An Innovative Nanocarrier for neuroprotection

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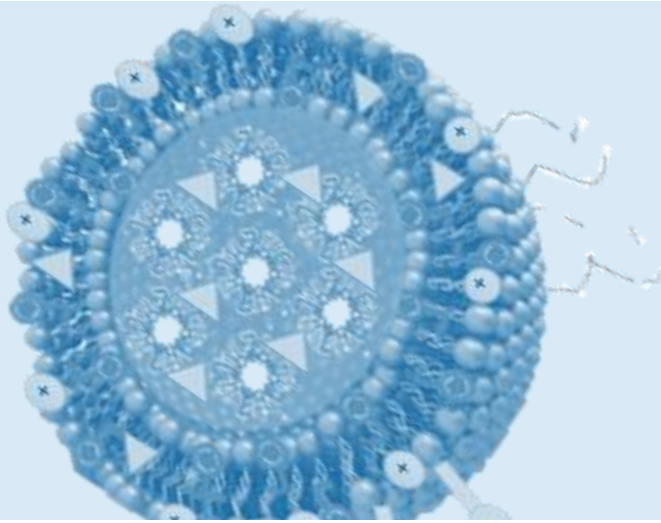
MSc in Biophysics and Bionanosystems



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Cofinanciado por:
COMPETE 2020
PORTUGAL 2020
UNIÃO EUROPEIA
Fundo Europeu de Desenvolvimento Regional
IAPME
Parcerias para o Crescimento

Presentation Overview

Step by step

Curcumin

Genesis and pharmacological targets

Neurodegenerative Disorders

Incidence and etiology

Main Goal

Encapsulation of commercial Curcumin

Selected Formulation

Liposomal formulation DODAB:MO(1:2)

Methodologies used in the characterization and development of formulations

Results presentation and discussion

Results regarding the characterization and development of formulations

Conclusions and work in progress

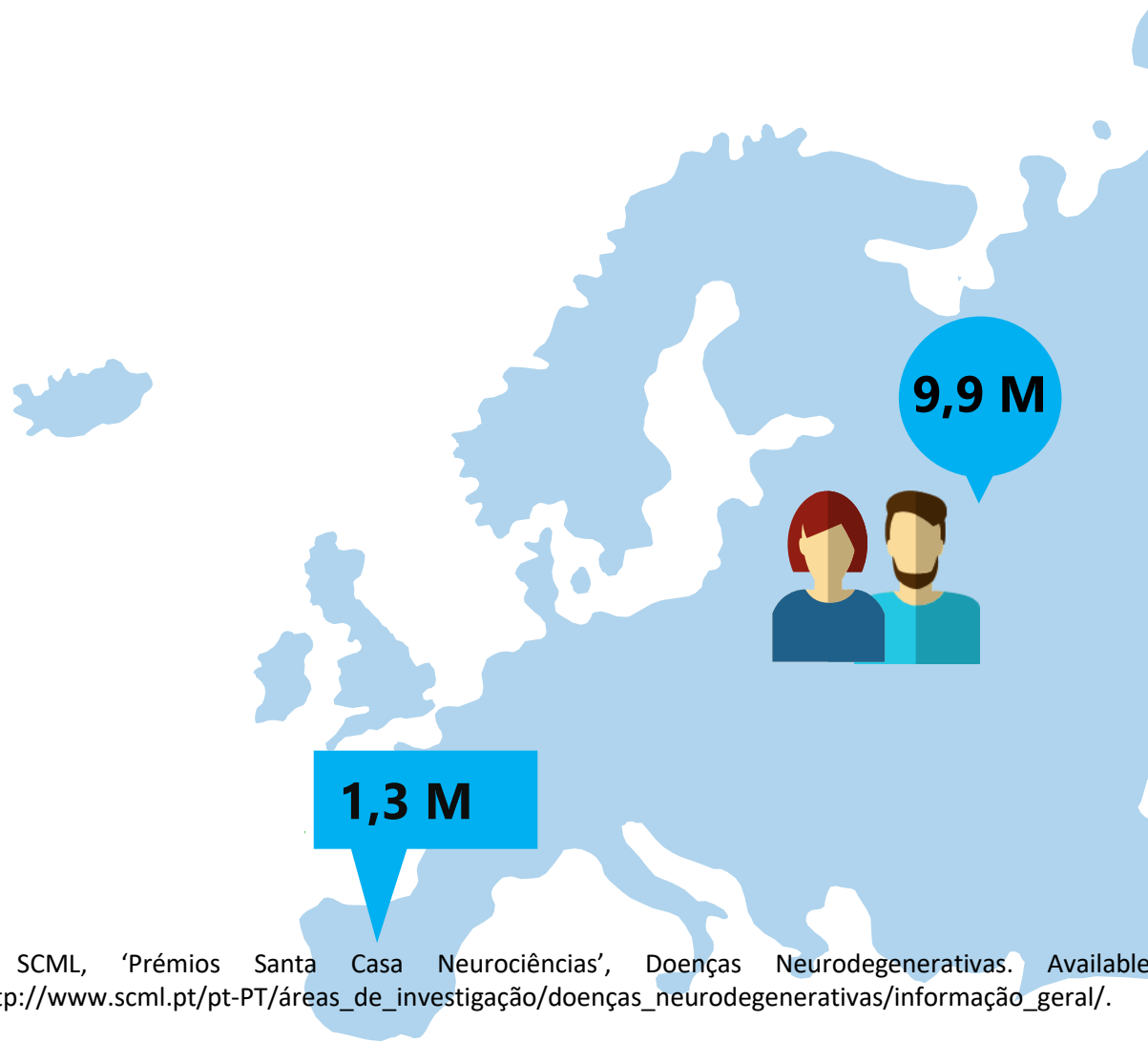
Summary of conclusions obtained and brief description of work in progress

Neurodegenerative Disorders

Incidence and etiology at European level

These are debilitating and time-consuming conditions, characterized as multifactorial disorders that promote irreversible dysfunctions resulting from the progressive degeneration of CNS nerve cells.

Telma Soares *et al., Progress in Neurobiology,* **2018**, *in press*



* SCML, 'Prémios Santa Casa Neurociências', Doenças Neurodegenerativas. Available http://www.scml.pt/pt-PT/áreas_de_investigação/doenças_neurodegenerativas/informação_geral/.

Curcumin

Genesis and importance on neurodegenerative diseases

Therapeutic utilities

Anti-cancer, anti-inflammatory and anti-oxidant properties.

Decrease of peptidic aggregation

Decrease of Aβ aggregation and ENF formation

Traditional uses

Seasoning, flavoring and coloring agent

Reduction of oxidative stress

Reduction of ROS and RNS

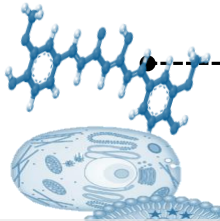
Curcuma genus

Zingiberaceae family
80 species

Decrease of neuroinflammation

Decrease of pro-inflammatory cytokines and glutamate-induced toxicity

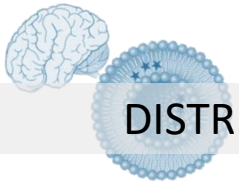
CURCUMIN ADMET PROPERTIES



ABSORPTION

Lipophilicity and cellular permeability

Derivative Spectroscopy



DISTRIBUTION

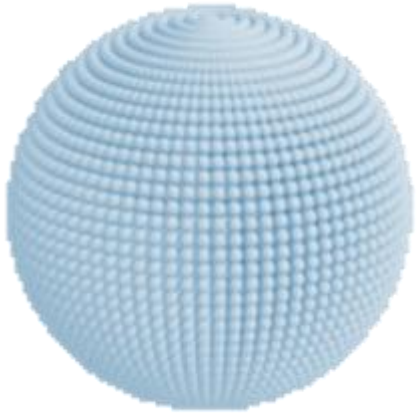
BBB permeability

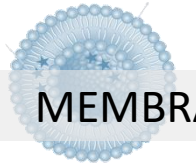
Derivative Spectroscopy

DISTRIBUTION/EXCRETION

Blood protein binding

Fluorescence, DLS and ELS





MEMBRANE TOXICITY

Changes in membrane biophysics

DSC, SAXS, WAXS and DLS

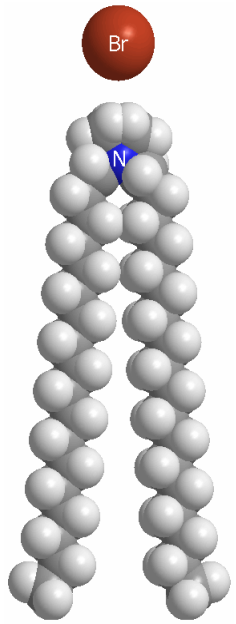
The main goal of this research

Formulation rational development and characterization

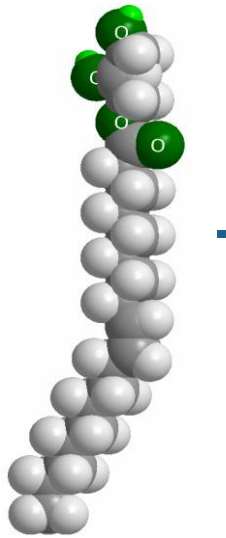
Development of a formulation loaded with curcumin for prevention and treatment of neurodegenerative disorders.

