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Confindustria Milano Monza e Brianza

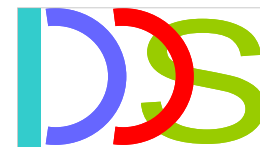
Produzione di nanoparticelle polimeriche con tecnologia industrialmente scalabile

Dipartimento di Scienze del Farmaco – Università di Pavia
Polymerix (*spin-off accademico*)

Speaker

Prof. Ida Genta, PhD

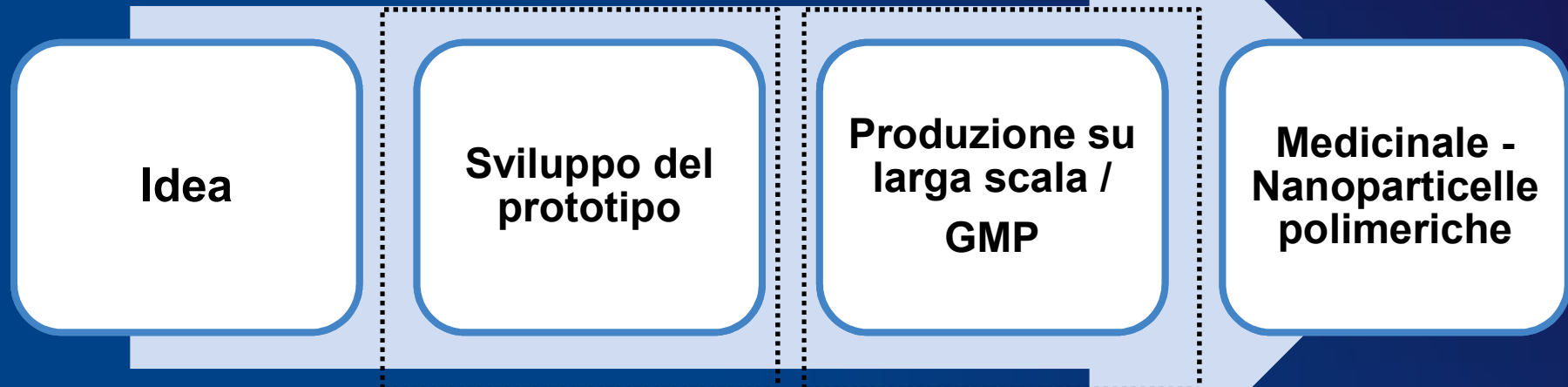
12 ottobre 2016



Processo produttivo di nanoparticelle polimeriche

Sfide

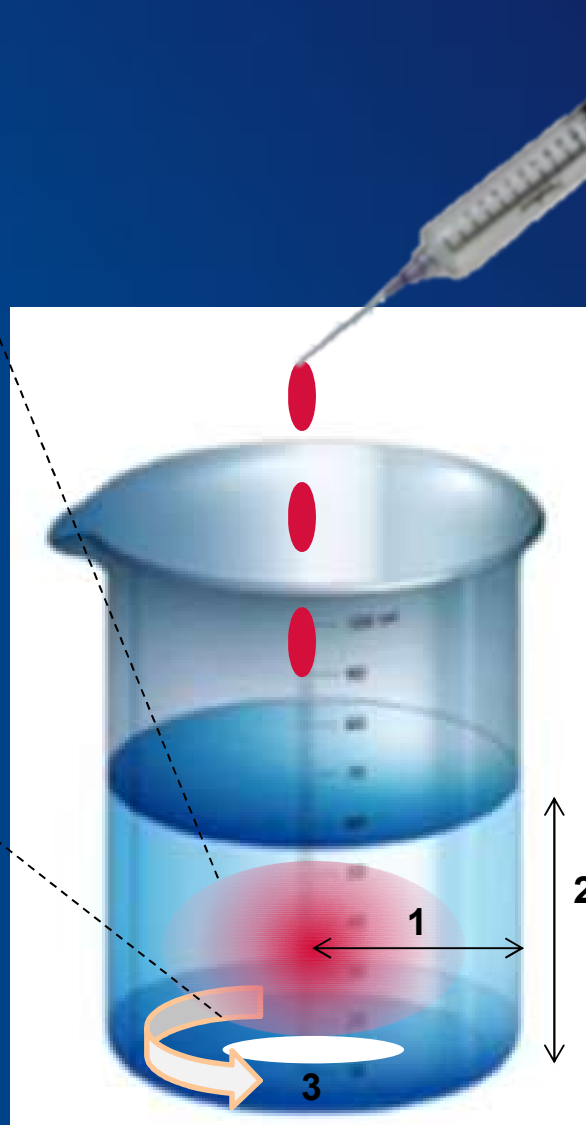
- ✓ Processo semplice
- ✓ Tempo di sviluppo (e produzione)
- ✓ Riproducibilità
- ✓ Facilità di *scale-up*



Metodo di produzione convenzionale: Nanoprecipitazione

Meccanismo *top-down*

Flusso turbolento



Scarso controllo sulle caratteristiche principali delle nanoparticelle

Parametri non controllabili

1. Gradiente di concentrazione del solvente
2. Volume di miscelazione
3. Velocità di agitazione

Metodo di produzione basato sulla microfluidica

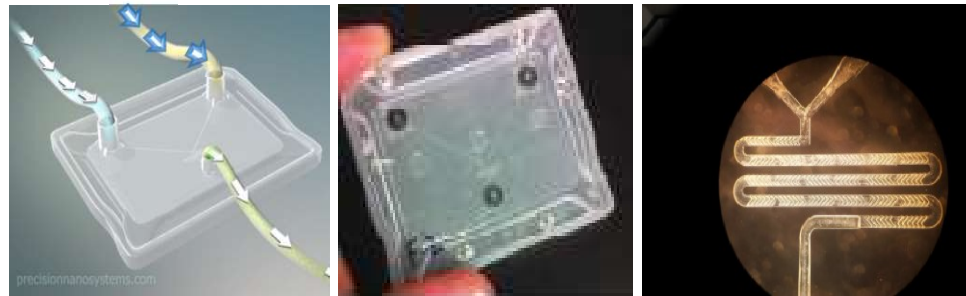
Cos'è la Microfluidica?

