2017/07/06, MI

Time-course size analysis of INP with High Throughput DLS analysis at different pH

EXPERIMENTAL PLAN

pH points

pH 4 - citrate phosphate buffer (0.05 M citric acid, 0.05 M TAPS, 0.05 M Na2HPO4)
pH 5 - citrate phosphate buffer
pH 6 - PBS, pH adjusted with HCl
~~pH 7.4 - PBS~~ pH 7, PH adjusted with HCl (Ramya prepared pH 7 instead)
pH 8 - PBS, pH adjusted with NaOH

Time points

t= 0 h - preparation
t= 1 h - 1st measurement
t = 2 h - 2nd measurement
T = 24 h - 3rd measurement
T = 48 h - 4th measurement

6 drugs grouped according to their pKa

Basic:
sorafenib
nilotinib

neutral:
paxitaxel
trametinib

acidic:
debrafenib
~~Glyburide~~ (This one is missing because Ramya did not prepare it)

Material's Ramya will prepare:

1. 50 ml of each buffer :
   All buffers need to be filtered with the smallest filter you have. At least using 0.22 um filter is necessary. The actual recommendation is using 0.02 um filter.
2. 500 uL of 1 mg/ml indocyanine nanoparticle solutions in 1.5 ml eppendorf tubes, resuspended in filtered water.

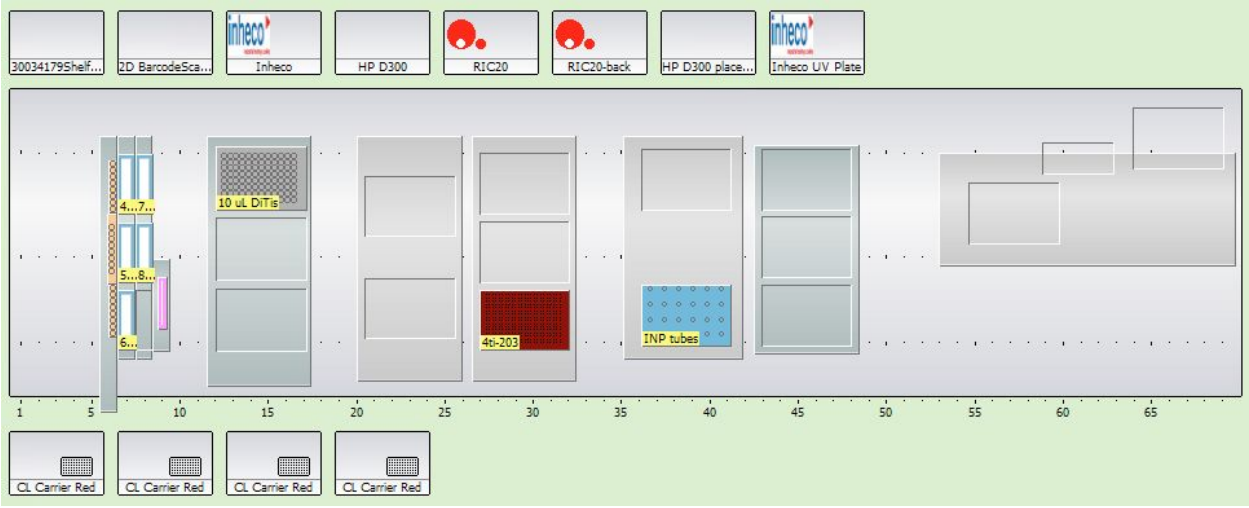
		pH 4			pH 5			pH 6			pH 7.4			pH 8											
Drugs		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
sorafenib	**A**																								
nilotinib	**B**																								
paxitaxel	**C**																								
trametinib	**D**																								
dabrafenib	**E**																								
glyburide	**F**																								
buffer	**G**																								
	H																								
	I																								
	J																								
	K																								
	L																								
	M																								
	N																								
	O																								
	P																								
time (min)		52.5																							

Plate: 384 well flat botttom black plate, [4ti-0203]
Sample volume: 60 uL

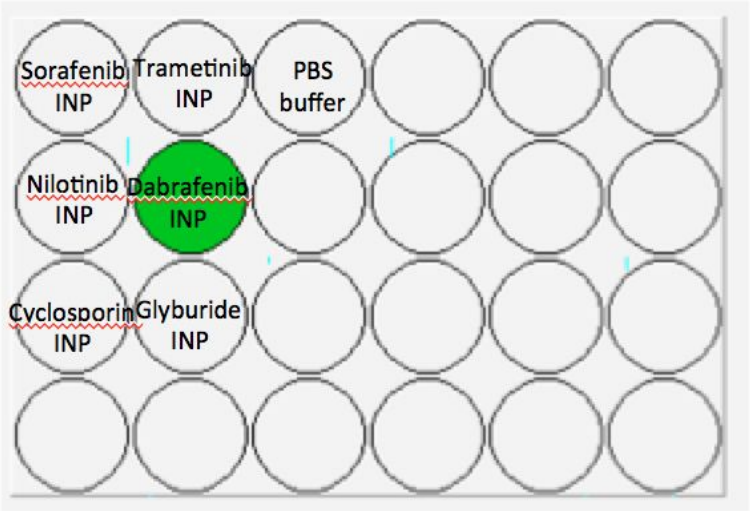
6 uL 1 mg/ml nanoparticle solution will be mixed with 54 uL new buffer with EVO.

EVO

EVO script: **MI_INP_DLS_pH_20170512**



Micrufuge Tube Block:



* Cyclosporin is replaced with paclitaxel!
* Glyburide row is empty!

2017/07/07, MI

Ramya resuspended the INPs in water at 9.45 AM.
I started running EVO script around 10:00 AM.
The plate was ready at 10:30 AM.

I centrifuged the plate at 710 g for 5 min, at RT.
First DLS measurement was started at 11.30 AM (t=1h measurement, labelled “exp_t1h”). It took ~ 45 min, so I added an 10 min wait time to DLS protocol.
First DLS measurement was started at 11.30 AM (t=2h measurement, labelled “exp_t2h”).