Selected Liposomal formulation

DODAB:MO (1:2)

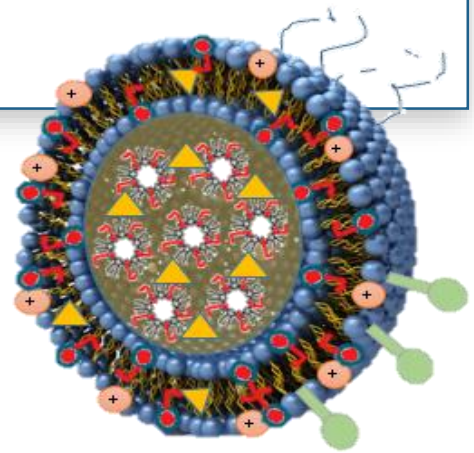


DODAB is a cationic lipid that tends to form lamellar phases.



MO is a neutral lipid that forms inverted non-lamellar phases.

- **STABILITY**: DODAB forms rigid membranes which ensure the stability of the formulation.
- **DRUG LOADING CAPACITY:** MO forms inside the liposomes a sponge-like structure with high lipid content and water, being able to charge both hydrophilic and lipophilic drugs.



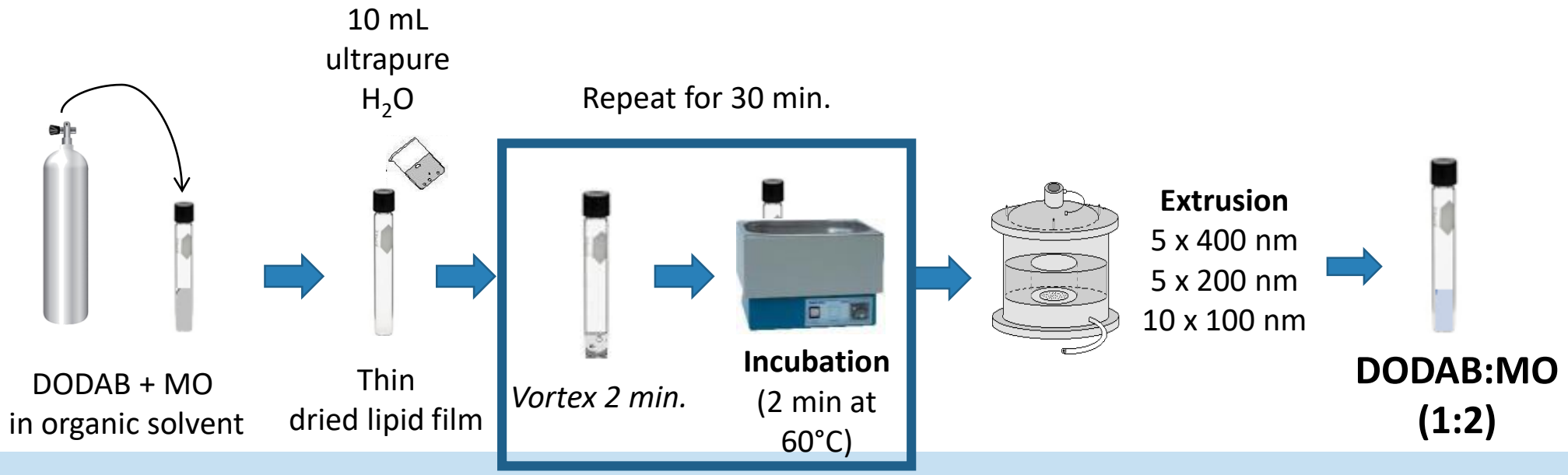
A.C. N. Oliveira *et al.*, *RSC Adv.*, **2016**, *6*, 57730.

Methodologies used in the development and characterization of formulations

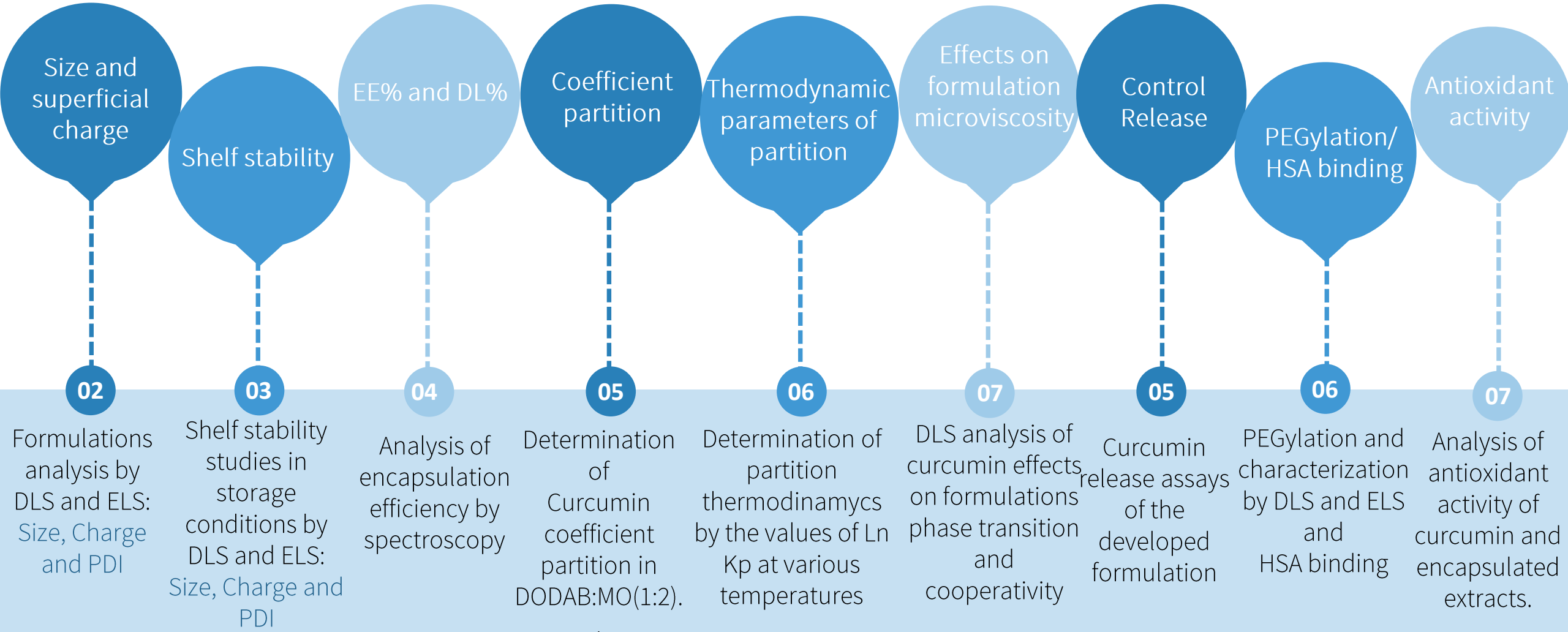
Formulations development

01

Preparation of formulations by different methods: Injection, Hydration and Incubation.

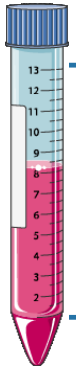


Methodologies used in in the development and characterization of formulations

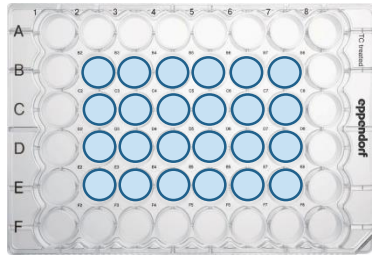


Methodologies used in the characterization and development of formulations

Cellular Assays



Suspension of **5 × 10^5 cells/well** in complete culture medium



On the next day after seeding ,cells were exposed 24h for the testing conditions

Cellular viability experiments:

- Neutral Red Uptake
- MTT Reduction

DODAB:MO (1:2) concentrations ranging from 1 µM to 500 µM

Curcumin concentration ranging from 1 µM to 100 µM

08

Analysis of concentration-toxicity curves of: (i) curcumin, (ii) empty nanocarrier and (iii) curcumin loaded nanocarrier.

Formulation characterization

SIZE < 200 nm and PDI < 0.25?

