Nano Innovation 2020
Rome, 15-18 September
Conference & Exhibition

Curcumin Nanocrystals for Intranasal administration: promising strategy to achieve brain targeting

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Rome, 18 September 2020

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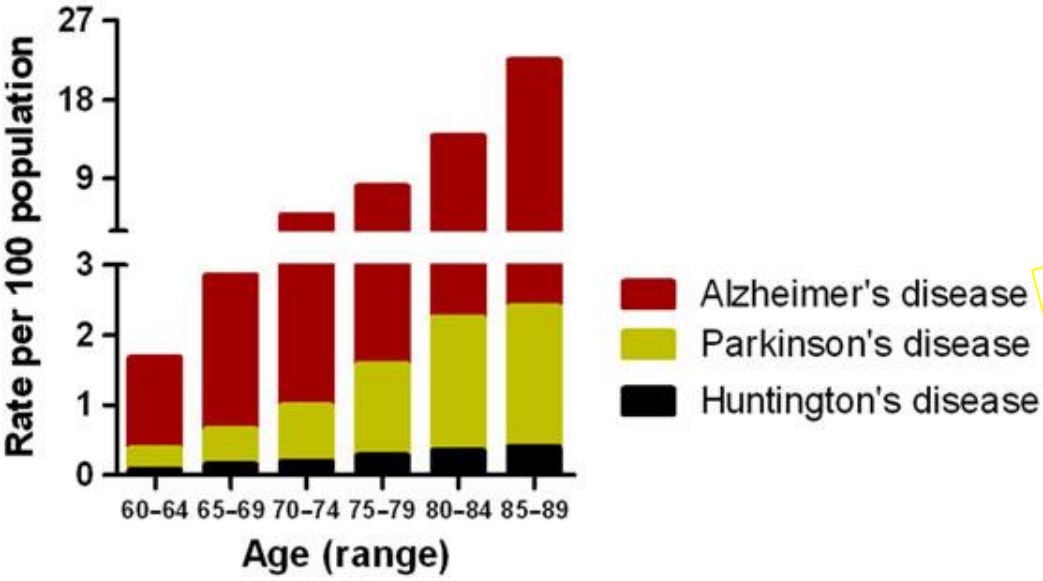
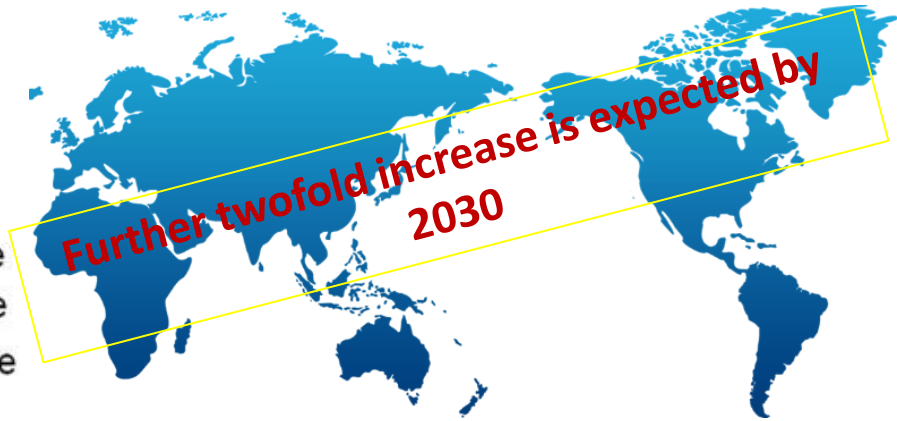
Neurodegenerative Disorders: Global Impact

The increase of individuals aged over 65 years has been paralleled with an exponential increase in the incidence of AD, PD and HD, as well as other neurodegenerative disorders.

Ageing-related increase in the incidence of specific neurodegenerative disorders





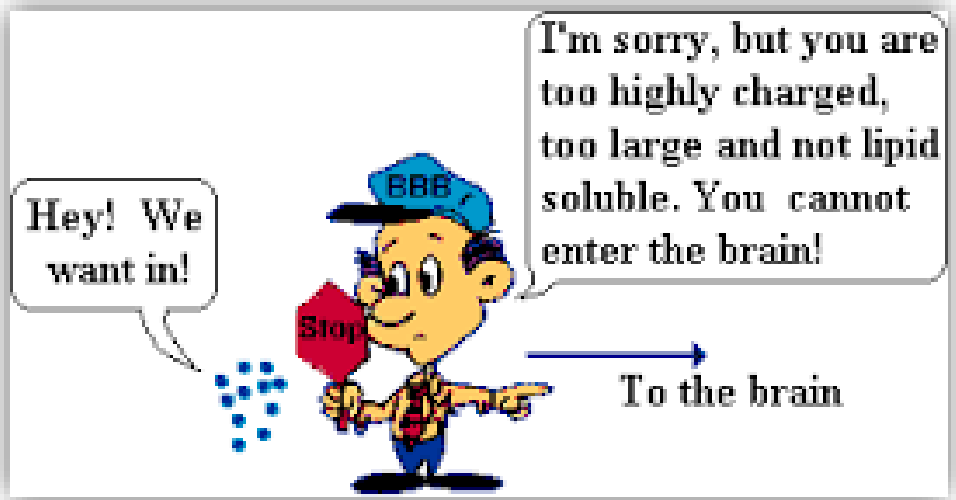
Fernández-Ruiz, J. (2019) *British Journal of Pharmacology*, 176: 1370– 1383. https://doi.org/10.1111/bph.14382

Brain Targeting

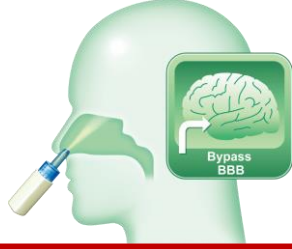
What are the causes for these high failure rates when attempting to develop efficacious neuroprotective therapies?

- ✓ Lack of understanding of the causes and mechanisms of neurodegenerative diseases;

