NanoGVA Symposium
Training session

Liposome and PLGA particles protocol preparation by Microfluidics and characterization by DLS, NTA, NanoFCM and AF4-DLS

Aim of the training session:

Synthesis of blank liposomes and PLGA nanoparticles via microfluidics varying various microfluidics NanoAssemblr® Ignite parameters (FRR – flow rate ratio and TFR – total flow rate). Assessing their size and Zeta-Potential in comparison of three orthogonal techniques (DLS, NTA and NanoFCM). Discuss about your findings and proceed to AF4-DLS session with the sample of choice additionally recovery of the sample and size.

Program:

8.30-12.00 microfluidics (liposomes and PLGA formulation), NTA and DLS (in separate groups)
12.00-13.00 Lunch
13.00-14.30 NanoFCM (everyone)
14.30-15.00 break
15.00-17.00 AF4-DLS (everyone)
17.00-18.00: Wrap-up session

Materials:

DPPC: 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine
CHOL: Cholesterol
TPGS: D-α-tocopheryl polyethylene glycol 1000 succinate
PLGA : poly-lactic-co-glycolic-acid (50:50)

Methods:

Microfluidics (PNS®), ZetaSizer Ultra (Malvern®), NTA (ZetaView® QUATT ParticleMetrix®), AF4 (Postnova®), Nano analyzer (NanoFCM®)

Contents

Protocol

You will be divided in 3 groups of 3 to 4 people, after a short introduction and having produced 2 samples per group with the microfluidics NanoAssemblr® Ignite each group will start a workshop on DLS, NTA or microfluidics. After 1 h the groups will exchange workshop. A wrap-up session will be planned in the afternoon in order to collect and compare all the datas.

1. Synthesis

a. Liposome synthesis:

i. Fill a syringe with NaCl 0.9% : this will be the aqueous phase (C)
ii. Fill a syringe with Ethanol : this will be the organic phase (R)
iii. insert the run parameters as in Table 1, in the **Quick Run** section of the NanoAssemblr® Ignite
iv. START waste: x mL (see Table 1), start waste: 0.50 mL, END waste: 0.05 mL, Total Volume: 4.0 mL
v. Charge the syringes and a new cartridge and run the sample

Table 1: Formulas of liposomes and parameters for microfluidics

Batch	Lipid compositions	Molar ratio	FRR (C:R)	TFR (mL/min)	Start Waste (mL)	C:R (mL)
Sample 1	DPPC:CHOL:TPGS	3:2:1	1:1	10	0.50	2,00:2,00
Sample 2	DPPC:CHOL:TPGS	3:2:1	1:1	4	0.50	2,00:2,00
Sample 3	DPPC:CHOL:TPGS	3:2:1	3:1	10	0.45	3,00:1,00
Sample 4	DPPC:CHOL:TPGS	3:2:1	3:1	4	0.45	3,00:1,00

b. PLGA particle synthesis:

i. PLGA is dissolved in acetonitrile at 5mg/mL. Fill organic phase syringe (R)
ii. Fill a syringe with PVA 0.1%, aqueous phase (C)
iii. Insert the run parameters as in Table 2, in the **Quick Run** section of the NanoAssemblr® Ignite
iv. START waste: see Table 2, END waste: 0.05 mL, Total Volume: 2.5 mL
v. Charge the syringes and a new cartridge and run the sample

Table 2: PLGA formulations parameters for microfluidics

Batch	Composition	FRR	TFR (mL/min)	Start Waste (mL)	C:R (mL) (mL)
Sample 5	PLGA RG502H	2:1	8	0.45	1,67:0,83
Sample 6	PLGA RG502H	4:1	8	0.45	2,00:0,50

2. Particle characterization

(1) Size (Zeta-Sizer Ultra, NTA)
(2) Zeta potential (Zeta-Sizer Ultra, NTA)
(3) NanoFCM
(4) AF4-DLS

Results interpretation

Table 3: Comparison of results between DLS, NTA and NanoFCM

Sample	DLS			NTA			NanoFCM
	Z-Average(nm)	PDI	Zeta-Potential (mV)	Mean size (nm)	Zeta-Potential -(mV)puls.	Zeta-Potential (mV)cont.	Size (nm)
Sample 1							
Sample 2							
Sample 3							
Sample 4							
Sample 5							
Sample 6							

Continue with the sample of choice, picked during the morning session.

Table 4: Parameters obtained by AF4-DLS

	AF4-DLS		
	Recovery (%)	Rg (nm)	Rh (nm)
Sample __			

Wrap-up session

Table 5: Size data comparison between DLS, NTA, AF4-DLS and NanoFCM

	z-average (nm) DLS	**(nm) NTA**	**Rg (nm) AF4-DLS**	**Rh (nm) AF4-DLS**	**(nm) NanoFCM**
Sample __					

Table 6: Sample concentration and volume comparison between DLS, NTA, AF4-DLS and NanoFCM

	DLS	**NTA**	**AF4-DLS**	**NanoFCM**
Concentration (mg/ml)				
Volume (ml)				

Notes