46

Optimization of diazepam-loaded nanostructured lipid carriers (NLC) for nose-to-brain delivery using the quality by design (QbD) approach

C. P. Costa[1]*, S. Cunha[1], J.N. Moreira[2,3], J.M. Sousa Lobo[1] and A.C. Silva[1,4]

1. UCIBIO, REQUIMTE, MEDTECH, Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal.
2. CNC – Center for Neurosciences and Cell Biology, Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Faculty of Medicine (Polo 1), Rua Larga, 3004-504 Coimbra, Portugal
3. UC – University of Coimbra, CIBB, Faculty of Pharmacy, Pólo das Ciências da Saúde, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal

[4]UFP Energy, Environment and Health Research Unit (FP-ENAS), Fernando Pessoa University, Porto, Portugal.

*Presenting author e-mail: claudiaspinacosta@gmail.com

Introdution

Epilepsy requires fast and effective treatment, targeting the brain. Herein, intranasal administration of nanostructured lipid carriers (NLC) has been suggested as a promising strategy [1]. In addition, the quality-by-design (QbD) approach is a useful tool for the optimization of manufacturing variables, resulting in effective and safe pharmaceutical products [2]. The aim of this work was to use the QbD approach to optimize a NLC formulation for the nose-to-brain delivery of diazepam, improving the emergency therapy of epilepsy. The studies began with screening of excipients and assessing lipid-drug compatibility. Afterwards, the central composite design was used to evaluate the effects of critical material attributes (CMAs) (ratio of solid and liquid lipids and amount of emulsifiers) on the critical quality attributes (CQAs) of the NLC formulation (particle size, polydispersity index (PDI), zeta potential (ZP) and encapsulation efficiency (EE)).

Experimental

Screening of excipients

- Solid lipids: Precirol® 5 ATO (glyceryl distearate), Imwitor® 900K (glyceryl stearate), Compritol® 888 ATO (glyceryl dibehenate), Gelucire® 43/01 (hard fat compounds), Gelucire® 44/14 (lauroyl polyoxyl-32 glycerides), Gelucire® 50/13 (stearoyl polyoxyl-32 glycerides), glyceryl monostearate, stearic acid, cetyl palmitate, Softisan® 100 (hydrogenated coco-glycerides), Softisan® 154 (hydrogenated palm oil), Dynasan® 118 (glyceryl tristearate), Apifil® (PEG-8 beeswax) and Witepsol® E85 (hard fat compounds).
- Liquid lipids: Miglyol® 812 (medium-chain triglycerides), oleic acid, isopropyl myristate, Cetiol® V (decyl oleate), vitamin E, Labrafac® W1349 (medium chain triglycerides), Capryol® 90 (propylene glycol monocaprylate) and Microcare® (cetyl dimeticone).
- Emulsifiers: non-ionic emulsifiers, such as Tween 80® (polysorbate 80), Lutrol® F68 (poloxamer 188), Lutrol® F127 (poloxamer 407); anionic emulsifiers, such as sodium deoxycholate and phospholipids, Phospolipon® 90 G and Phospolipon® 90 H.

Compatibility between solid and liquid lipids

- The compatibility between lipids were evaluated by screening different ratios of solid and liquid lipids, i.e. 60:40, 70:30, 80:20 and 90:10, which were heated 5-10ºC above the melting point of the solid lipid and cooled to room temperature. Afterwards, the mixture was placed in a hydrophilic filter paper, followed by visual observation to determine the presence/absence of oil droplets on the filter.

Lipid-drug solubility

- To evaluate the lipid-drug solubility, an excess of diazepam (5-10 %, w/w) was added to the lipid and heated 5-10 ºC above its melting point, under continuous stirring during 60 minutes. Afterwards, was observed the presence/absence of insoluble drug crystals. The same procedure was performed for the liquid lipid.