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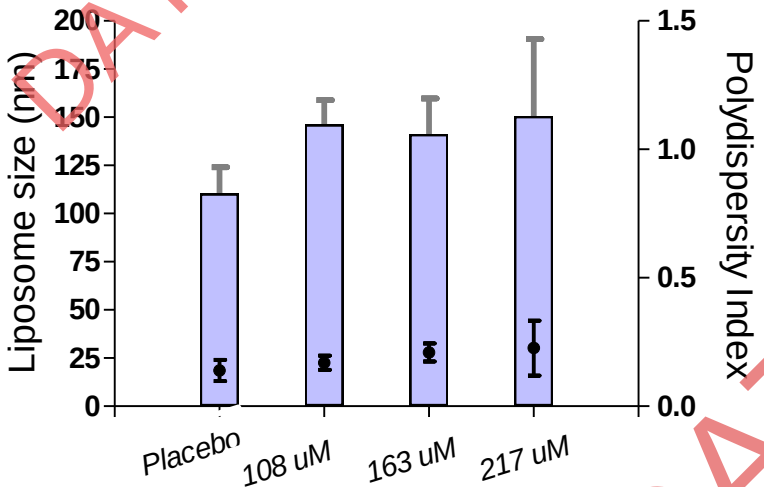
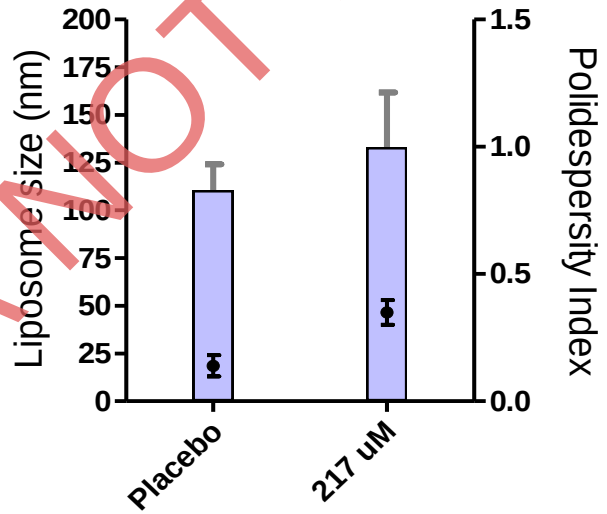
LUVs DODAB:MO (1:2)

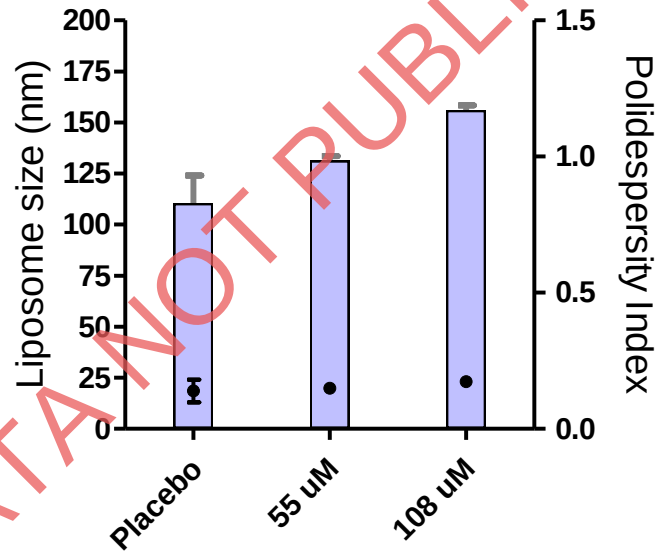
INCUBATION



HYDRATION



INJECTION



Formulation characterization

SURFACE CHARGE > +30 mV?

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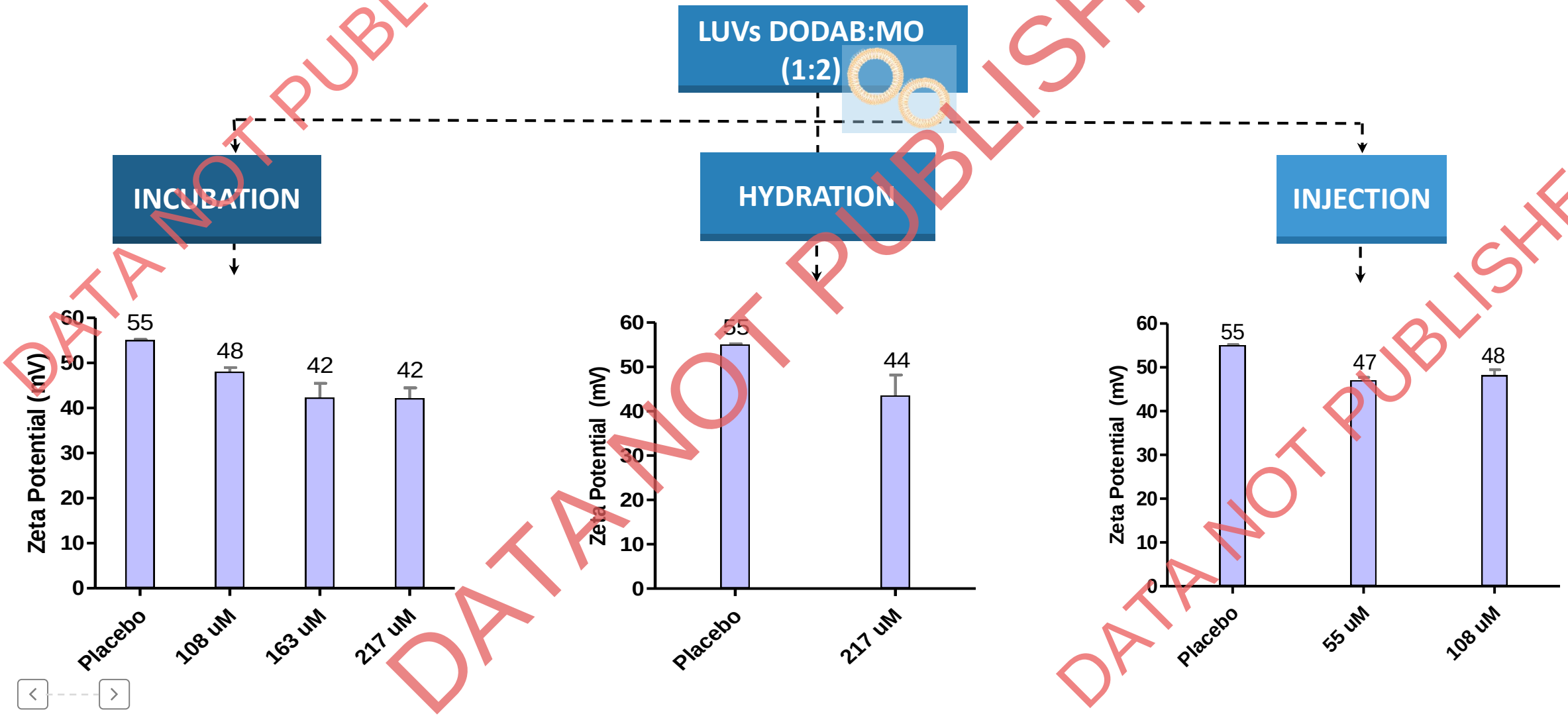
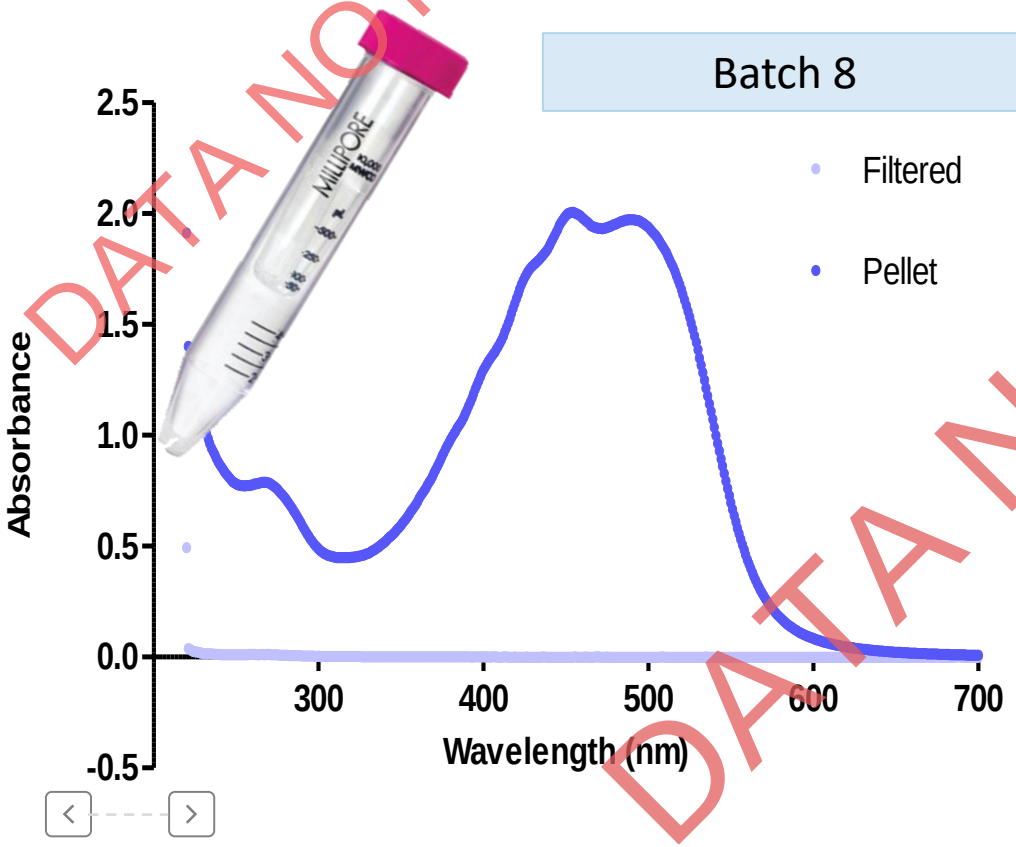


