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Solubility Studies in the Development of Phenytoin Nanoemulsions for Intranasal Delivery

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Introduction

Phenytoin (PHT), an antiepileptic drug, must reach the central nervous system to act. The oral administration route is the most common one but it is not an optimal option for PHT. This happens because this drug has low water solubility as well as an erratic gastrointestinal absorption profile [1]. Given the existence of a direct nasal-brain connection [2] and since nasal delivery presents itself as a non-invasive and convenient administration route with promising results, it can be explored for this type of drugs. The chosen pharmaceutical form was the nanoemulsion, since it may enhance PHT's solubility and absorption profile. As part of a rational formulation design process, an analysis of literature was used in order to choose the oil phase excipients and solubility studies were performed to determine the most promising one for the nanoemulsion formulation.

Methods

Bibliographic Analysis

A bibliographic search was conducted in the PubMed database. Studies describing the intranasal administration of nano/microemulsions and presenting pharmacokinetic data were selected for analysis.

$$\text{Drug Targeting Efficiency, DTE (\%)} = \frac{(AUC_{brain}/AUC_{blood})_{in}}{(AUC_{brain}/AUC_{blood})_{iv}} \times 100 \quad (1)$$

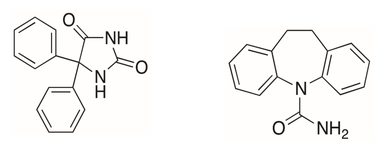
$$\text{Direct Transport Percentage, DTP (\%)} = \frac{B_{in} - B_x}{B_{in}} \times 100, \quad B_x = \frac{B_{iv}}{P_{iv}} \times P_{in} \quad (2)$$

$$\text{Bioavailability, BA (\%)} = \frac{AUC_{brain\ in}}{AUC_{brain\ iv}} \times 100 \quad (3)$$

Assay

For the determination of the solubilized PHT in the selected oil phase excipients, a spectophotometric assay using 7,7,8,8-tetracyanoquinodimethane (TCNQ) and High-Performance Liquid Chromatography (HPLC) were performed and compared. The TCNQ method is based on the formation of a charge-transfer colored complex whose absorvance can be measured at 842 nm [3]. The chromatographic conditions were:

- Column: LiChroCART Purospher® Star C18 (55 mm x 4 mm x 3 µm)
- Column temperature: 30°C
- Mobile phase: phosphate buffer (10 mM) with 0.25 % triethylamine (pH 3.0) – methanol (64:36, V/V)
- Elution type: isocratic
- Flow rate: 1 mL/min
- Detection: DAD at 215 nm
- Run time: 16 min
- Injection volume: 20 µL



Phenytoin (PHT) **10,11-Dihydrocarbamazepine (IS)**

Fig.1: Chemical structure of the analyte (PHT) and internal standard (10,11-Dihydrocarbamazepine).

Solubility Study

In the PHT solubility study, the solutions were kept at 25°C with a constant agitation of 60 rpm and sampled at three time points (4, 24 and 48h).

<u>Preparation of saturated PHT solutions at 25°C:</u>

25 mg PHT + 1 g excipient → Store solutions in the orbital shaker → Vortex solutions (30 s) every hour during the first 4 h

<u>Sample preparation and analysis of PHT concentration by HPLC:</u>

Vortex and collect 150 µL of each sample → Centrifuge samples at 18400 g and 25°C (15 min) → 20 mg of supernatant + 20 µL IS + 960 µL MeOH; mix thoroughly with vortex → Rest in ice (15min) → Centrifuge samples at 18400 g and 4°C (15 min) → 780 µL of mobile phase + 20 µL of supernatant and freeze at -20°C → Thaw samples at room temperature → Centrifuge samples at 18400 g and 4°C (15 min) and transfer 100 µL to a vial for HPLC analysis

Results

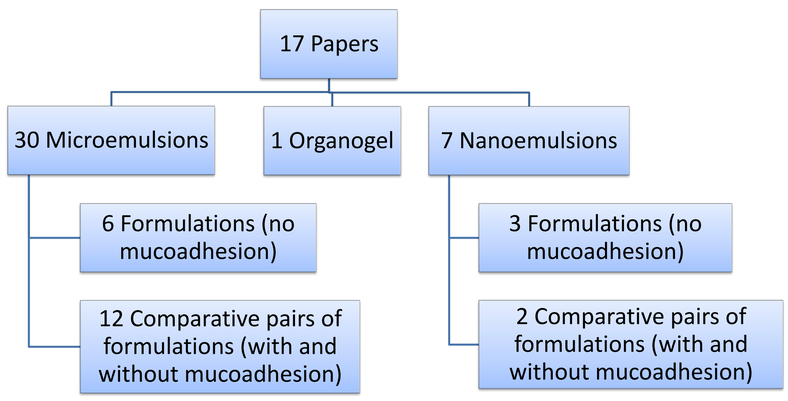


Fig.2: Formulation distribution in the analyzed research papers.