Scienza che studia il comportamento dei fluidi in microcanali che hanno almeno una dimensione < 1 mm



NanoAssemblr™ Benchtop
(Precision Nanosystem)

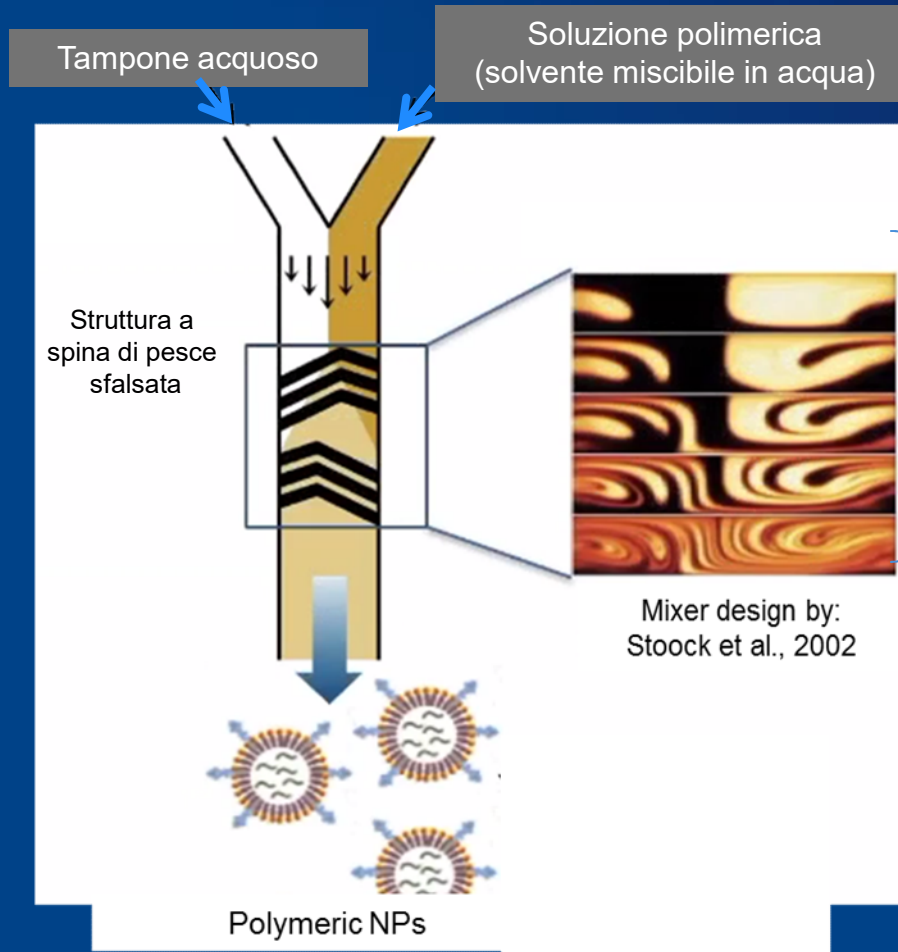
Cartuccia



Schematic representation of microfluidics process of the manufacture of liposomes and polymeric nanoparticles **(a)**, microfluidics cartridge **(b)**, SHM with dimensions of 200 μm in width and 79 μm in height as seen under a microscope at 10X magnification **(c)**

Microfluidica

Meccanismo *bottom-up*



FASE DI MISCELAZIONE

Parametri di processo critici

Step 1: Nucleazione

- ✓ Velocità totale di flusso (TFR)

Step 2: Rapporto di velocità di flusso (FTR)

- ✓ Concentrazione della soluzione

Step 3: Ulteriore crescita (più lenta)

- ✓ Composizione della fase acquosa

Step 4: Crescita finale veloce (non-solvente)

Miscelazione prevedibile e riproducibile

- ✓ Flusso laminare (diffusion mixing)
- ✓ Tempo di miscelazione (millisecondi)
- ✓ Piccoli volumi di miscelazione
- ✓ Automazione completa del sistema