- ✓ **Difficult to deliver therapeutic agent to the brain.**

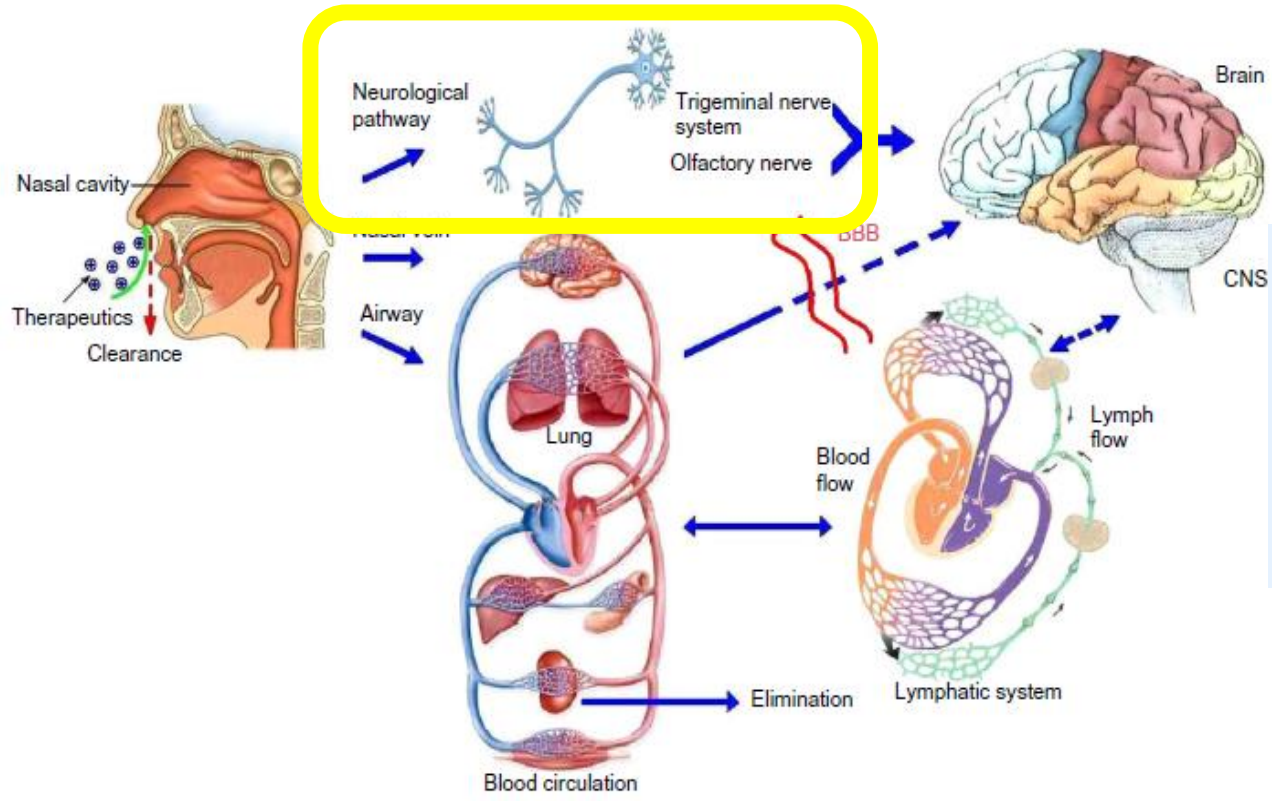


Nose-to-brain delivery



"The nose is the only natural corridor where the brain meets the outside world"

(by Dr. G. Williams)



For nose-to-brain delivery, the dose must be deposited in the olfactory region

The selection of drug transport pathway depends upon the nature of the drug, the formulation parameters, the physiological condition, the type of delivery devices, etc.

Colloids and Surfaces B: Biointerfaces 154 (2017) 297–306

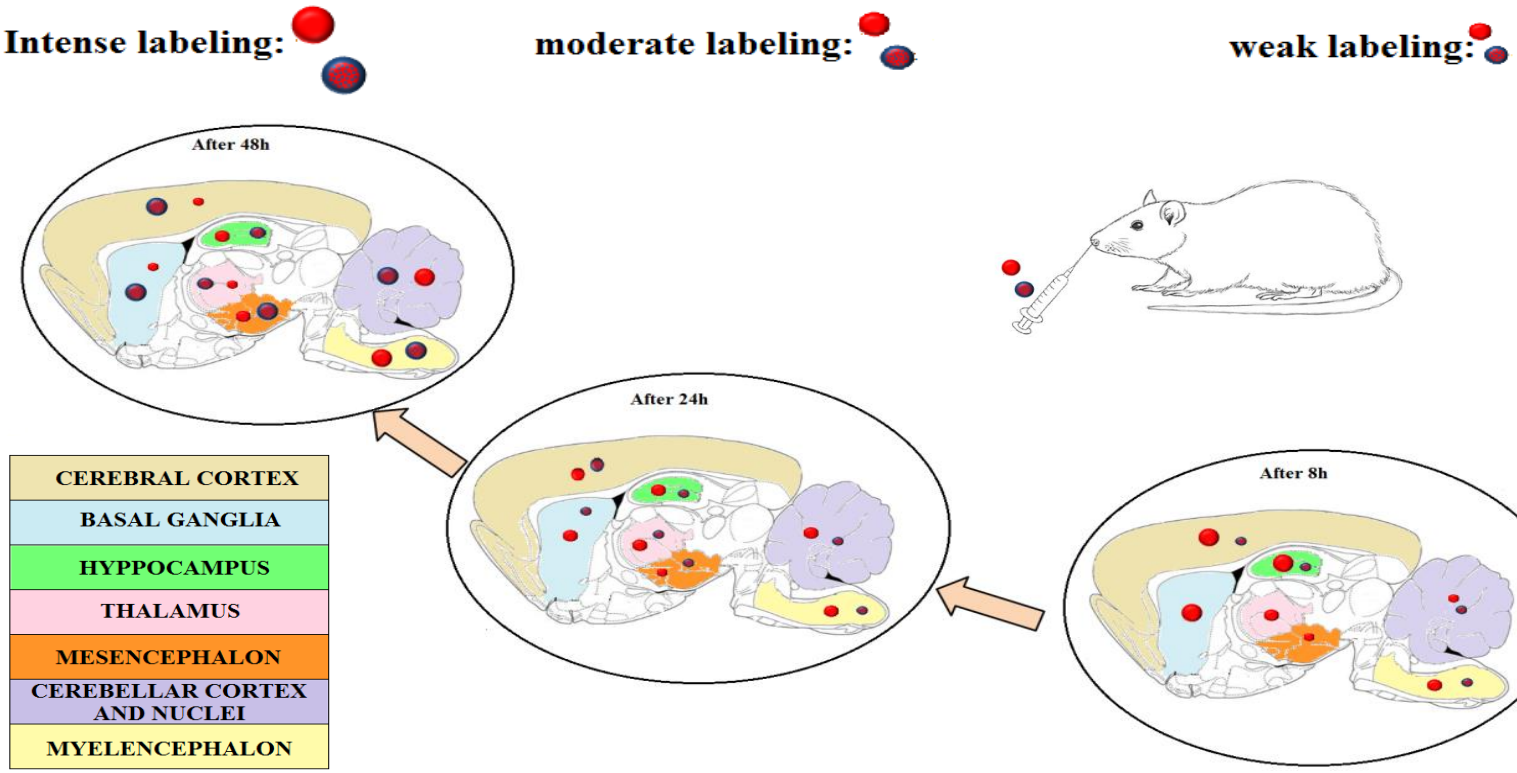
Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Nose to brain delivery in rats: Effect of surface charge of rhodamine B labeled nanocarriers on brain subregion localization

A. Bonaccorso[a], T. Musumeci[a,*], M.F. Serapide[b], R. Pellitteri[c], I.F. Uchegbu[d], G. Puglisi[a]



The rapid appearance of the fluorescent signal in rostral brain regions at early time points for **PLGA NPs** suggested the **olfactory transport.**

The uptake of **CS- PLGA NPs** was different, since we found a weak fluorescent signal in all areas at early time points and a strong fluorescent intensity in caudal brain after 48h suggesting the involvement of the **trigeminal nerve transport.**

Bonaccorso et al., Colloids and Surfaces B: Biointerfaces 154 (2017) 297–306; doi: 10.1016/j.colsurfb.2017.03.035.