Formulation characterization

Encapsulation Efficiency (EE%) and Drug Loading (DL%)

$$EE\ (\%) = \frac{[Curcumin]\ loaded}{[Curcumin]\ initially\ added} \times 100$$

$$DL\ (\%) = \frac{[Curcumin]\ loaded}{[Nanocarrier]} \times 100$$



		INCUBATION		HYDRATION		INJECTION	
		EE%	DL %	EE%	DL%	EE%	DL%
55 µM	Batch 1	-	-	-	-	100	2.03
	Batch 2	-	-	-	-	100	2.03
	Batch 3	-	-	-	-	100	2.03
108 µM	Batch 4	88.19	0.73	-	-	97.48	3.9
	Batch 5	99.30	0.83	-		100	4.05
	Batch 6	96.15	0.80		-	100	4.05
163 µM	Batch 7	100	3.36	-	-	-	-
	Batch 8	100	3.37	-	-	-	-
	Batch9	99.61	3.34	-	-	-	-
217 µM	Batch 10	99.89	2.23	98.69	5.62	-	-
	Batch 11	100	2.15	88.10	1.49	-	-
	Batch 12	99.61	2.17	84.01	1.07	-	-

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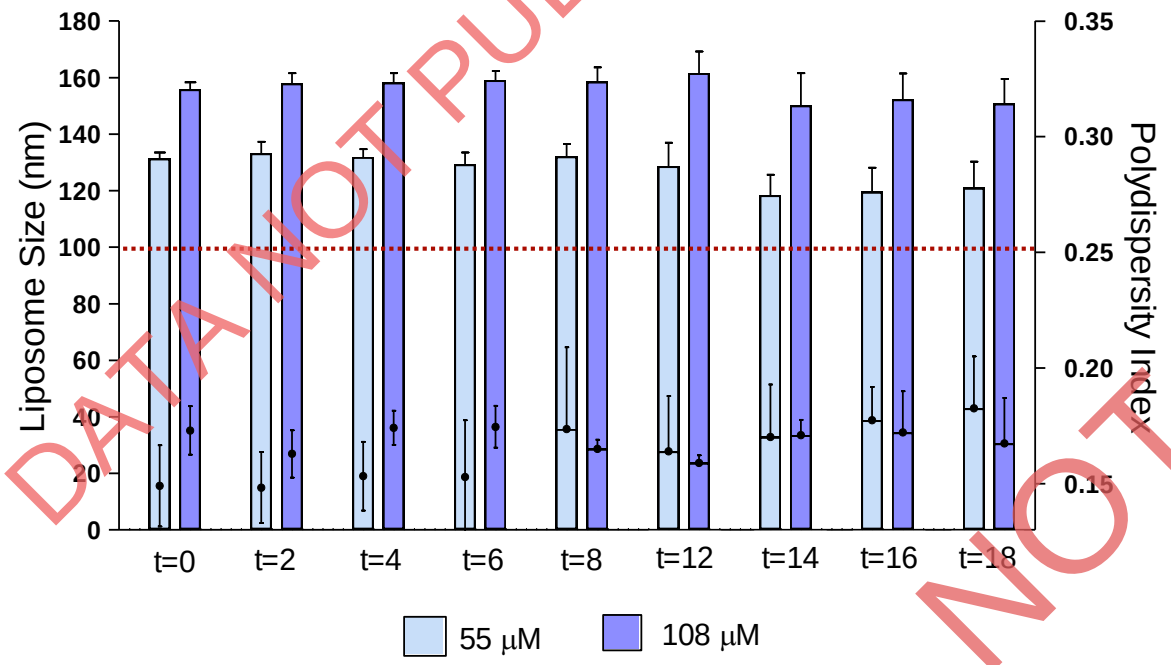
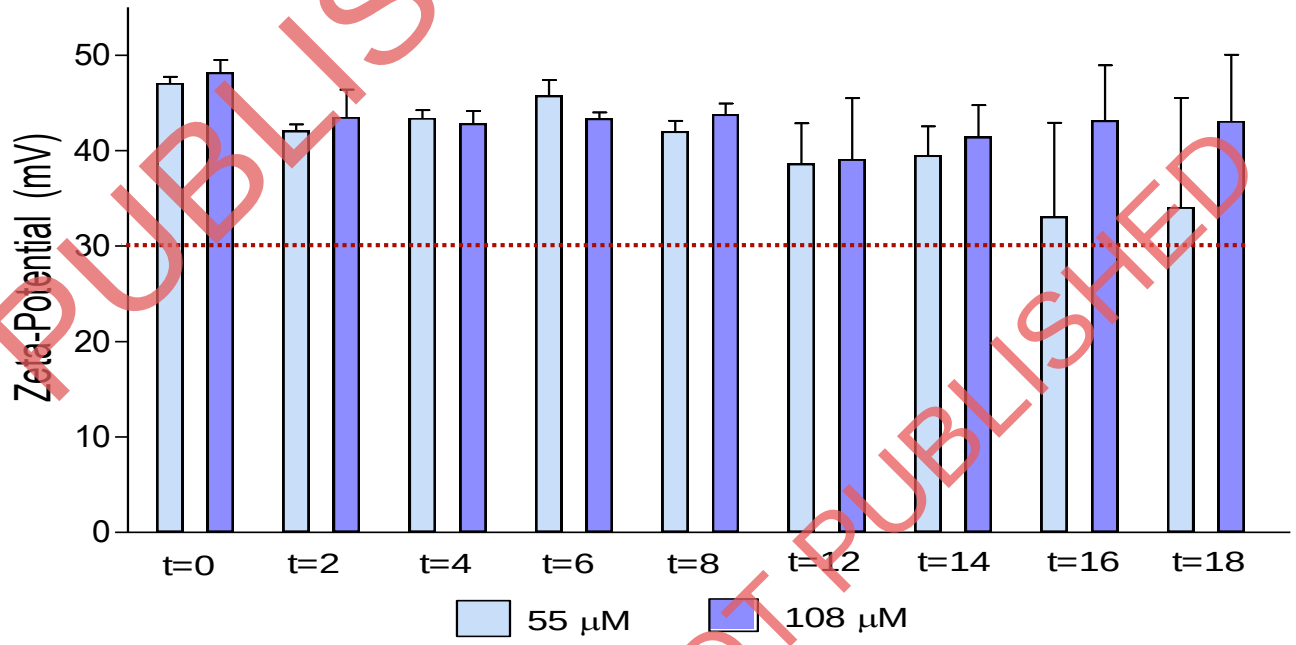
INJECTION

Is the developed formulation stable?





SIZE AND PDI

Stable for at least 4 months !

ZETA POTENTIAL

Stable for at least 4 months !