Preparation and characterization of diazepam-loaded NLC

- Diazepam-loaded NLC were prepared from the method previously employed by Silva et al [3]. Based on the results of the lipid-drug solubility and the compatibility between lipids. Precirol® 5 ATO and Cetiol® V were selected as the solid lipid (SL) and liquid lipid (LL), respectively. Tween 80® and sodium deoxycholate were selected as emulsifiers. Particle size was evaluated by laser diffractometry (Mastersizer 3000, Malvern) and dynamic light scaterring (DLS), using a Malvern nanozetasizer (Malvern, UK). PDI and ZP were evaluated using the same Malvern nanozetasizer. Diazepam was quantified by high pressure liquid homogenization (HPLC), with a wavelenght of 254 nm. The encapsulation efficiency (EE) of diazepam in the NLC was calculated according to the following equation:

  EE (%) = [(total amount of drug – free drug) / total amount of drug] x 100.

Table 1: Composition of diazepam-loaded NLC formulation.

Composition	(w/w%)
Precirol® 5 ATO	6.65
Cetiol® V	2.85
Diazepam	0.50
Tween 80®	4.20
Lutrol® F68	0.30
Benzalkonium chloride	0.02
Ultrapure water	q.s. 100.00

Quality by Design (QbD)

- The QbD was applied to optimize the diazepam-loaded NLC formulation, improving manufacturing process and ensuring the quality and safety of the final product. Quality target profile product (QTPP) and critical quality attributes (CQAs) were identified and a risk assessment analysis was conducted to detect the critical material attributes (CMAs) and critical process parameters (CPPs).
- The effects of the CMAs and CPPs on the CQAs of the final formulation is schematically presented in Figure 1.

