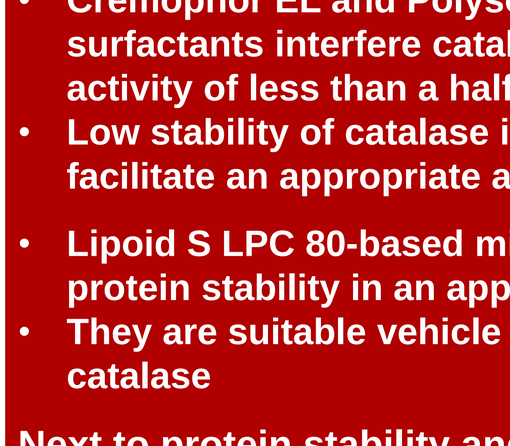
PEG formulation with well-known PEGylated surfactants.

Enzymatic Activity Assay

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

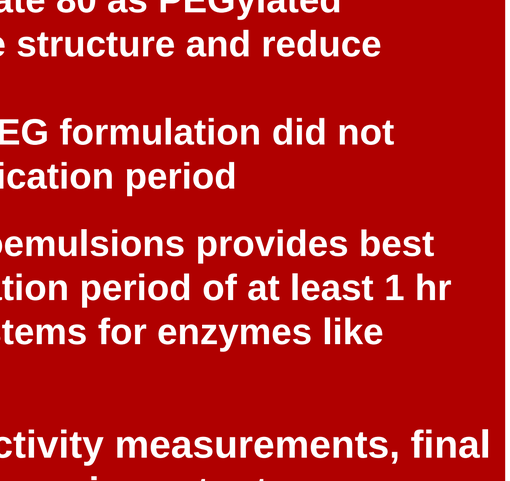
Description: $2\ H_2O_2 \xrightarrow[A_{240}]{\text{Catalase}} 2\ H_2O + O_2$

NanoDSF



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 according to Prometheus NT.48 product information).

Size Exclusion Chromatography (SEC)



Catalase subunits or aggregates could be separated according to their molecular size. Dissolved smaller molecules move more slowly through the column, whereas large molecules move quickly and elute earlier.

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

[1] Frokjaer, S.: Nature Reviews Drug Discovery **2005**, 4(4): 298-306.
[2] Kogan, A., Garti, N.: Adv. Coll. Interface Sci. **2006**, 123: 369-385.
[3] Paolino, D. *et al*.: Int. J. Pharm. **2002**, 244(1): 21-31.
[4] Beers, R.F.Jr., Sizer, I.W.: J. Biol. Chem. **1952**, 195: 133-140.

Acknowledgements

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

DPhG Annual Meeting 2016

Munich, 4th – 7th October