European Journal of Pharmaceutics and Biopharmaceutics 133 (2018) 309–320

Contents lists available at ScienceDirect

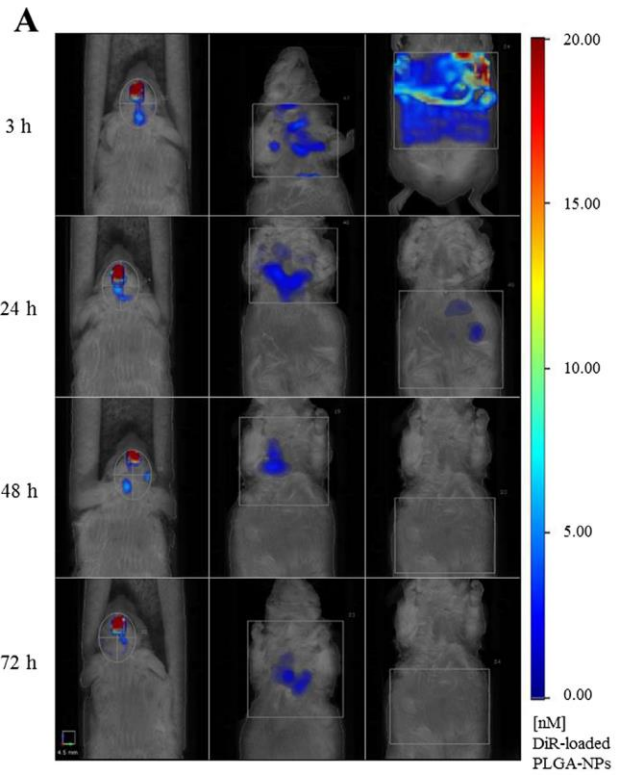
European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Oxcarbazepine free or loaded PLGA nanoparticles as effective intranasal approach to control epileptic seizures in rodents

Teresa Musumeci[a,*], Maria Francesca Serapide[b], Rosalia Pellitteri[c], Alessandro Dalpiaz[d], Luca Ferraro[e], Roberta Dal Magro[f], Angela Bonaccorso[a], Claudia Carbone[a,g], Francisco Veiga[g], Giulio Sancini[f], Giovanni Puglisi[a]



- ✓ 3 h after a single IN administration, more than 5% of the instilled dose of the NPs was detectable in the brain (Fig. A and B).
- ✓ Repeated IN administrations provided a significant increase of NP-associated fluorescence in the brain (>8%).
- ✓ Repeated IN administrations did not affect NP accumulation in other organs and tissues (Fig. C).
- ✓ Less than 10% of the instilled dose was found in extracerebral organs 24 h after a single and repeated instillations .

DiR-loaded PLGA NPs biodistribution and biovailability in the brain. Representative image of DiR-loaded PLGA NP biodistribution in mice at different times after the second IN administration. Fluorescence was detected by a FMT1500 and the amount of fluorophore (DiR) in the regions of interest (ROI) was quantified using TrueQuant Software (A). Quantification of fluorescence associated with DiR loaded PLGA NPs in the brain (B) and body (C) of CD-1 mice after single and repeated IN administrations. Values of repeated IN administrations are related to the % of fluorescence measured in the brain 3, 24, 48 and 72 h after the second instillation. Data are expressed as % of the instilled NPs. ***$p < 0.001$ by Student's t test (B).

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Review

Epilepsy Disease and Nose-to-Brain Delivery of Polymeric Nanoparticles: An Overview

Teresa Musumeci *, Angela Bonaccorso and Giovanni Puglisi

Table 1. Advantages and disadvantages of Intranasal (IN) drug delivery.

Advantages	Disadvantages
Rapid, safe, non-invasive and convenient method	Rapid elimination of drug substances from nasal cavity due to mucocilliary clearance
Avoids drug degradation in the gastrointestinal (GI) tract, first-pass metabolism allowing enhanced bioavailability	Nasal congestion due to cold or allergic condition may interfere with this technique of drug delivery
Reduction of systemic side effects	Suitable for potent drugs since only a limited volume can be sprayed into the nasal cavity
Bioavailability for low molecular weight drugs	Frequent use of this route may lead to mucosal damage and/or irritation of nasal mucosa
Rapid drug absorption via highly vascularized mucosa	Mechanical loss of the dosage form could occur due to improper administration technique
Easy administration	Mechanisms of drug transport are still unclear

The drug dose should fit into a volume of roughly 100–200 µL when both nostrils are sprayed.

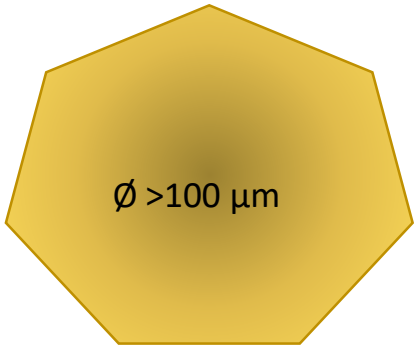
High dose in low volume (expecially for hydrophobic drug)

Direct delivery via the olfactory and trigeminal neural pathways

Musumeci T, Bonaccorso A, Puglisi G. Pharmaceutics. 2019;11(3):118. doi: 10.3390/pharmaceutics11030118.

Nanocrystals

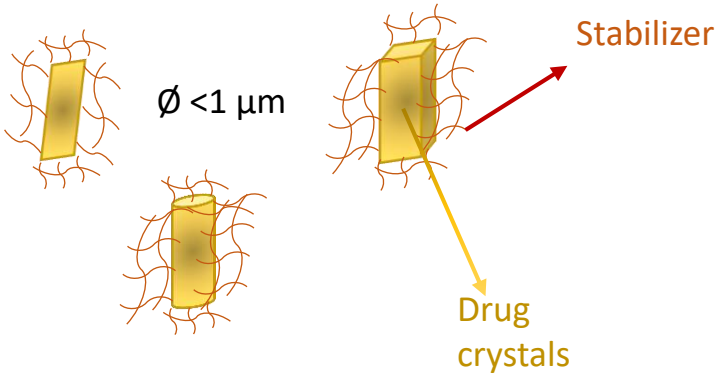
Carrier-free colloidal system in the nanometer range, with a theoretical drug loading of 100%, with surfactants or polymeric steric stabilizers.