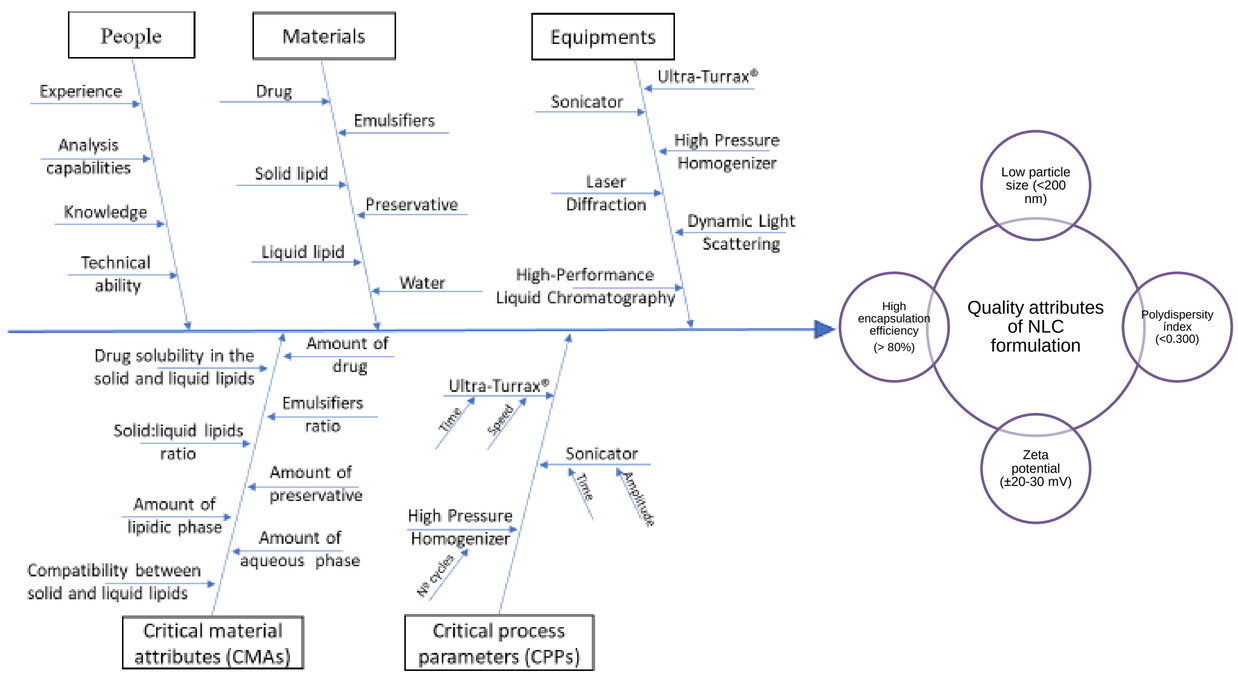


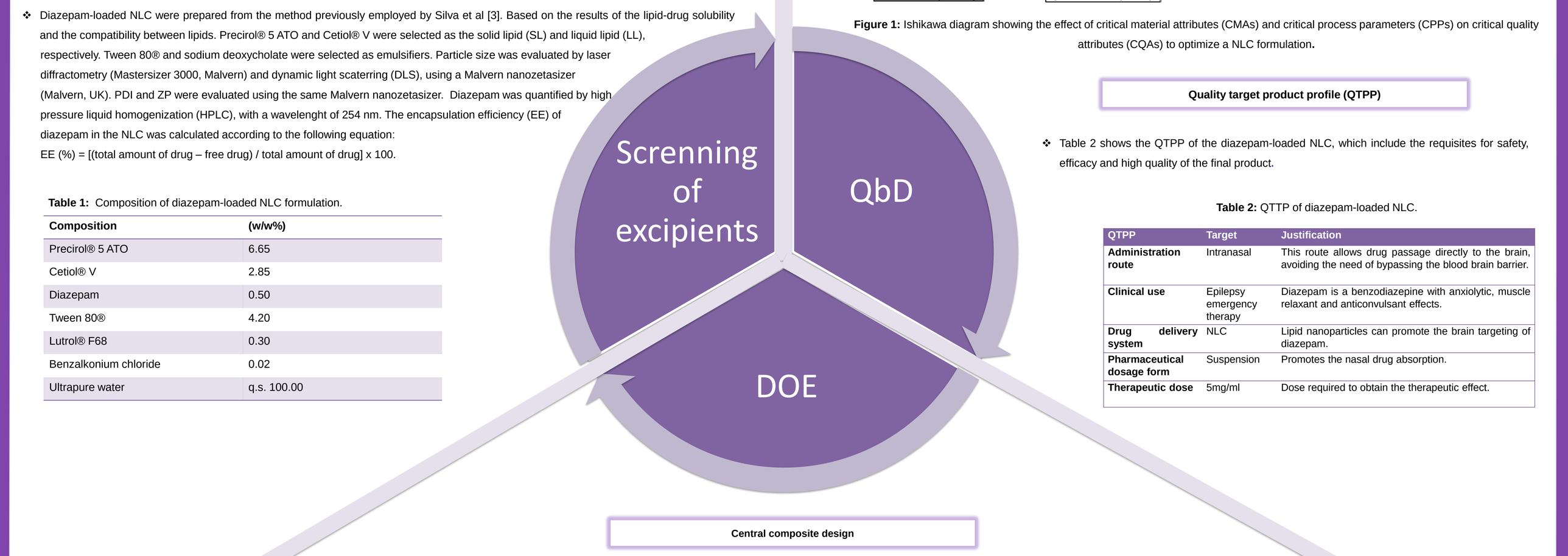
Figure 1: Ishikawa diagram showing the effect of critical material attributes (CMAs) and critical process parameters (CPPs) on critical quality attributes (CQAs) to optimize a NLC formulation.

Quality target product profile (QTPP)

- Table 2 shows the QTPP of the diazepam-loaded NLC, which include the requisites for safety, efficacy and high quality of the final product.

Table 2: QTTP of diazepam-loaded NLC.

QTPP	Target	Justification
Administration route	Intranasal	This route allows drug passage directly to the brain, avoiding the need of bypassing the blood brain barrier.
Clinical use	Epilepsy emergency therapy	Diazepam is a benzodiazepine with anxiolytic, muscle relaxant and anticonvulsant effects.
Drug delivery system	NLC	Lipid nanoparticles can promote the brain targeting of diazepam.
Pharmaceutical dosage form	Suspension	Promotes the nasal drug absorption.
Therapeutic dose	5mg/ml	Dose required to obtain the therapeutic effect.