Riproducibilità: una sfida

Metodo convenzionale

1. Variabilità dell'operatore
2. Scarso controllo sulla formazione delle nanoparticelle
3. Polidispersità elevata

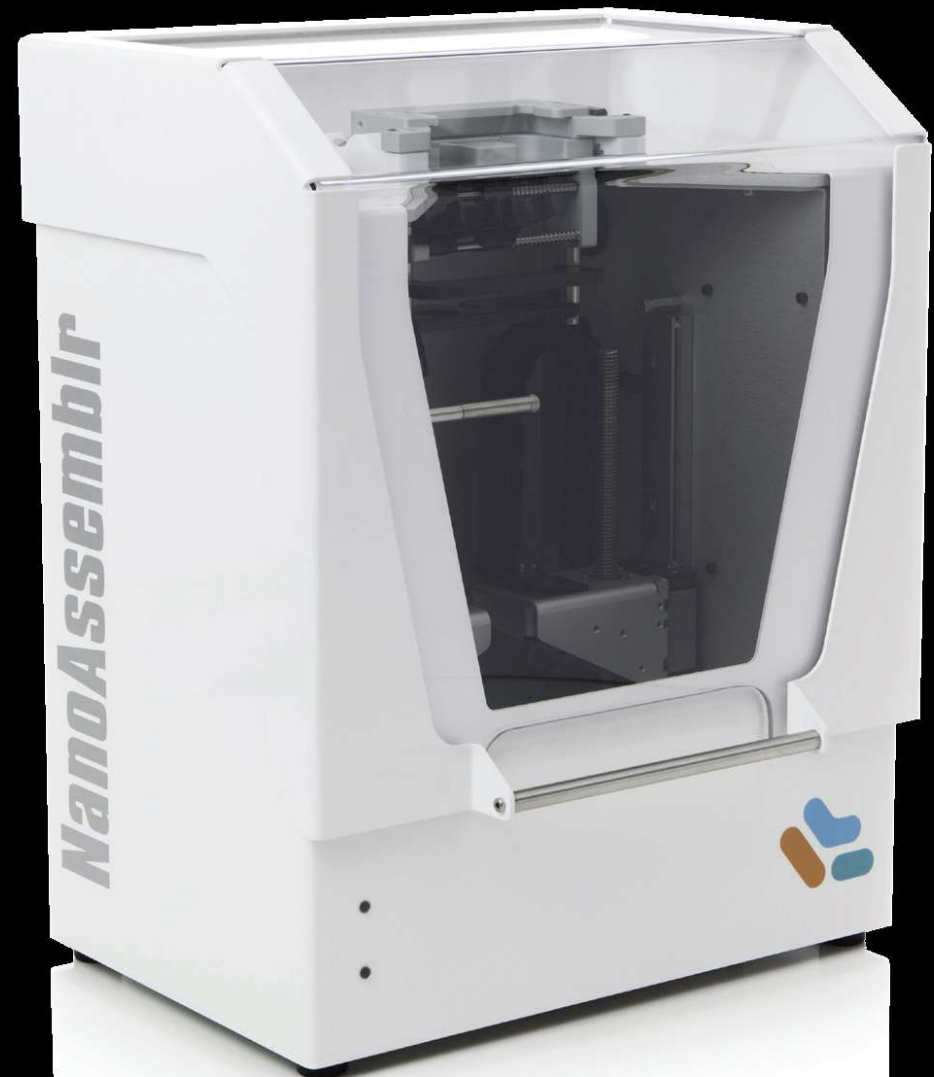
← Scarsa riproducibilità

Ottima riproducibilità →



Microfluidica

1. Automazione del processo
2. Veloce miscelazione, basso volume di miscelazione, preciso controllo del processo di miscelazione ed **efficace rimozione del solvente**
3. Fine modulazione della dimensione particellare con basso PDI



Specifiche del sistema Nanoassemblr

Dimensioni (larghezza x profondità x altezza) 31 x 23 x 38 cm

Velocità di flusso 2 to 12 mL/min

Controllo della temperatura 20 – 70 °C (with heating block)

Compatibilità delle siringhe 1, 3, 5, 10 ml BD Luer-Lok™ or
Slip-Tip siringhe disposable

Max volume per corsa 15 mL

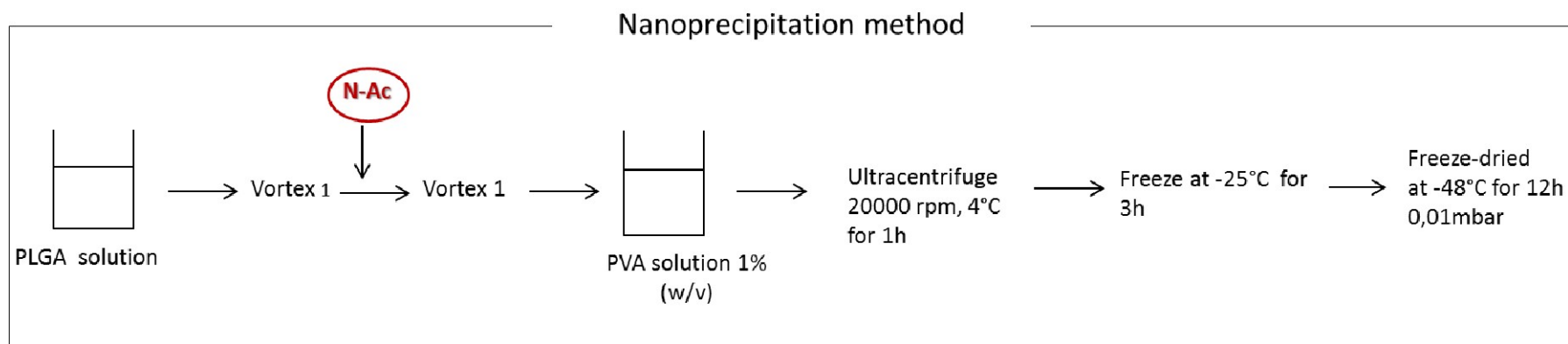
Min volume raccomandato per corsa 1 mL

Tempo di miscelazione <3 ms

Flusso Laminare

Raccolta dello scarto e del prodotto 15 mL Falcon Conical Tubes

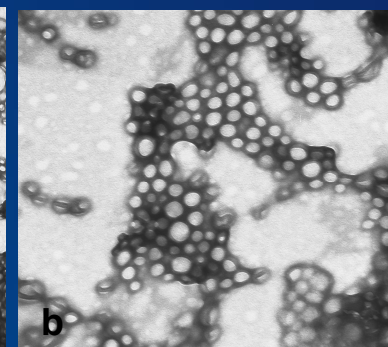
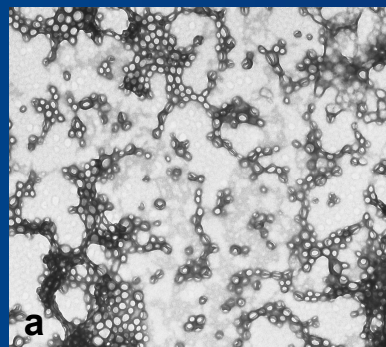
Nanoprecipitazione tradizionale vs Nanoassembly



Preparation protocols of PLGA nanoparticles following a **conventional nanoprecipitation method**

Size, surface charge of PLGA nanoparticle batches, prepared by nanoprecipitation method.

Solvent	Polymer concentration (mg/ml)	External aqueous phase	Size (nm)	PDI	Zeta-potential (mV)
Acetone	15	PVA (1%)	473.2± 197.3	0.374	-4.1
DMSO	15	PVA (1%)	167.2 ± 124.9	0.558	-3.6



TEM micrographs of PLGA nanoparticles at 20000X (a) and 50000X (b)