Poorly water soluble drug

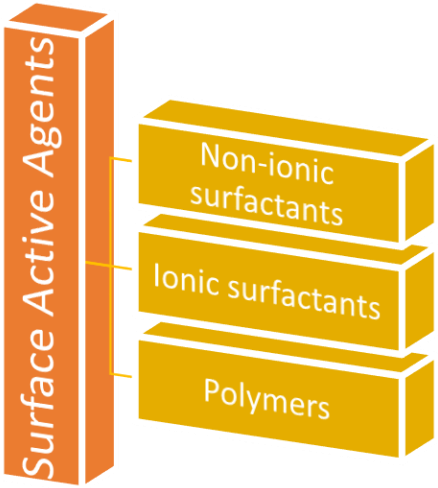
Nanonization

Nanotechnology



Nanocrystals

- Improving drug bioavailability
- Improving drug physico-chemical characteristic and stability



The main role of the stabilizers is to prevent the unstable particles from aggregation and/or Ostwald ripening during storage of the drug nanocrystal suspensions

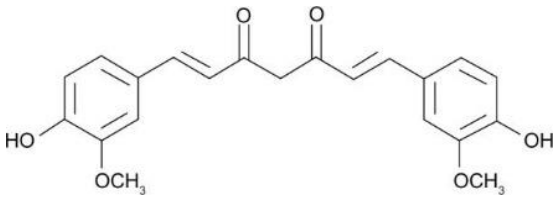
<u>General features of drug nanocrystals:</u>

(1) **increased saturation solubility** due to increased dissolution pressure of strongly curved small nanocrystals;

(2) **increased dissolution rate** due to increased surface area;

(3) **increased adhesiveness** of nanomaterial due to increased contact area of small versus large particles.

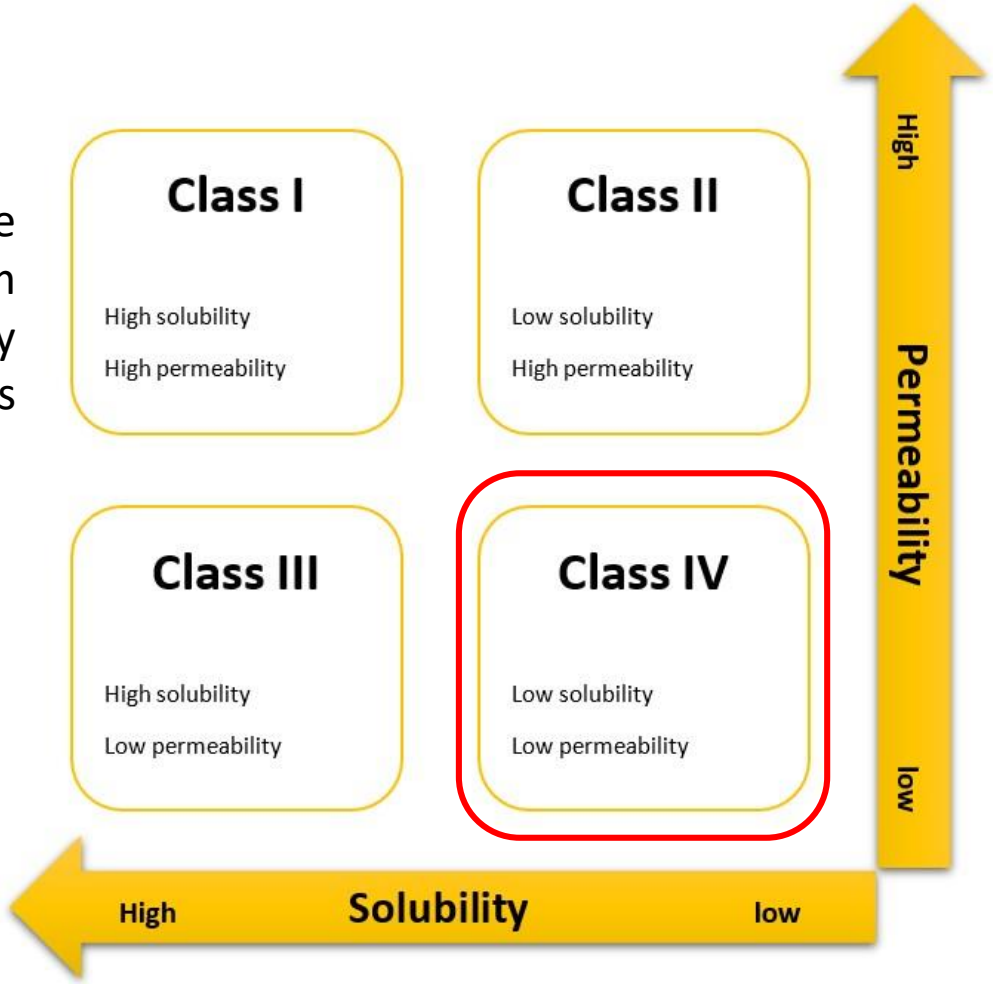
Modified from: Gigliobianco, M.R. et al.,. Pharmaceutics. 2018 Sep; 10(3): 134. doi: 10.3390/pharmaceutics10030134.



Curcumin

- for its nature, belonging to class IV of the biopharmaceutics classification system (BCS) (poorly soluble and poorly permeable) which is a prerogative for NCs preparation

- Multiple pharmacological effects (i.e., antioxidant, anti-inflammatory, antimicrobial, anticancer);



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Article

Optimization of Curcumin Nanocrystals as Promising Strategy for Nose-to-Brain Delivery Application

Angela Bonaccorso [1,*], Maria Rosa Gigliobianco [2], Rosalia Pellitteri [3], Debora Santonocito [1], Claudia Carbone [1], Piera Di Martino [2], Giovanni Puglisi [1] and Teresa Musumeci [1]

Design and optimization of Curcumin NCs

- Box Behnken design

Preparation and technological characterization

- Sonoprecipitation method
- Mean size, ZP, PDI, pH, osmolarity, thermotropic behavior (DSC), the degree of crystallinity (XRPD, FT-IR), morphology.

Biological study

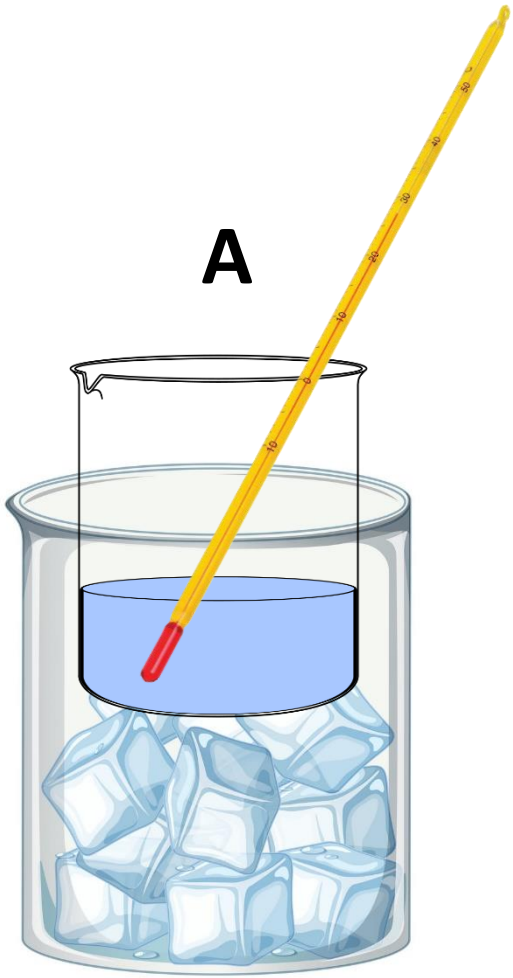
- Cellular uptake on Olfactory Ensheathing Cells (OECs)

Bonaccorso et al., Pharmaceutics 2020, 12(5), 476; https://doi.org/10.3390/pharmaceutics12050476

Sonoprecipitation: Solvent-Antisolvent

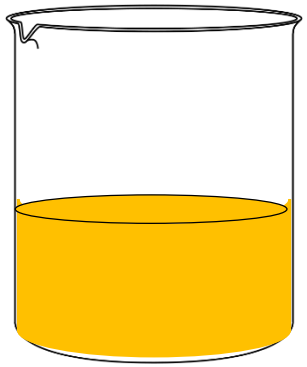
Process parameters such as solvent/antisolvent ratio, mixing type and rate, solvent as well as stabilizer selection are critical to achieve a homogenous nanosuspension.