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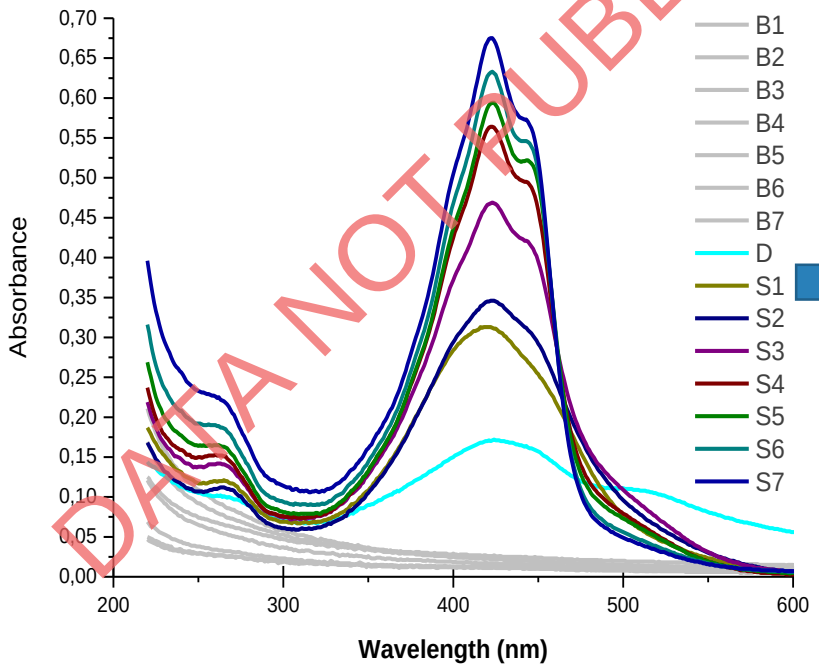
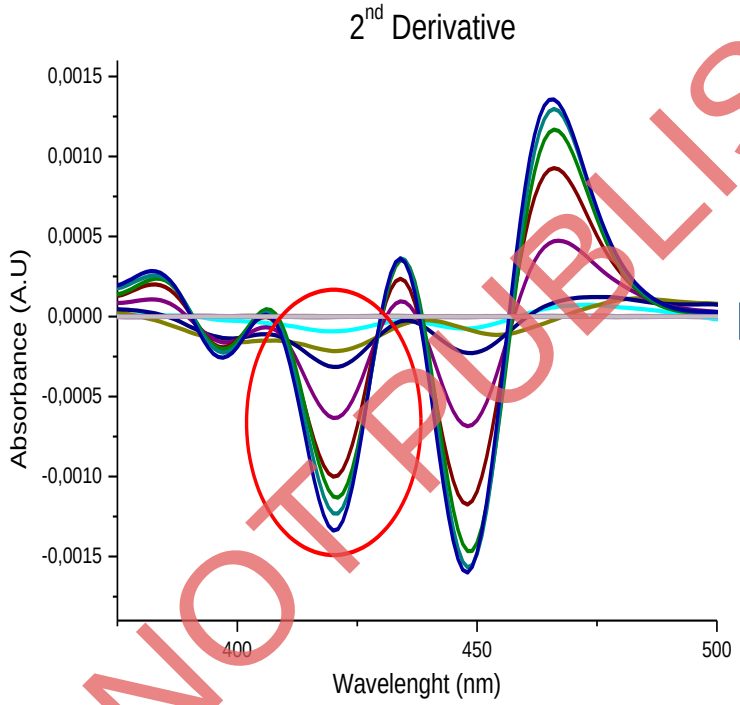
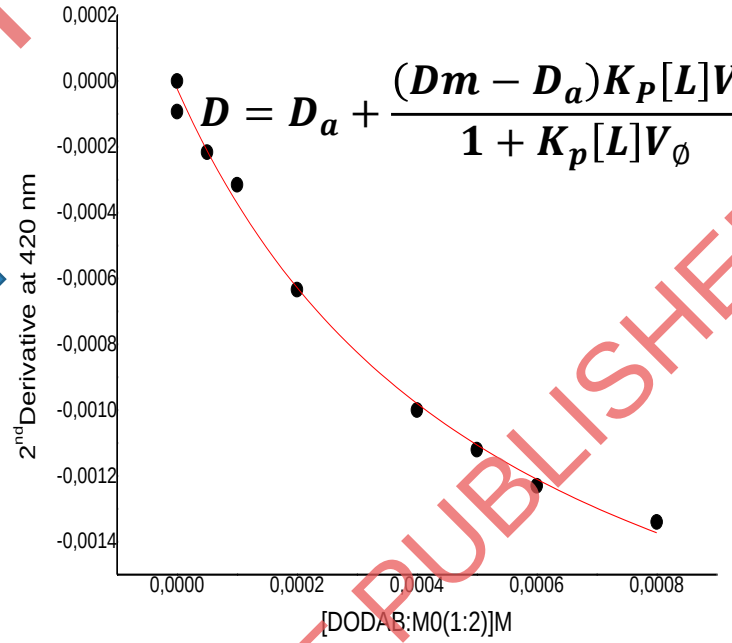
Curcumin partition coefficient in DODAB:MO(1:2)

Kp determination by Derivative spectroscopy







Log $P_{Liposome/water}$
2.74 ± 0.33

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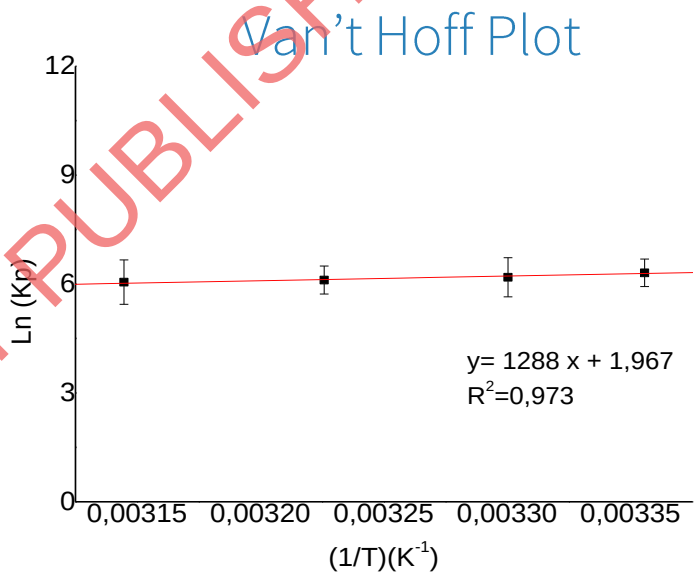
Determination of partition thermodynamics parameters



	KJmol^{-1}
$\Delta H_{AQ \rightarrow MEM}$	-10,71
$\Delta S_{AQ \rightarrow MEM}$	0,0164
$\Delta G_{AQ \rightarrow MEM}$	-10,7101

$$Ln\,(Kp) = -\frac{\Delta H}{RT} + \frac{\Delta S}{R}$$

$$\Delta G = \Delta H - T\Delta S$$

$\Delta H_{AQ \rightarrow MEM}$

ΔH<0 → **Exothermic partition process** → **Hydrogen bonds formation**

$\Delta G_{AQ \rightarrow MEM}$

ΔG<0 → **Spontaneous partition process** → **Efficient encapsulation method**

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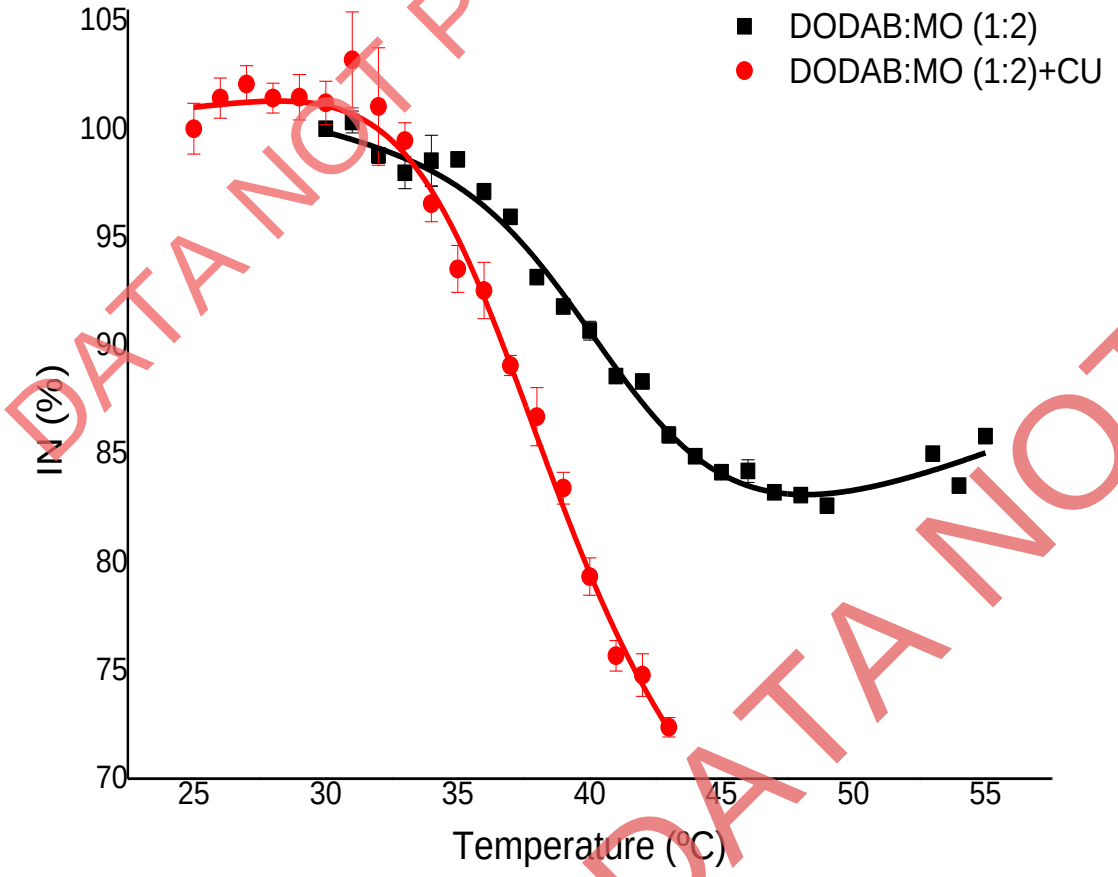
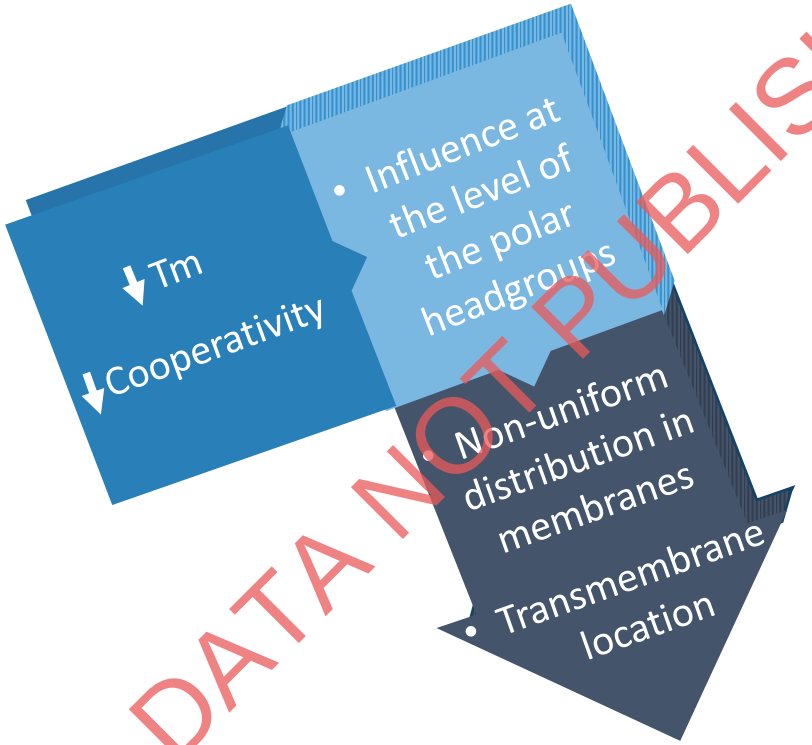
Curcumin effects on DODAB:MO(1:2) microviscosity