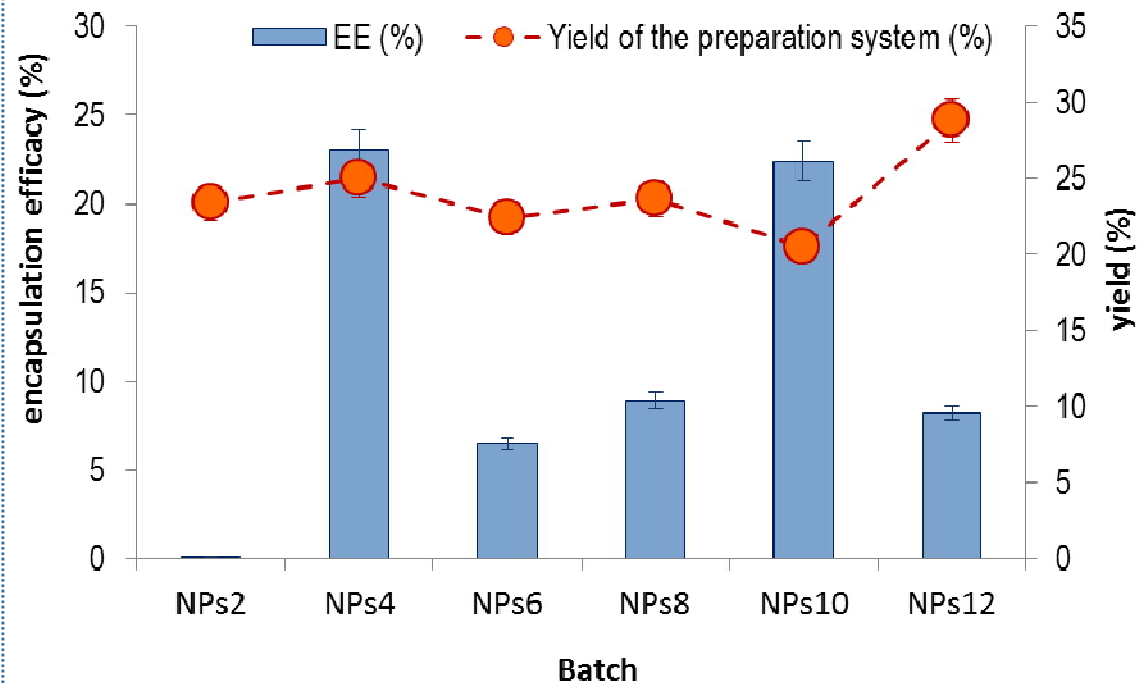


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N-Ac loading (traditional method)

Optimization of NPs preparation protocol to improve and N-Ac loaded into NPs.

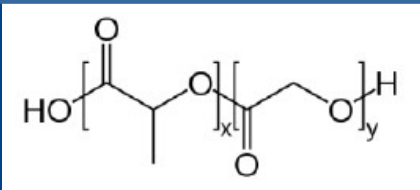
Batches #	Composition		External Phase	
	Drug (mg)	Polymer (mg)	% NaCl (w/v)	pH
NP-2	7,5	75	-	6.8
NP-4	7,5	75	3	6.8
NP-6	7,5	75	-	3.24
NP-8	7,5	75	3	3.24
NP-10	7,5	75	5	6.8
NP-12	15	75	3	6.8



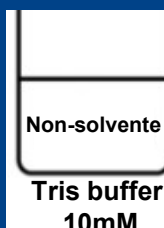
N-Ac encapsulation efficiency and yield of process (%) for N-Ac loaded PLGA-PEG NPs. The encapsulation efficiency was determined by quantification of not-entrapped drug in the supernatant by HPLC analysis performed by ZORBAX Eclipse Plus C18 column, 4,8x150 5µm, mobile phase KH_2PO_4 (pH 3,00): Acetonitrile (88:12)).

The yield defines the amount of polymer employed in the preparation process which effectively reacts to form nano-systems. It was evaluated by gravimetric determination of freeze-dried (Lio 5P, Cinquepascal s.r.l., MI, Italy) nanoparticles compared to the initial amount of polymers.

Nanoassemblr™



PLGA uncapped , DLG 75:25 3A (25kDa)



N-Ac

Soluzione
polimerica

*Micromixer
chip*



Nanoparticelle

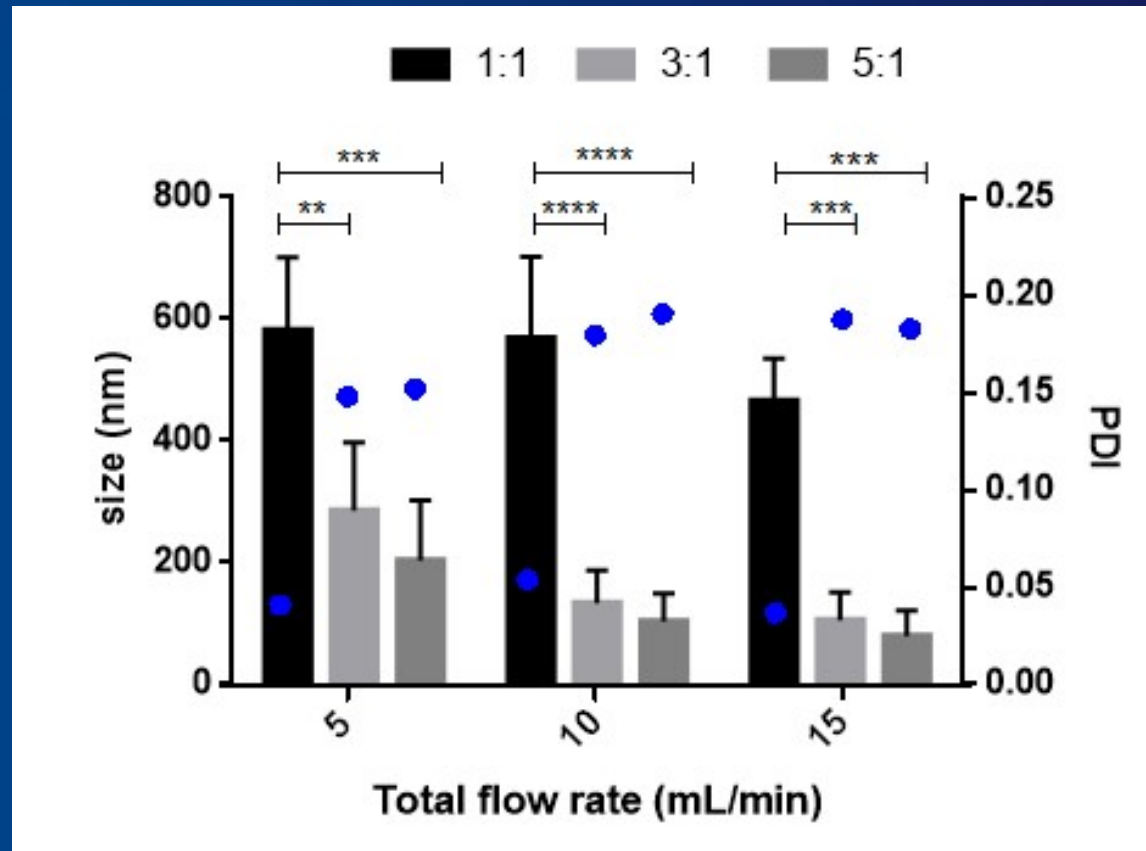
Variabili di processo

Velocità totale di flusso (TFR)	5, 10, 15 mL/min
Rapporto velocità di flusso (FRR , fase acquosa /sol.polimerica)	1:1; 3:1; 5:1 v/v
Concentrazione soluzione polimerica (PLGA)	5 -10 - 15 mg/mL
Rapporto N-Ac : PLGA (F/P)	1:10; 1:5 p/p