A



The antisolvent phase (A) was prepared by dissolving different conc. and types of stabilizer in water.
The antisolvent was cooled in an ice water bath (~1°C).

S

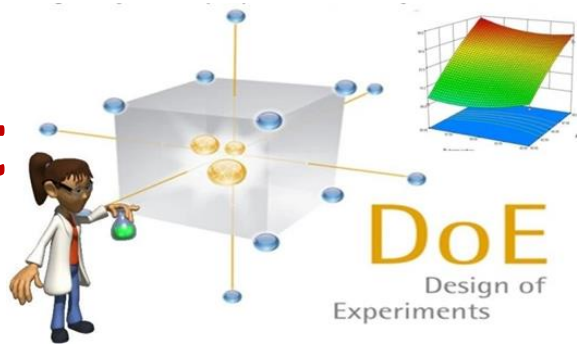


Curcumin was dissolved in a 60% ethanol/water solution of different conc. (Solvent (S) phase) according to the above-mentioned experimental design.

S/A

The S phase was added into the precooled (~1 °C) A phase containing a stabilizer (poloxamer 188, PVP or Tween 80 at a specific conc.) under intense sonication using an ultrasonic probe.

Design of Experiment



The relevant factors are changed simultaneously and systematically, reducing the number of tests needed to establish the multifactorial influences of the variables among them obtaining the **maximum and the best quality of information with minimum effort.**

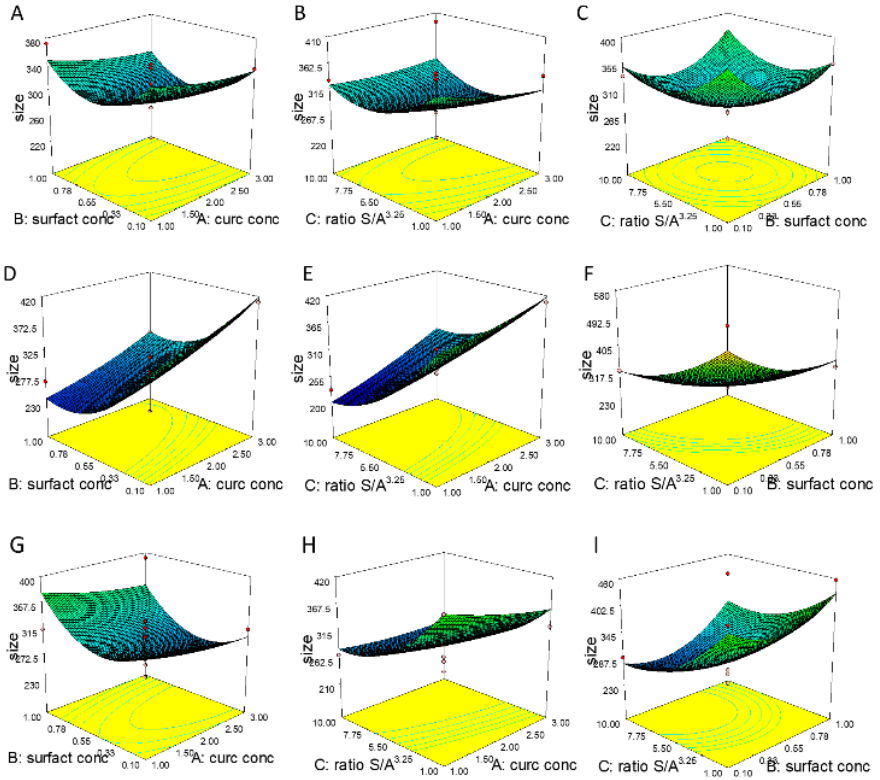
The three-level Box–Behnken experimental design with categorical and numeric factors was employed to optimize the size of curcumin NCs (response)

Factors and the corresponding levels investigated by the Box Behnken design.

Independent Variables	Type	Coded factors	Levels	
			low	high
Curc conc. (mg/ml)	Numeric	X_1	1	3
Surfactant conc (% w/v)	Numeric	X_2	0.1	1
S/A ratio (v/v)	Numeric	X_3	1:1	1:10
Surfactant type	Categoric	X_4	PVP	
			TWEEN 80	
			POLOXAMER 188	

Bonaccorso et al., Pharmaceutics 2020, 12(5), 476; https://doi.org/10.3390/pharmaceutics12050476

Effect of Independent Variables on NC Size and NC Optimization



NC size was found to be in the range of 216.1 ± 13.63 to 572.8 ± 39.89 nm.

The model's F-value of 4.76 and p-value less than 0.0001 indicate that the suggested model is significant.

-Surfactant conc.
-S/A ratio even in combination with the surfactant type.

Desirability: 0.872

Error %: 0.28%

NCs optimization

	GOAL	Lower limit	Upper limit
Curc conc (mg/ml)	maximize	1	3
Surfactant conc (% w/v)	is in range	0.1	1
S/A ratio (v/v)	minimize	1	10
Surfacant type	is in range	Poloxamer 188	Tween 80
Size (nm)	minimize	216.1	572.8

Final formulation with a high dose of the drug.