Results obtained by dynamic light scattering



System	B	T_m (°C)	R^2
DODAB:MO (1:2)	272.44	41.96 ± 0.070	0.987
DODAB:MO (1:2) + CU	227.34	38.00 ± 0.213	0.997



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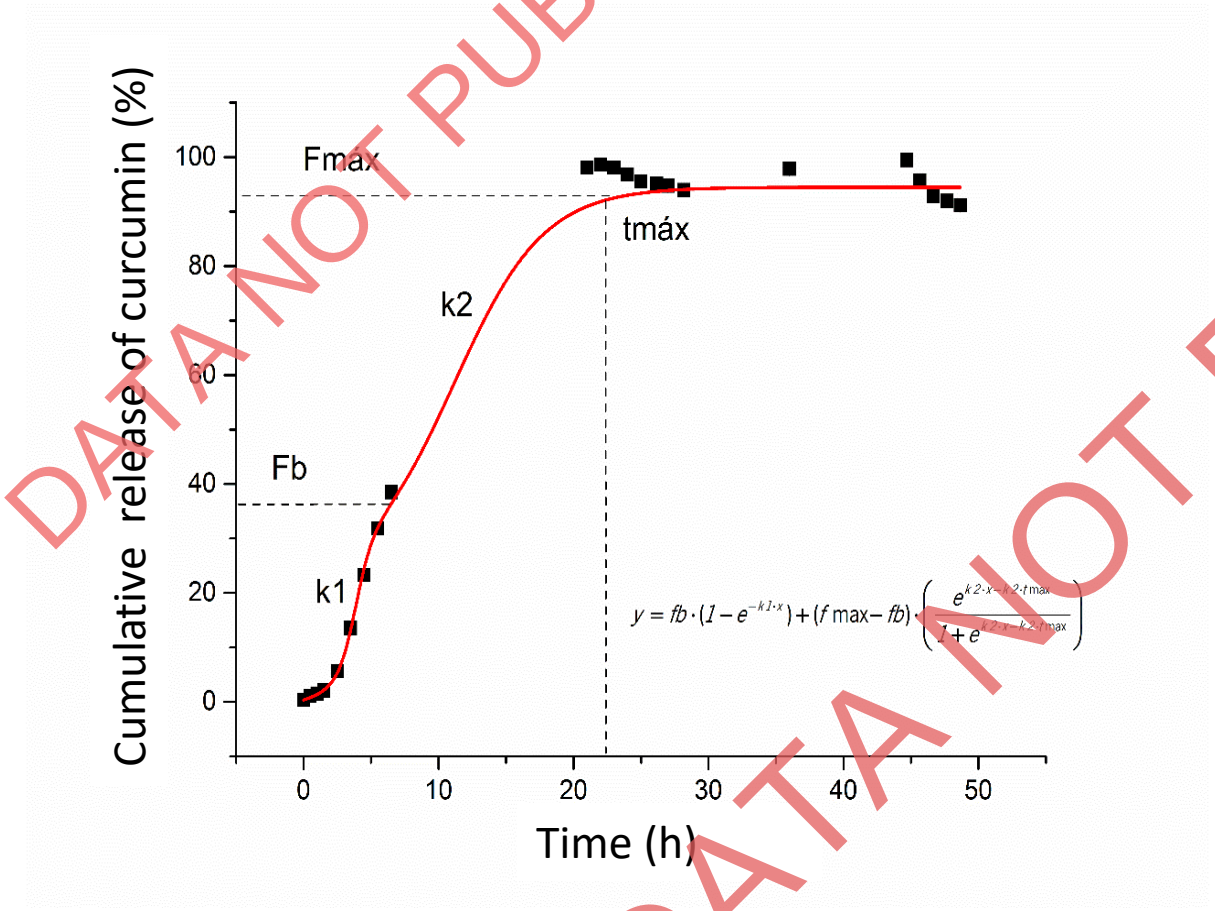
Controlled release assays

In vitro kinetics of curcumin release from DODAB:MO (1:2) liposomes



Release profile

'Burst' Phase – 33 %

Maximum fraction released (Fmax) – 94.47 %

Time required until Fmax released – 22.25 h

DEVELOPED FORMULATION PROMOTES CURCUMIN CONTROLLED RELEASE!

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PEGylation and HSA binding

Liposomal characterization after PEGylation and HSA binding assays using fluorescence quenching and DLS techniques

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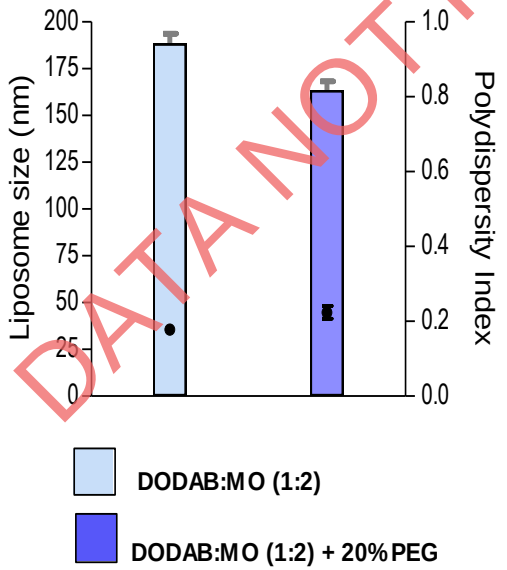
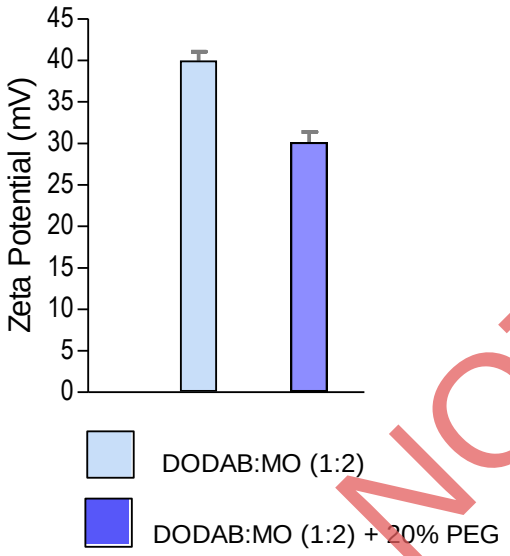
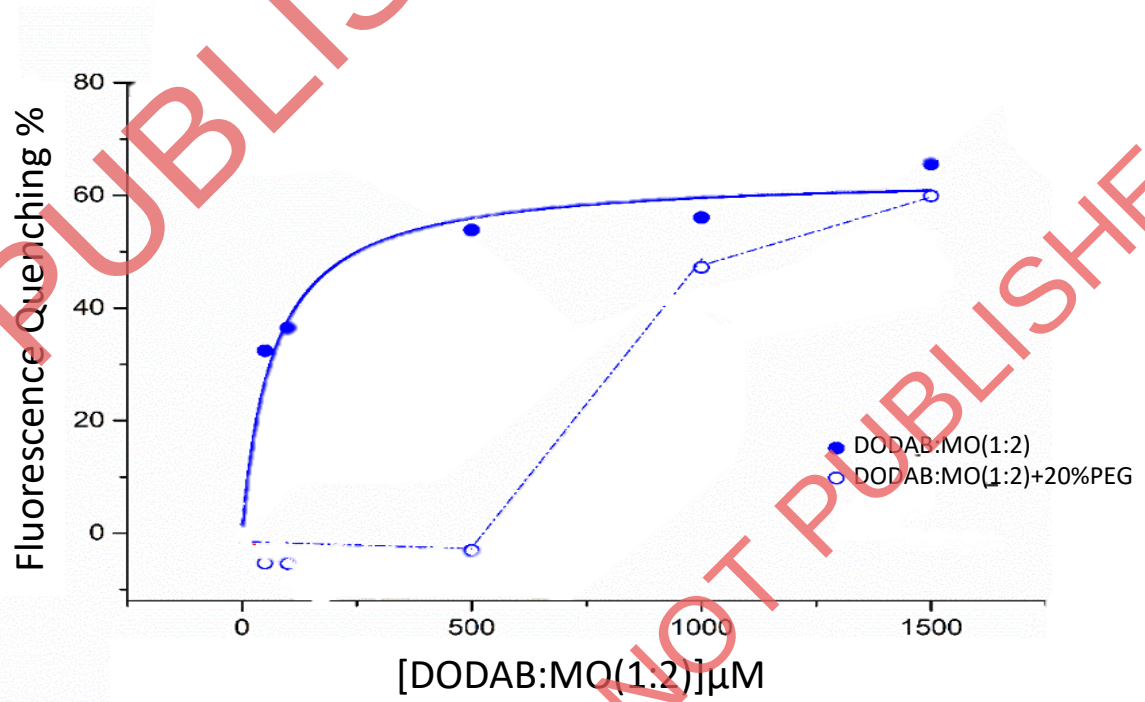
Characterization