Physical characterization

(Nanoassemblr™ method)

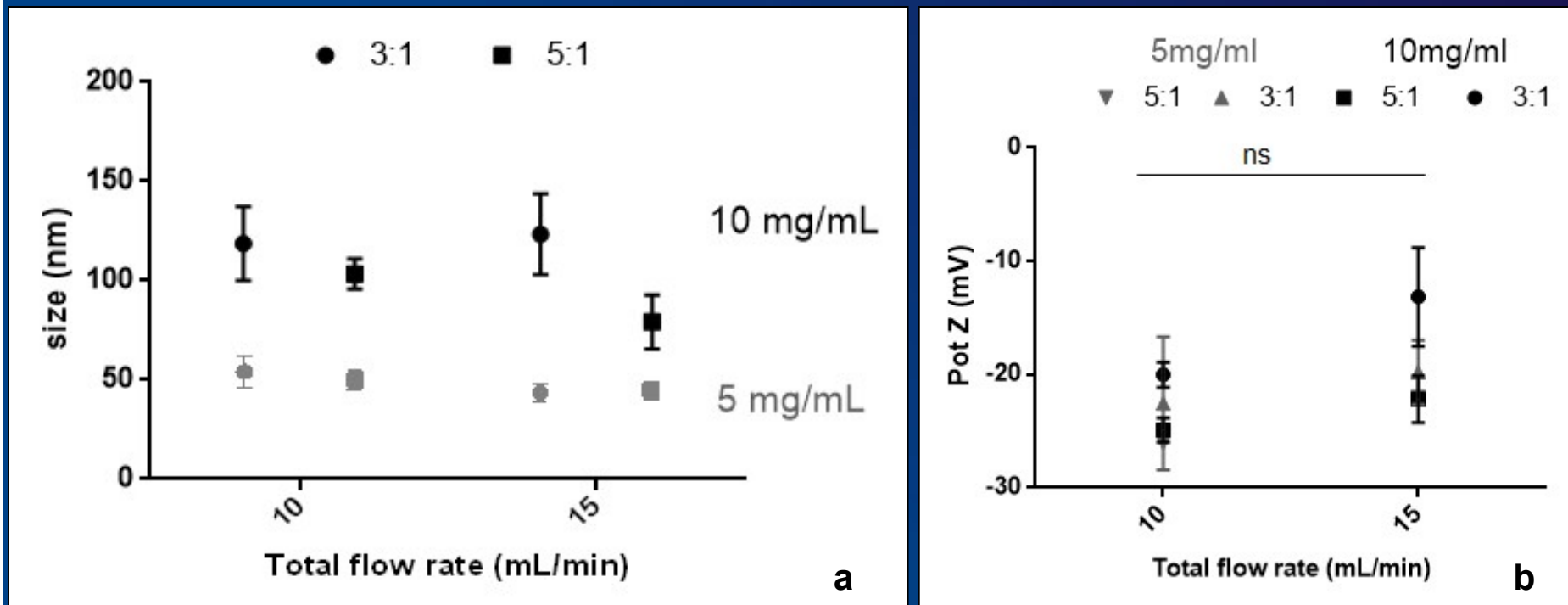
Effect of instrument parameters



Effect of varying total flow rate and ratio flow rate on size and polydispersity index (PDI).
Statistical analysis significant difference (**) for $p < 0.01$, (***) for $p < 0.001$, (****) for $p < 0.0001$.

Physical characterization (Nanoassemblr™ method)