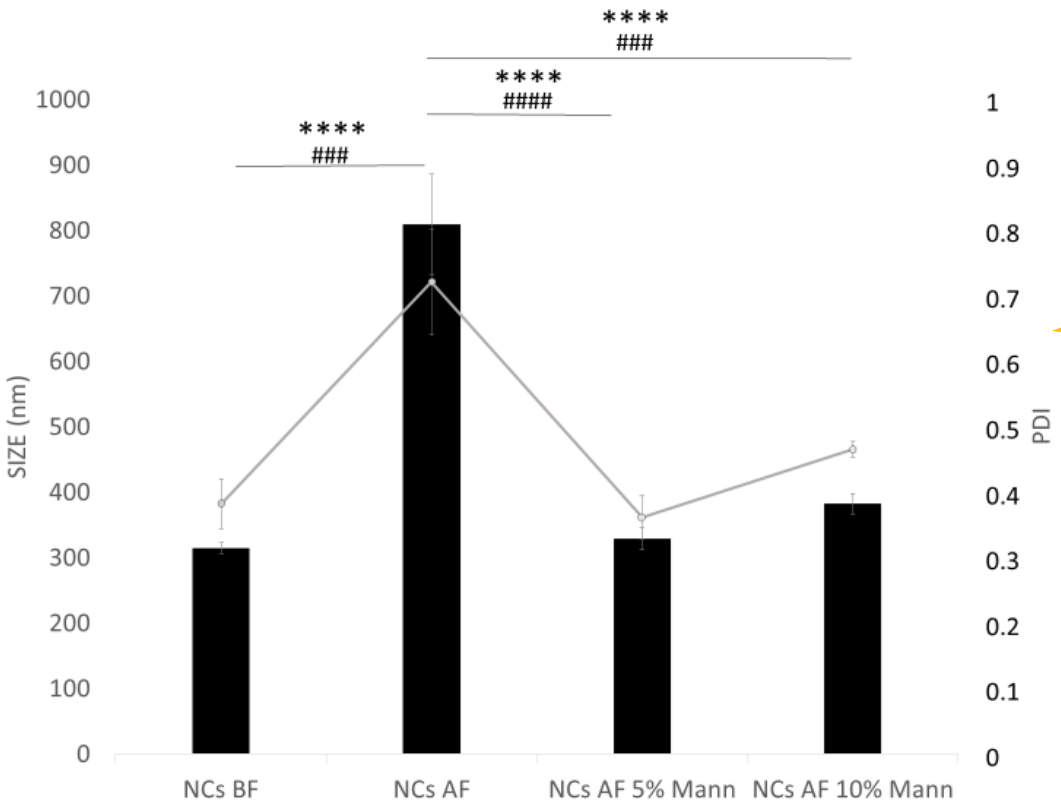
The interaction of particles with cells is known to be strongly influenced by their size.

Bonaccorso et al., Pharmaceutics 2020, 12(5), 476; https://doi.org/10.3390/pharmaceutics12050476

Freeze-Drying Process for NCs Long Term Storage

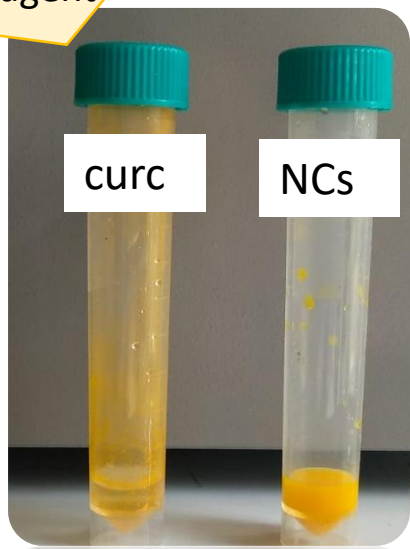
Improve drug stability

Avoid aggregation and sedimentation phenomenon



After lyophilization, NCs revealed an increase in particle size (Sf/Si = 2.57) and PDI values compared to the results before freeze–drying.

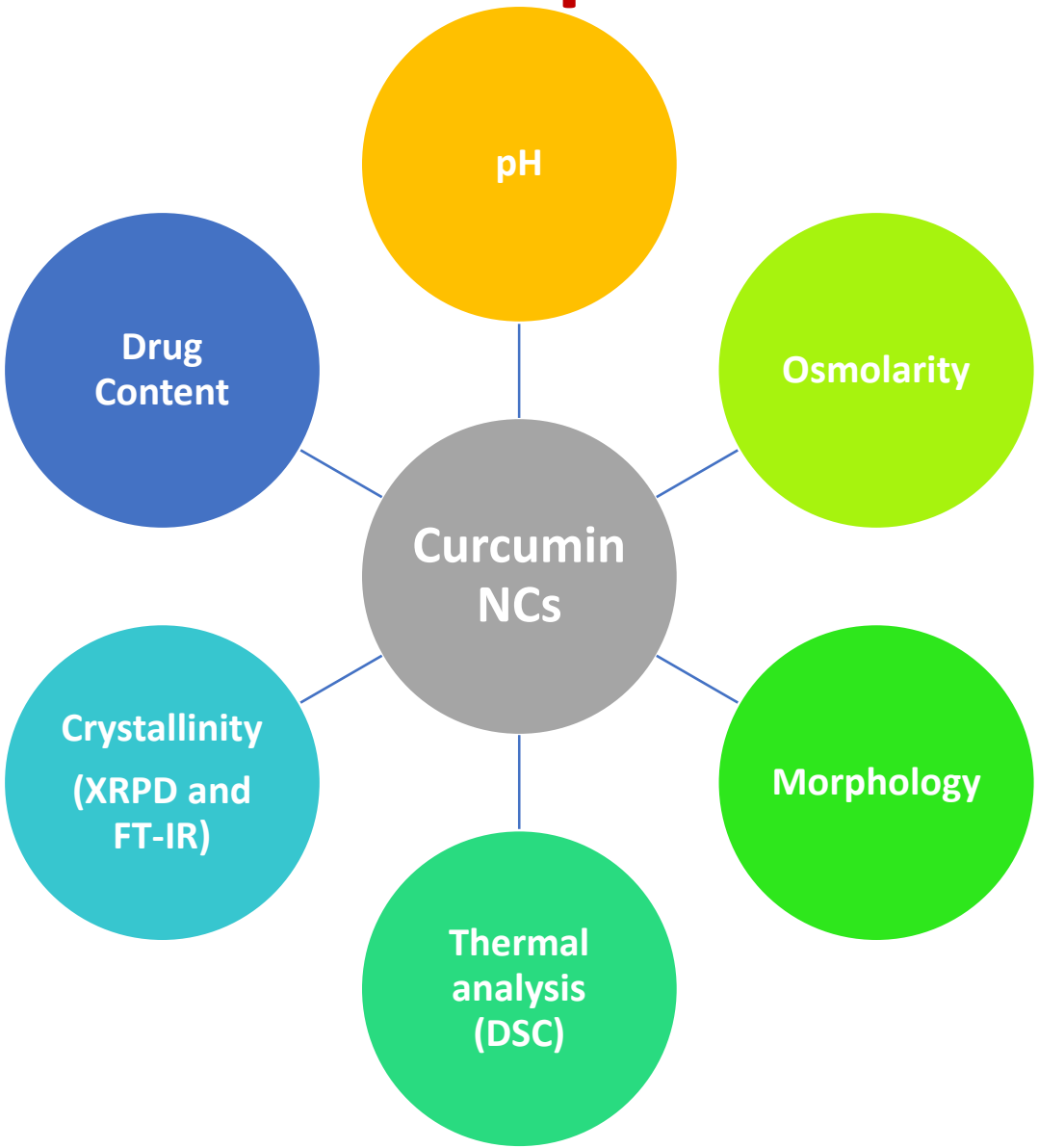
mannitol was selected as cryoprotectant agent



Mean size and PDI of NCs before and after freeze-drying without and with mannitol as cryoprotectant. BF = before freeze-drying; AF = after freeze-drying; 5% Mann = 5% (w/V) mannitol; 10% Mann = 10% (w/V) mannitol. Tukey's test for NC size and PDI. The * symbol denotes statistical significance dierence for size; The # symbol denotes statistical significance dierence for PDI. Significance was defined as **** p <0.0001