Size and Zeta potential decrease

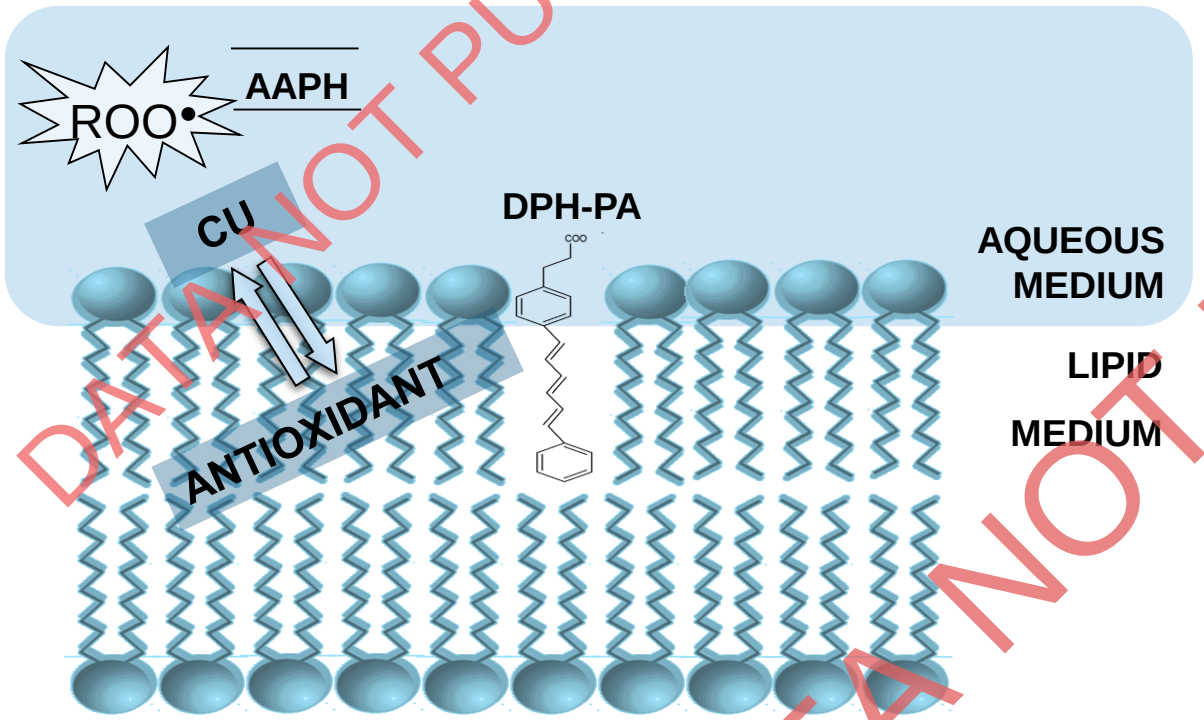
HSA binding



HSA binding decrease

Antioxidant activity

Antioxidant activity analysis measured by peroxidative degradation of the DPH-PA probe



	CURCUMIN
AUC_{CU}	2109
AUC_{AAPH}	1380
$[(AUC_{CU} - AUC_{AAPH})/ AUC_{AAPH}$	0.53

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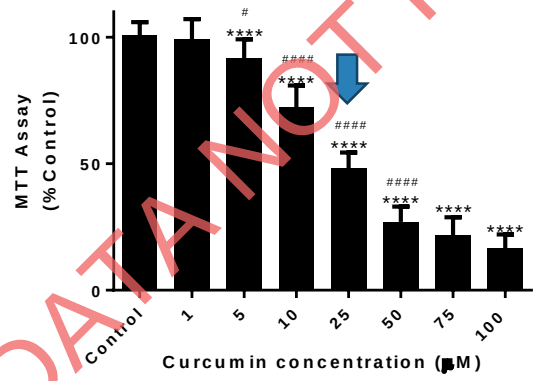
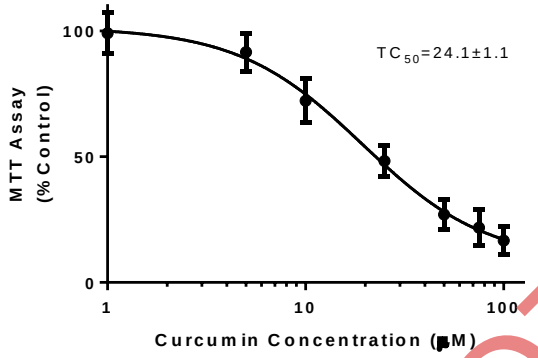
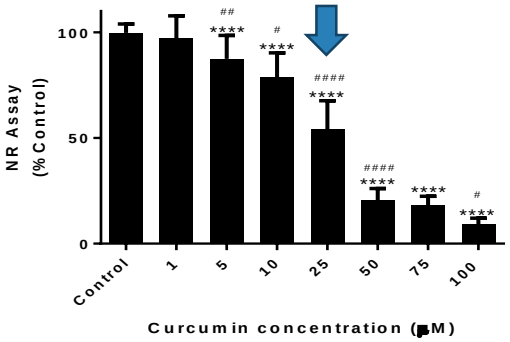
Concentration-toxicity curves in Human SH-SY5Y neurons

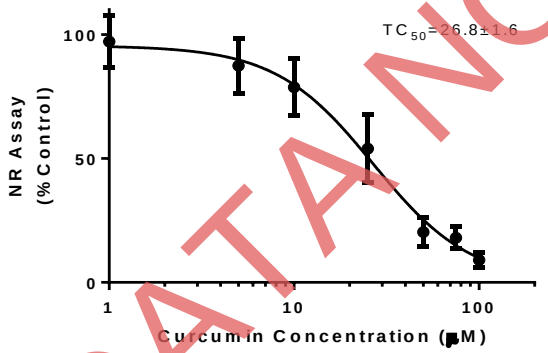
Using two cytotoxicity assays: dimethylthiazol diphenyltetrazolium (MTT) reduction and neutral red (NR) uptake.