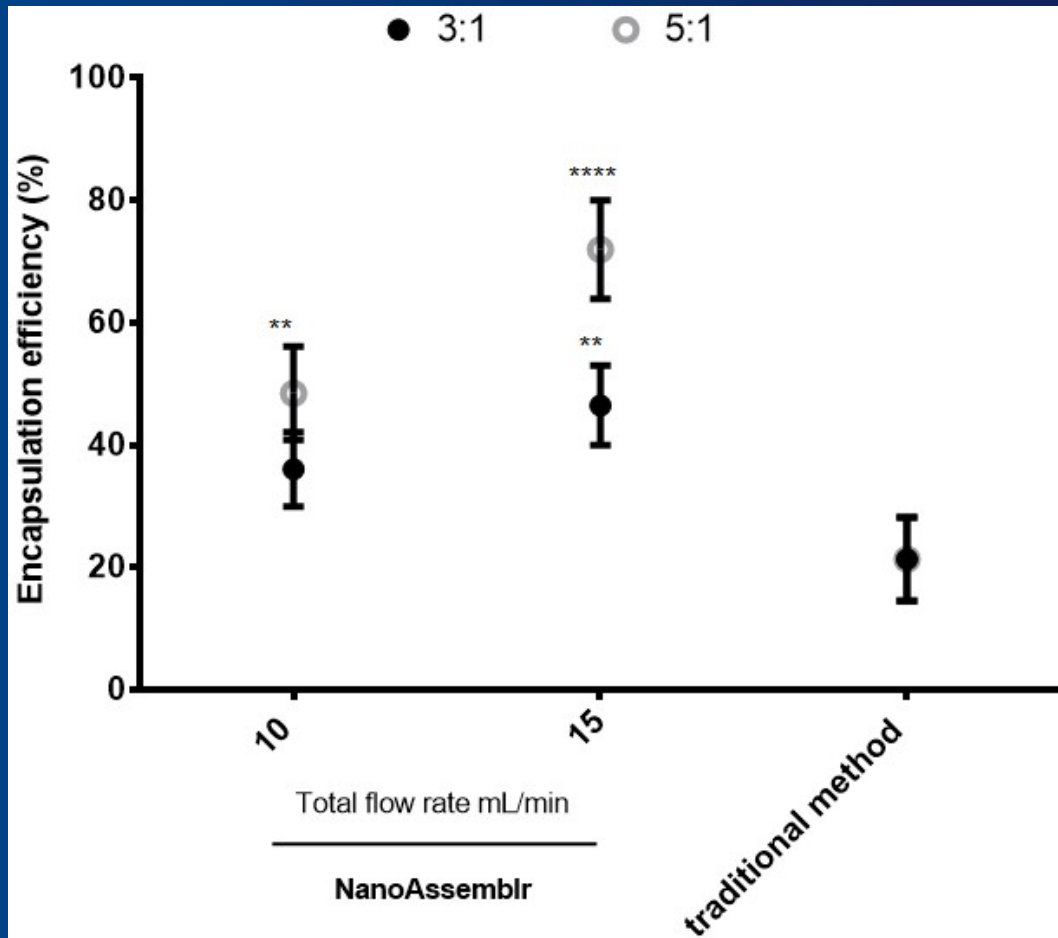
Effect of PLGA concentration



Effect of varying PLGA concentration on physical characteristics (a) size and (b) Zeta-potential. PDI

N-Ac loading

(Nanoassemblr™ method **vs** traditional nanoprecipitation method)

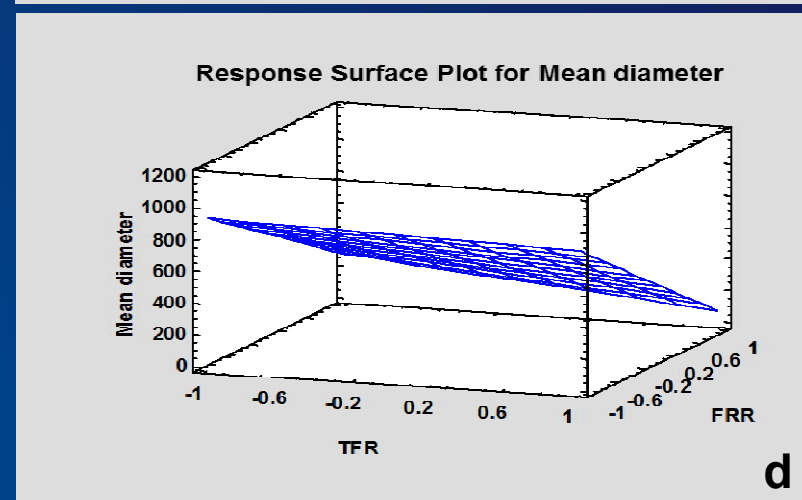
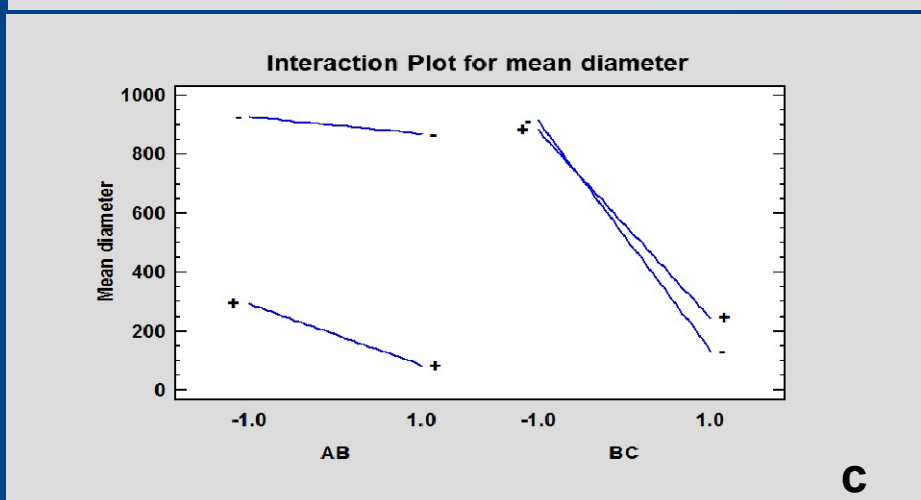
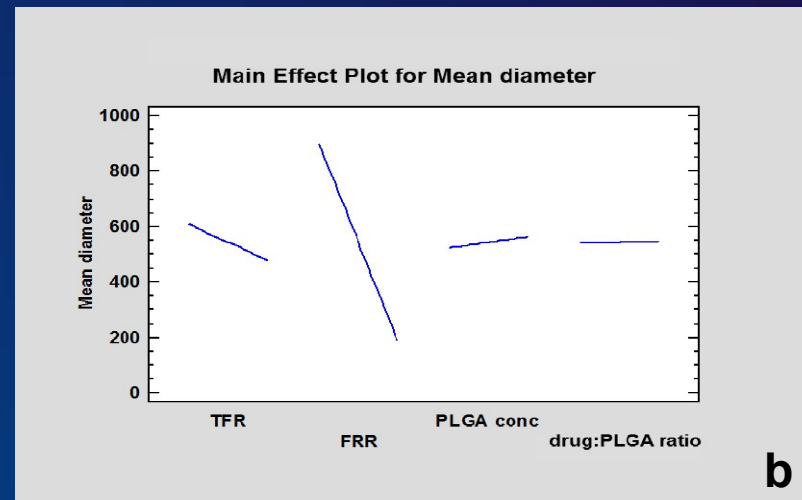
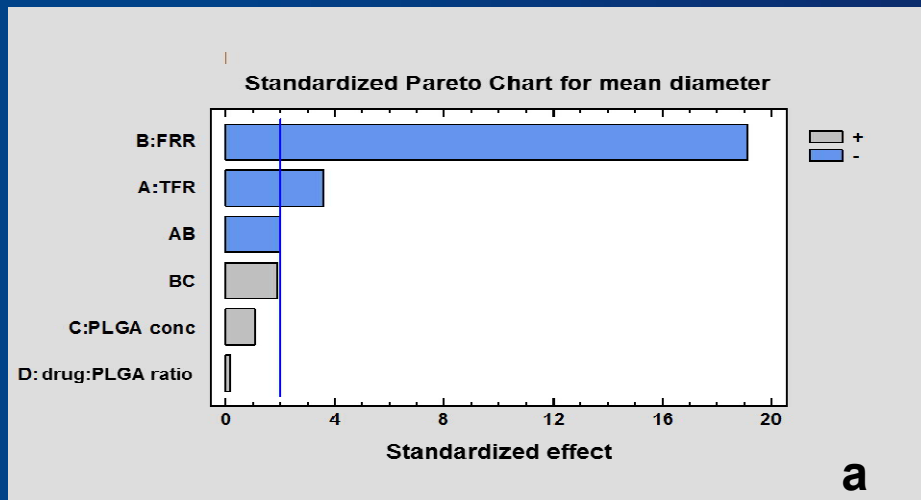