Bonaccorso et al., Pharmaceutics 2020, 12(5), 476; https://doi.org/10.3390/pharmaceutics12050476

Physico-chemical and technological characterization of optimized Curcumin



Characterization of optimized Curcumin NCs

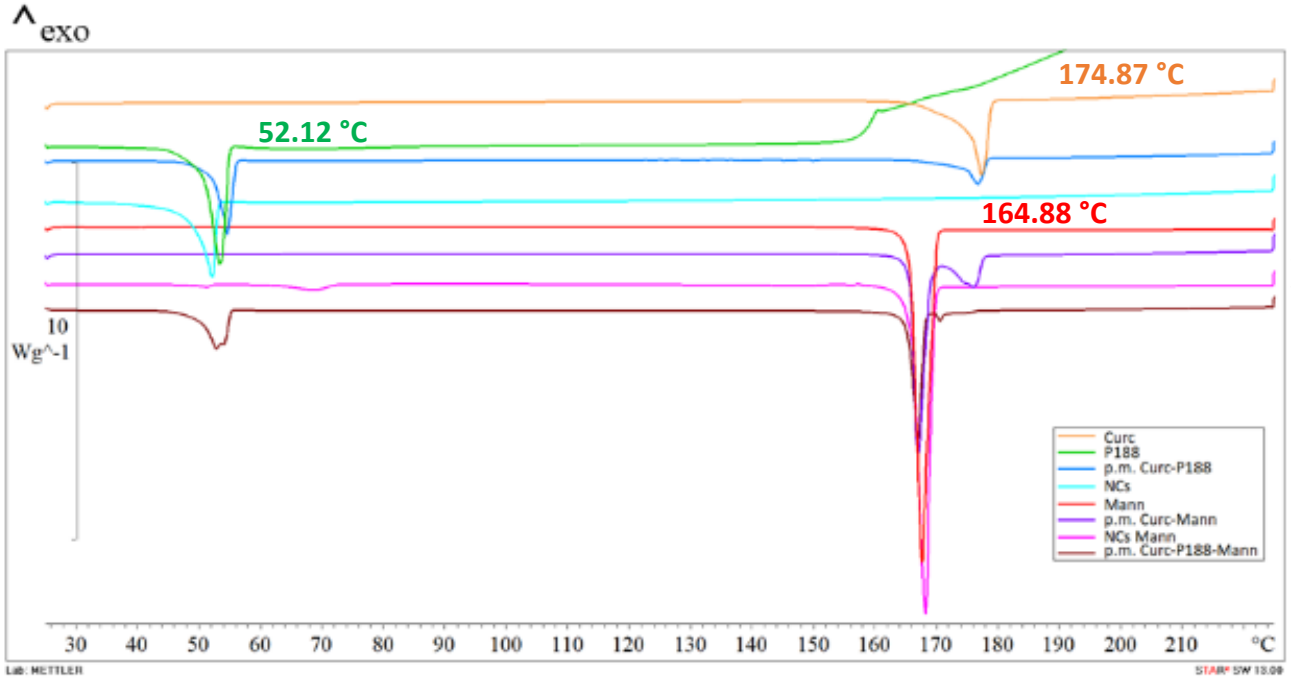
Table 4. Phisico-chemical properties of optimized NCs

Size (nm) ± SD	PDI ± SD	ZP (mV) ± SD	Drug content (%) ± SD	pH ± SD	Osmolarity (mOsm/L) ± SD
328.7 ± 16.87	0.361 ± 0.03	-36.7 ± 0.45	100 ± 0.03	6.1 ± 0.02	145.1 ± 0.51

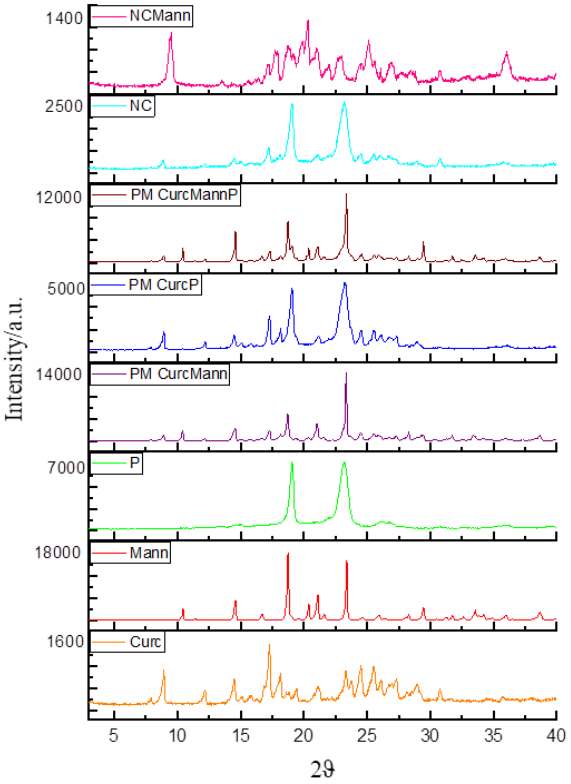
- ✓ Optimized NCs were homogeneous and had a suitable diameter for the IN route;
- ✓ The high and negative ZP value observed is probably due to the presence of P188, which forms a sterically stabilized polymer layer;
- ✓ No drug loss was found during the preparation method;
- ✓ The NC formulation pH was found to be within the ideal range; tissue damage, and irritation can occur outside the physiological range.
- ✓ Nasal formulations with osmolarity values within the range of 85.47–341.88 mOsmol/L can be considered acceptable because they do not harm the nasal cilia.

Bonaccorso et al., Pharmaceutics 2020, 12(5), 476; https://doi.org/10.3390/pharmaceutics12050476

Technological characterization of Curcumin NC



DSC Thermograms



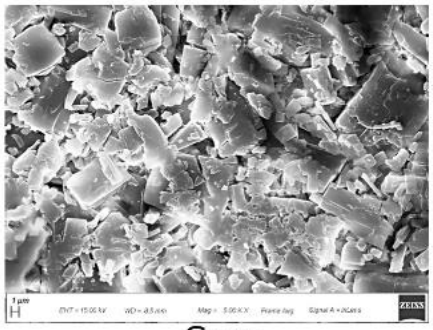
XRPD spectra of the samples.

The absence of the Curc melting endotherm in both NC formulations with or without mannitol revealed drug amorphization which make the drugs dissolving more rapidly.

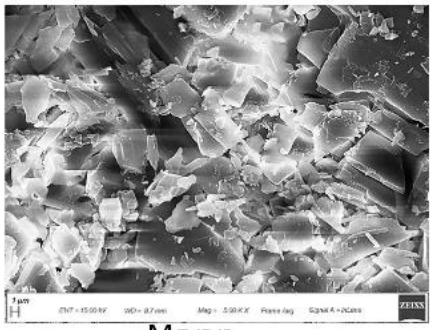
Even if a certain tendency to a crystallinity decrease can be assumed, the powder is still crystalline and no complete amorphization occurred during processing.