Curcumin



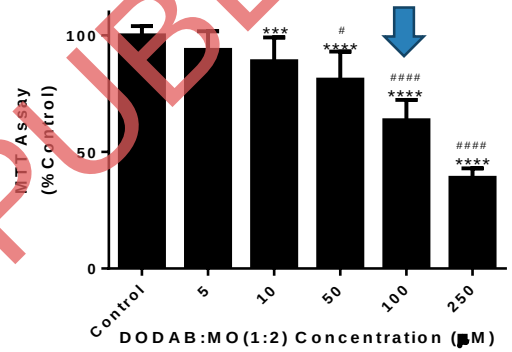


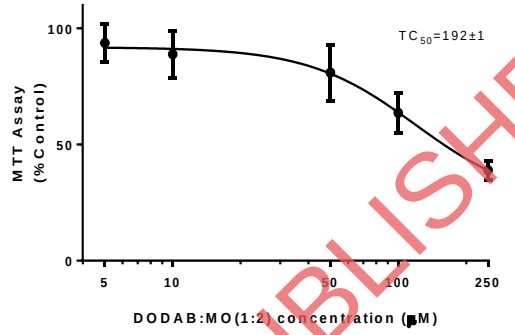


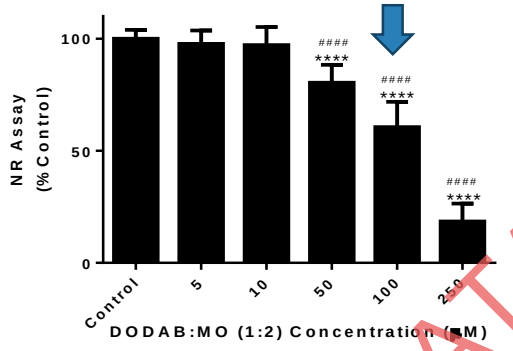


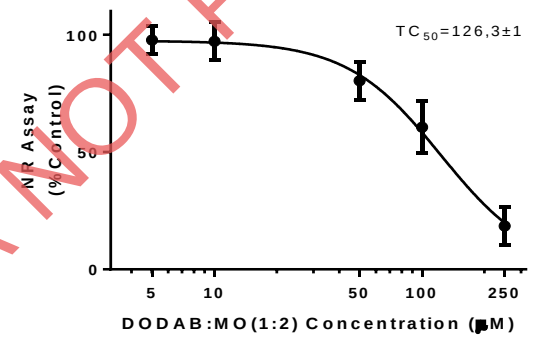
Vs

DODAB:MO (1:2) loaded with 22% Curcumin









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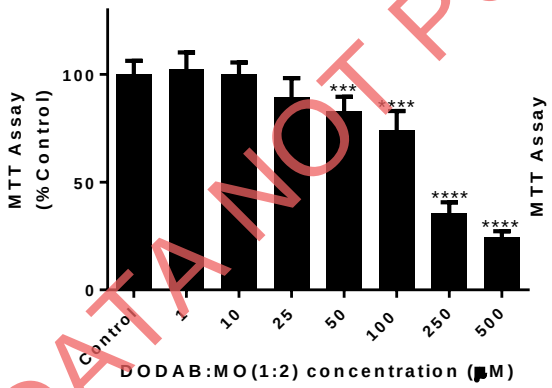
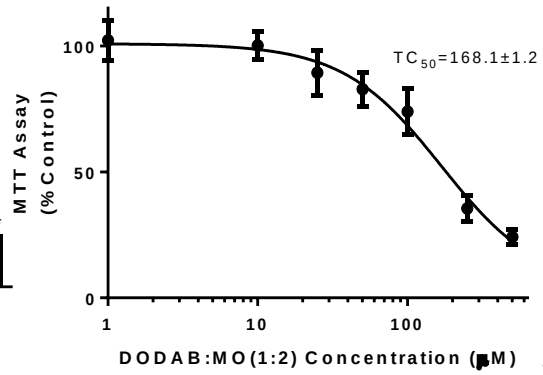
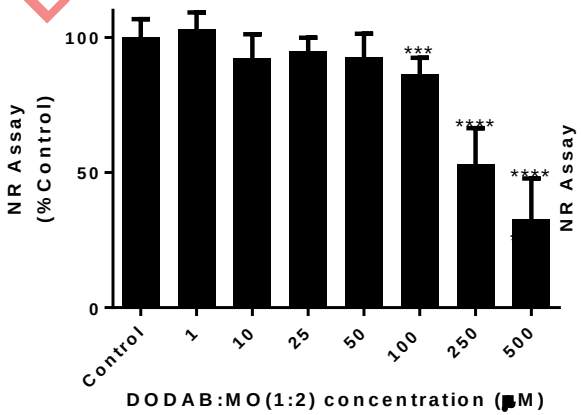
Concentration-toxicity in Human SH-SY5Y cells

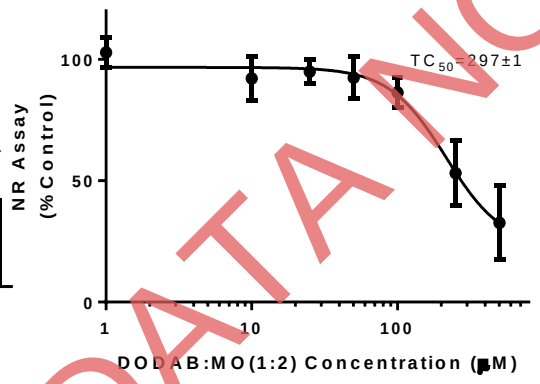
Using two cytotoxicity assays: dimethylthiazol diphenyltetrazolium (MTT) reduction and neutral red (NR) uptake.

DODAB:MO (1:2)



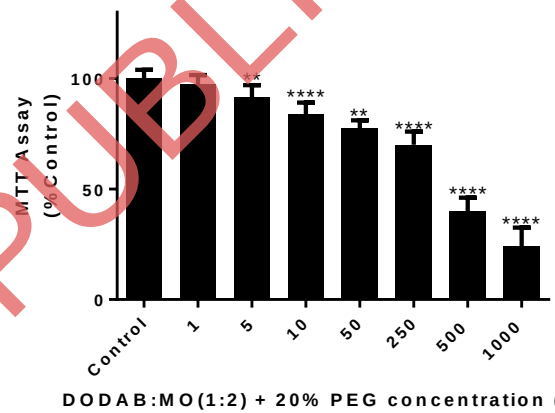


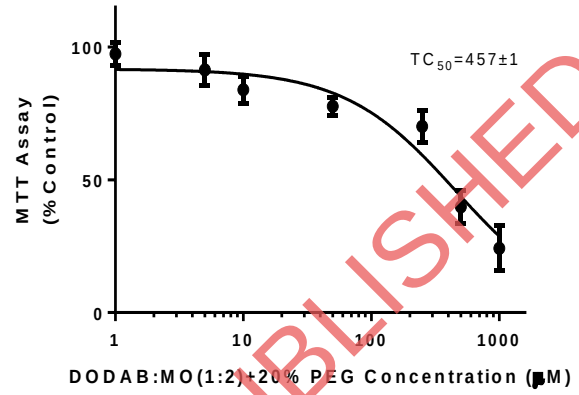


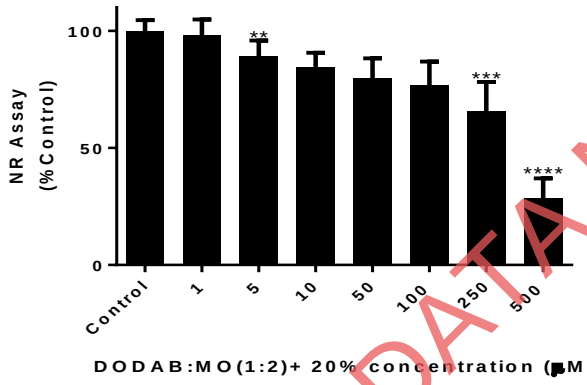


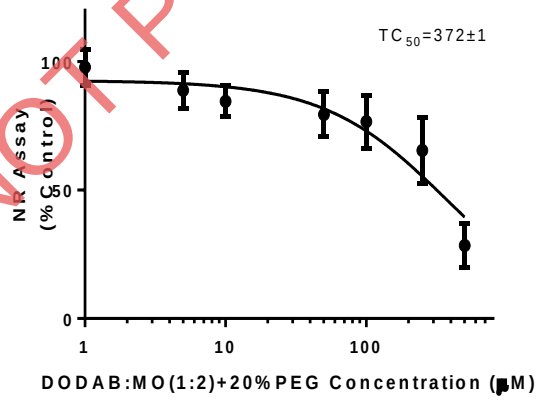
Vs

DODAB:MO(1:2) with 20% PEG









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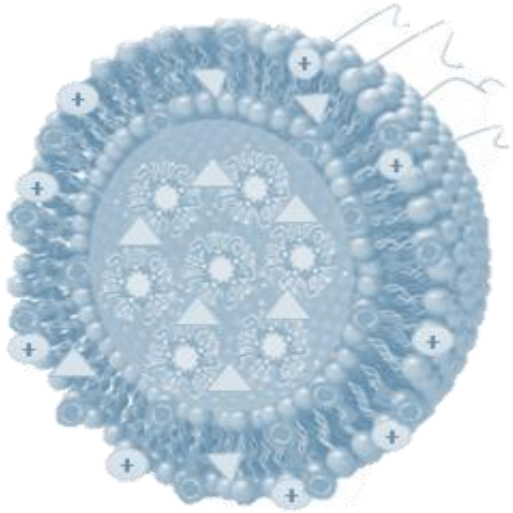
Conclusions and work in progress

Suitable characteristics for BBB penetration

Size, Charge and Stability

Efficient PEGylation

Adequate liposomal size and charge
Decreased HSA binding



Efficient Encapsulation

Curcumin was efficiently encapsulated in liposomes of DODAB:MO (1:2).

Neuroprotective Properties

Antioxidant activity

Controlled release

DODAB:MO (1:2) liposomes promote the curcumin controlled release

Encapsulation is advantageous

Lower curcumin toxic-concentration
PEGylation promotes a decrease in NC toxicity

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Thank you for your attention

Any question?