L'incapsulazione
del farmaco
NON modifica le
dimensioni delle
nanoparticelle

Comparison between traditional and NanoAssemblr PLGA NPs preparation method

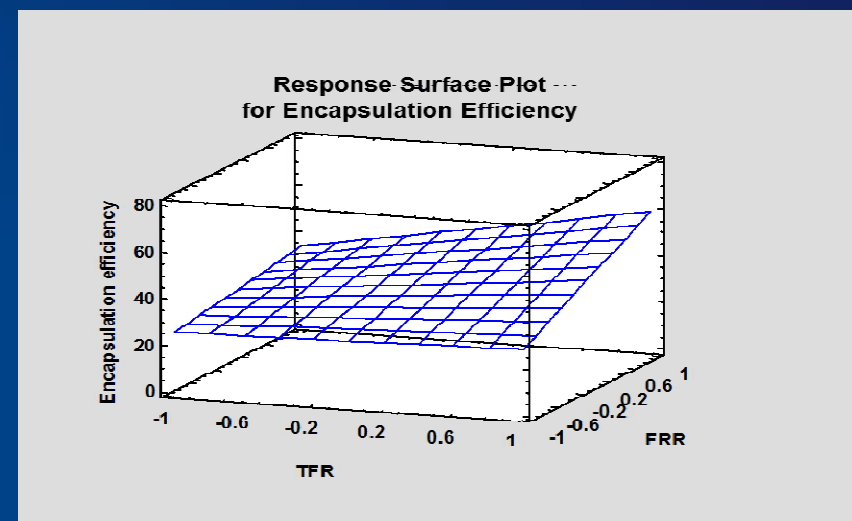
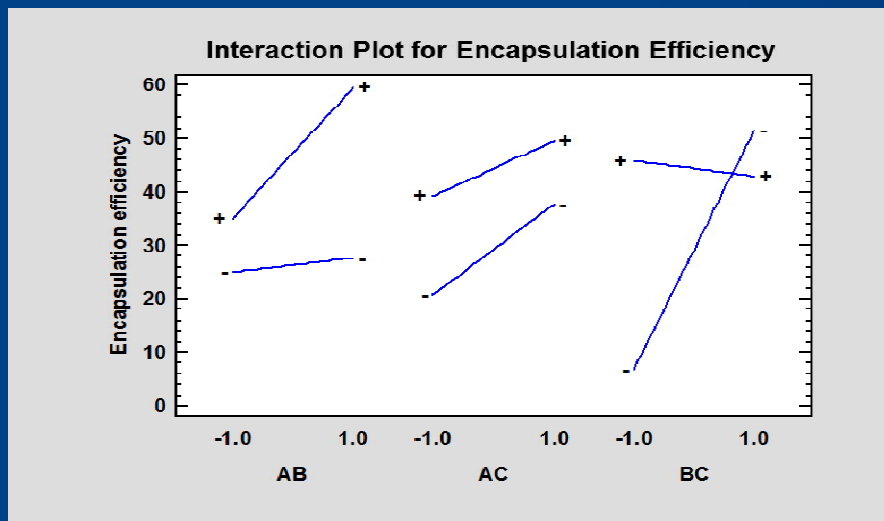
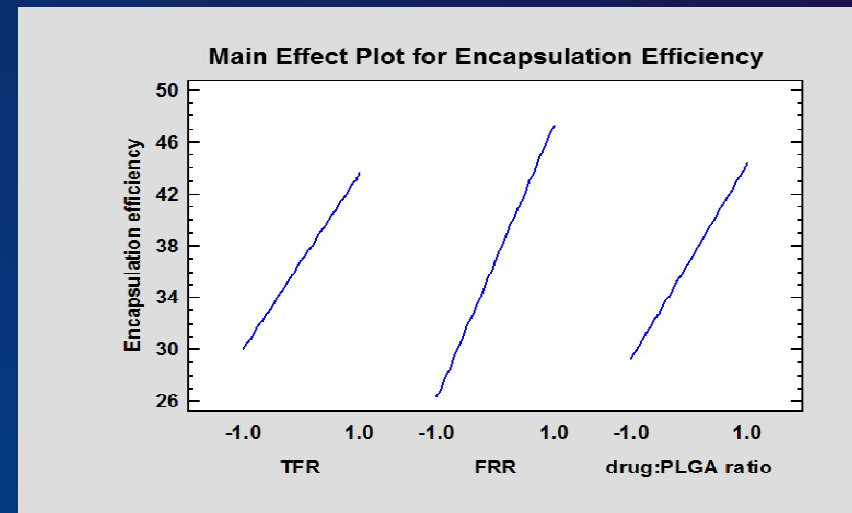
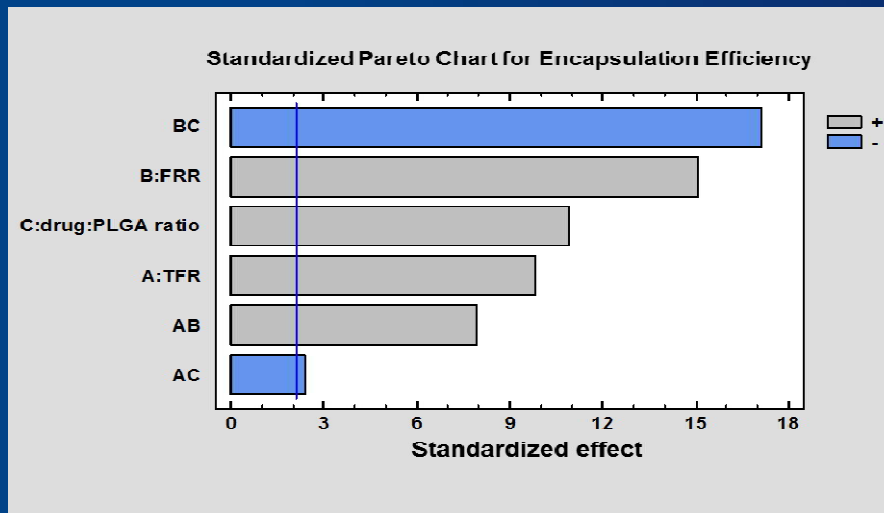
Design of experiments (DoE)

$$\text{Diametro medio} = 542,739 - 66,4875 \cdot \text{TFR} - 355,112 \cdot \text{FRR} + 19,1208 \cdot [\text{PLGA}] + 3,40417 \cdot \text{F/P} - 37.7333 \cdot \text{TFR} \cdot \text{FRR} + 35,375 \cdot \text{FRR} \cdot [\text{PLGA}]$$