Central composite design

- Central composite design was used to evaluate the effects of CMAs and CPPs on the CQAs of the NLC formulation (Table 3).

Table 3: Selection of the central composite design variables and respective levels.

CMAs	-2	-1	0	1	2
Ratio SL:LL	5:5	6:4	7:3	8:2	9:1
Ratio E1:E2	2.4:0.1	2.9:0.1	3.3:0.2	3.7:0.3	4.2:0.3

SL: Precirol® 5 ATO; LL: Cetiol® V; E1: Tween 80®; E2: sodium deoxycholate

Table 4: Effect of the CMAs on the CQAs (size (D50, D90 and Z-Ave), PDI, ZP and EE).

CMA75	D(50) nm	D(90) nm	Z-Ave (nm)	PDI	ZP (mV)	EE (%)
A1	57.100±0.001	167.000±0.003	129.300±46.960	0.179±0.000	-16.100±7.240	93.960±0.001
A2	60.600±0.000	146.000±0.004	107.000±44.690	0.188±0.000	-20.200±7.710	94.770±0.001
A3	53.700±0.002	141.000±0.002	140.300±48.400	0.205±0.000	-18.000±6.440	92.720±0.003
A4	55.300±0.000	145.000±0.003	93.980±47.430	0.180±0.000	-19.200±9.440	94,470±0.001
A5	77.200±0.000	126.000±0.007	109.600±40.260	0.157±0.000	-20.600±8.500	94,600±0.002
A6	68.700±0.001	192.000±0.005	113.900±47.660	0.185±0.000	-16.200±8.660	94.430±0.000
A7	55.700±0.003	155.000±0.009	158.400±45.660	0.164±0.000	-14.100±9.500	92.000±0.003
A8	53.300±0.000	137.000±0.003	84.920±45.750	0.178±0.000	-18.200±7.220	95.480±0.001
A9	75.500±0.005	134.000±0.007	110.900±40.080	0.153±0.000	-18.400±8.340	94.750±0.002
A10	75.200±0.001	133.000±0.008	110.500±42.760	0.151±0.000	-18.100±8.890	94.790±0.002

- From Table 4 and Figure 2, it can be observed that the most adequate ratios of lipids and emulsifiers were 6.65:2.85 and 4.2:0.3 (%, w/w), presenting values of 84.92 nm, 0.178, -18.20 mV and 95.48% for particle size, PDI, ZP and EE, respectively.