Table 1: Comparison of the quantification capacity of PHT by TCNQ and HPLC methods.

Method	Recuperation (%) (mean ± SD)	Calibration curve	r^2	Concentration range (µg/mL)
TCNQ	Cetiol V	91 ± 4	0.0679x + 0.0781	0.9492	2.5 - 20
	Miglyol 812	105 ± 3	0.0815x + 0.1077	0.9573	
	Imwitor 948	75 ± 9	0.0662x + 0.0497	0.9685	
HPLC	Cetiol V	90 ± 7	0.3131x + 0.0421	0.9998	0.125 - 30
	Miglyol 812	96 ± 18			
	Imwitor 948	91 ± 2			

- A strong interference from Span 80 in the spectrophotometric assay was verified.
- HPLC was chosen for the solubility study on the basis of its higher linearity over a wider range of PHT concentrations.

- 5 classes of oil excipients were identified

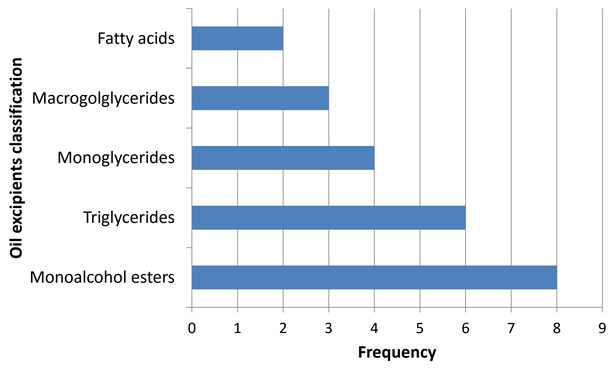


Fig.3: Classification of the oil phases used in the included research papers.

- 4 oil phase candidates were tested: Cetiol V (monoalcohol ester), Miglyol 812 (triglyceride), Imwitor 948 (monoglyceride) and Span 80 (lipophilic surfactant).
- Span 80 was excluded due to a troublesome sample preparation.

- The mucoadhesive polymers enhanced the nano/microemulsion's Drug Targeting Efficiency (DTE), brain drug Direct Transport Percentage (DTP) and Bioavailability (BA) (p= 0,0184; p=0,0375 and p=0,0003, respectively).

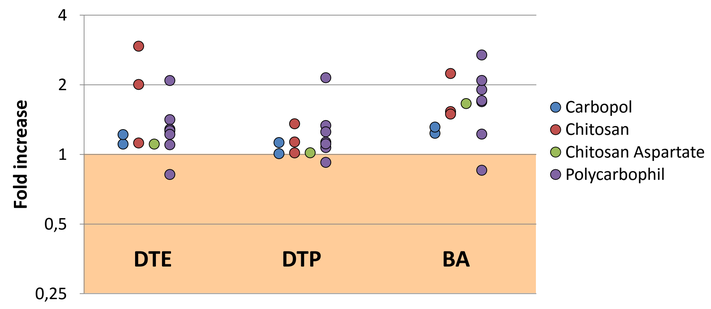
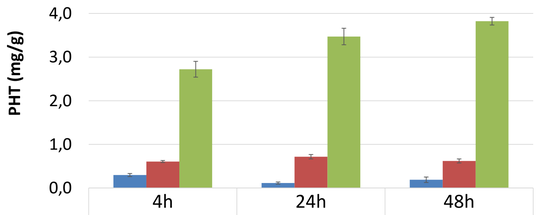


Fig.4: Ratio of pharmacokinetic parameters (mucoadhesive:non-mucoadhesive formulations). The difference from 1 was evaluated by One Sample t-test, on Prism 6,01 (GraphPad Software Inc.).

- Solubility was significantly higher in Imwitor 948 (p<0,0001)



	4h	24h	48h
Cetiol V	0,30	---*	---*
Miglyol 812	0,61	0,72	0,62
Imwitor 948	2,72	3,47	3,82

Fig.5: PHT solubility in oils at 25°C (mg/g of excipient). Mean ± SD (n=3). *[PHT] below the limit of quantification. (statistical significance was evaluated by t-test on Prism 6,01 (GraphPad Software Inc.).

Conclusions

- The inclusion of a mucoadhesive polymer in the nanoemulsions is an important factor to consider.
- Imwitor 948 was selected as the oil excipient.
- The next stages of this study will be to select a combination of surfactant and co-surfactant/co-solvent and to characterize the resulting nanoemulsions in regard to their droplet size, polydispersity index and zeta potential.

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References: [1] Atef E. *et al*. *Eur J Pharm Sci*. **2008**, 35, 257-263. [2] Dhuria S. *et al*. *J Pharm Sci*. **2010**, *99(4)*, 1654-1673. [3] Saleh G. *Talanta*. **1998**, 46, 111-121.

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