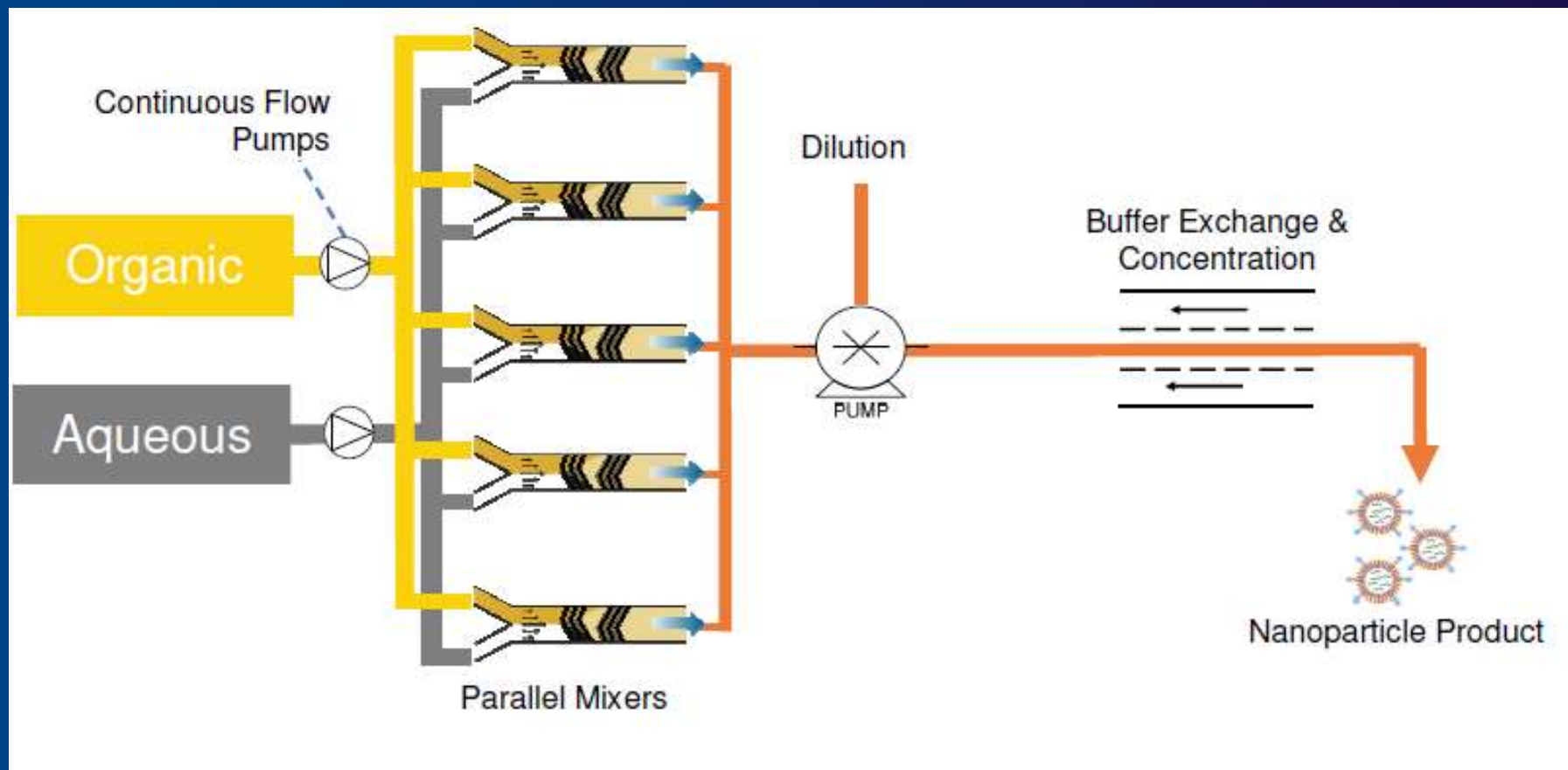


Design of experiments (DoE)

$$\text{Efficienza di incapsulazione} = 36.7836 + 6.7914 \cdot \text{TFR} + 10.4525 \cdot \text{FRR} + 7.57151 \cdot \text{F/P} + 5.47907 \cdot \text{TFR} \cdot \text{FRR} - 1.65013 \cdot \text{TFR} \cdot \text{F/P} - 11.8764 \cdot \text{FRR} \cdot \text{F/P}$$



Facile scale -up



Idoneo per la produzione di:

- Nanoparticelle polimeriche (polimeri sintetici e naturali)
- Liposomi
- SLN

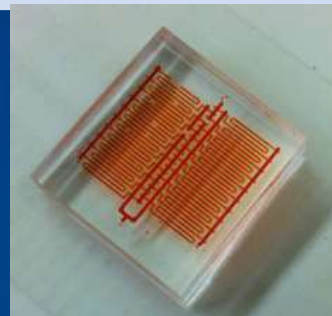
Produzione di nanoparticelle polimeriche con tecnologia semplice, riproducibile, industrialmente scalabile

Idea

**Sviluppo del
prototipo**

**Produzione su
larga scala /
GMP**

**Medicinale -
Nanoparticelle
polimeriche**



**NanoAssemblr™
Benchtop**



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Prof. Bice Conti

Prof. Tiziana Modena

Dr. Rossella Dorati

Dr. Enrica Chiesa

Dr. Antonella De Trizio

Dr. Silvia Pisani

The limitation of current [nanoparticles] delivery technology ... are substantial barriers to... growth

Frost & Sullivan, RNAi Markets 2006

Grazie per l'attenzione



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