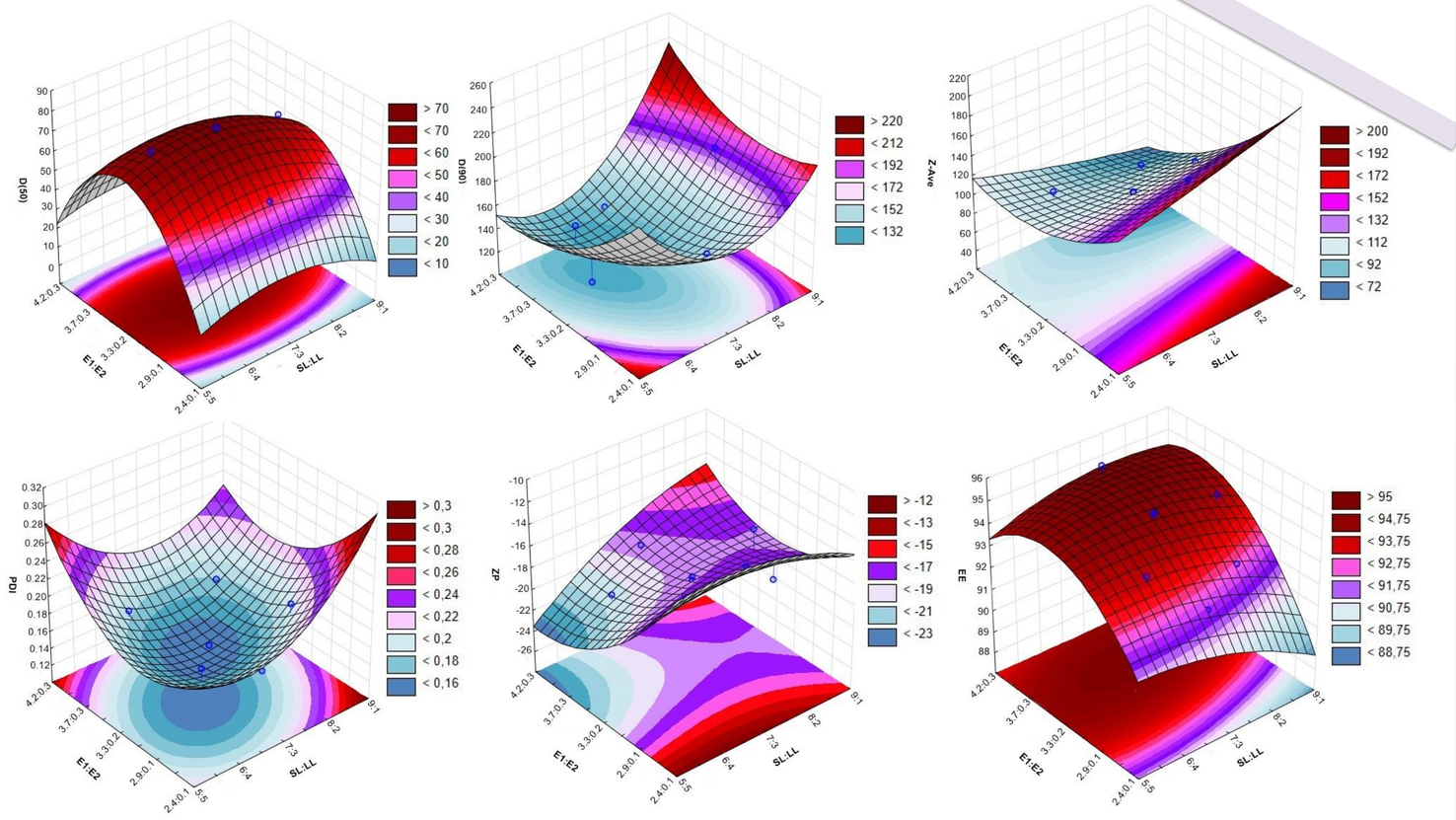


Figure 2: The 3-D surface plots portraying the effect of the ratio between the solid and liquid lipids and the two emulsifiers on the size (D(50), D(90), Z-Ave), PDI, ZP and EE.

Conclusion

The formulation with the best CQAs was selected for the second part of the optimization, which is related to selection of the best CPPs through the same design of experiment. The final formulation will be tested *in vitro* and *in vivo*.

References

[1] Costa, C. et al. Nose-to-brain delivery of lipid-based nanosystems for epileptic seizures and anxiety crisis, Journal of Controlled Release. 187-200 (2019). [2] Cunha, S., et al., Using the quality by design (QbD) approach to optimize formulations of lipid nanoparticles and nanoemulsions: A review. Nanomedicine, 2020]. [3] Silva AC, *et al*. Preparation, characterization and biocompatibility studies on risperidone-loaded solid lipid nanoparticles (SLN): High pressure homogenization versus ultrasound. Colloids and Surfaces B: Biointerfaces, 86, 158 – 165 (2011).

Acknowledgements This work was supported by Fundação para a Ciência e a Tecnologia (FCT), Portugal (SFRH/BD/136177/2018) and by the Applied Molecular Biosciences Unit-UCIBIO which is financed by national funds from FCT/MCTES (UIDMulti/04378/2019).