Bonaccorso et al., Pharmaceutics 2020, 12(5), 476; https://doi.org/10.3390/pharmaceutics12050476

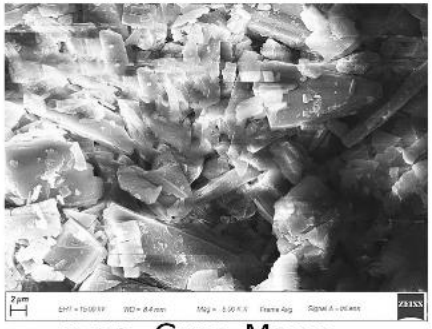
Curcumin NCs: Morphology


Curc

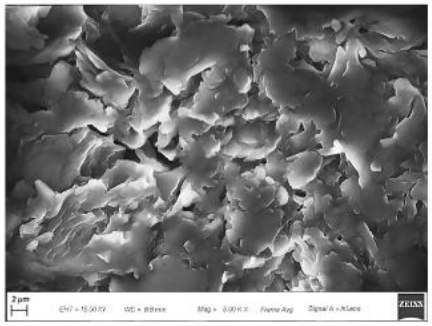

P188


Mann


p.m. Curc-P188


p.m. Curc-Mann


p.m. Curc-Mann-P188


NCs


NCs Mann

Scanning electron microscopy of different samples (X5000).

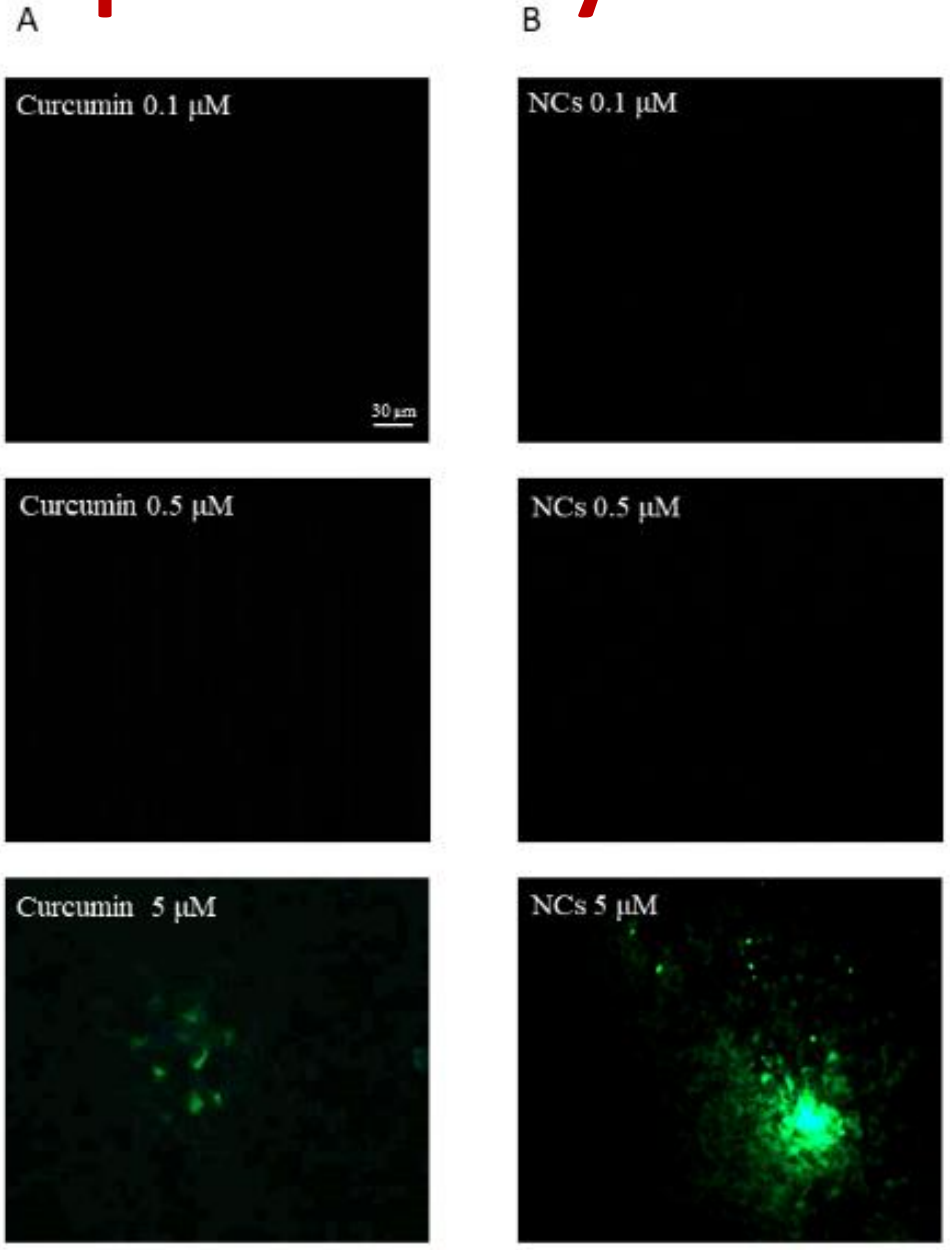
Crystallization by sonoprecipitation technique resulted orthorhombic forms.

curcumin NCs with mannitol seems as monoclinic orthorhombic form with flattened structures.

Bonaccorso et al., Pharmaceutics 2020, 12(5), 476; https://doi.org/10.3390/pharmaceutics12050476

In vitro Cell Uptake Study

- ✓ Free curcumin and curcumin NCs at the lowest concentrations did not exhibit any fluorescence.
- ✓ Curcumin NCs (5 uM) showed a high-intensity intracellular green fluorescence, proving that the OECs efficiently took up the NCs compared to the free curcumin at the same concentration
- ✓ NCs can enhance drug permeability.



The internalization and uptake of curcumin (**A**): 0.1 µM; 0.5 µM; 5 µM; and Curcumin NCs (**B**): 0.1 µM; 0.5 µM; 5 µM into OECs. Scale bar: 30 µm.

Bonaccorso et al., Pharmaceutics 2020, 12(5), 476; https://doi.org/10.3390/pharmaceutics12050476

Conclusion

- DOE is a reliable approach to design and optimize a new pharmaceutical formulation
- NCs represent a useful strategy to overcome drug poorly water solubility expecially for hydrophobic drug with central action
- NCs can overcome the limit of the small volume for intranasal administration
- *In vivo* study should be perform to confirm that NCs together with IN dosing may improve drug brain targeting

Acknowledgments

Prof.ssa Di Martino Piera
Dott.ssa Maria Rosa Gigliobianco

Dott.ssa Rosalia Pellitteri

Prof.ssa Claudia Carbone
Prof.ssa Teresa Musumeci
Prof. Giovanni Puglisi

Thanks for your attention

Ricerca di Ateneo 2020-2022, Piano di incentivi per la ricerca (PIA.CE.RI.), Linea di intervento 2; Nas**O**, Nanomedicina e Neu**R**oterapie: Le 3 N per il t**A**rget **C**erebrale di moleco**LE** bioattive (3N-ORACLE). Responsabile Prof.ssa Teresa Musumeci, Progetto interdipartimentale
CUP 57722172124