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Flow field-flow fractionation for the characterization of lipid-based colloidal formulations

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Outline

Asymmetrical flow field-flow fractionation (AF4)

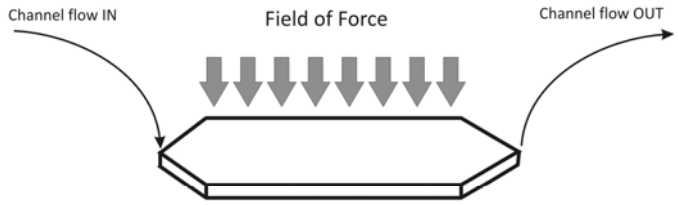
- principle
- separation channel
- promises of AF4 for the characterization of colloidal formulations

Multi-angle laser light scattering (MALLS) for size determination

Selected results of current projects

1. drug release and transfer of a water-insoluble model drug from liposomes
2. Evaluation of the effect of autoclaving on poloxamer-stabilized trimyristin dispersions

Field-flow fractionation

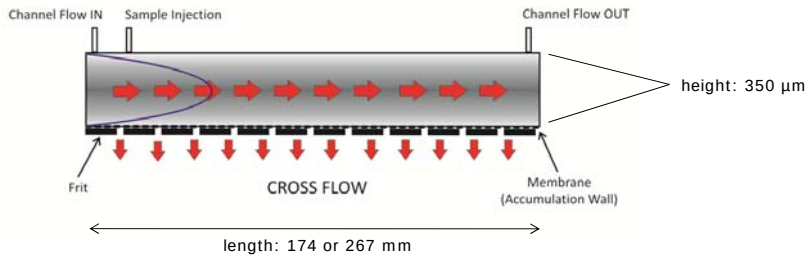


Temperature gradient
Sedimentation field
Electrical field
Magnetic field
Cross flow

→ Flow field-flow fractionation

Cölfen & Antonietti, Adv. Polymer Sci. 150 (2000).

Asymmetrical flow field-flow fractionation (AF4)

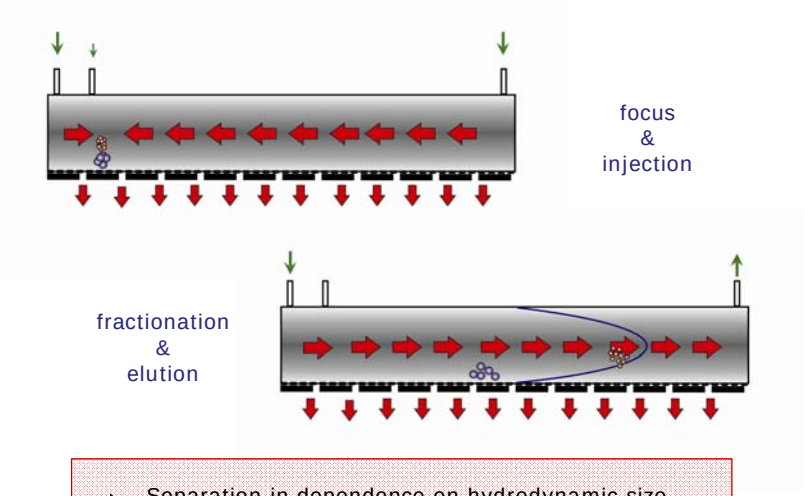


closed cover plate

spacer
→ defines channel geometry (trapezoidal)

membrane (accumulation wall)
→ usually PES or RC with MWCO 5-10 kDa

Separation principle



Separation in dependence on hydrodynamic size
(smallest particles eluting faster than bigger ones).

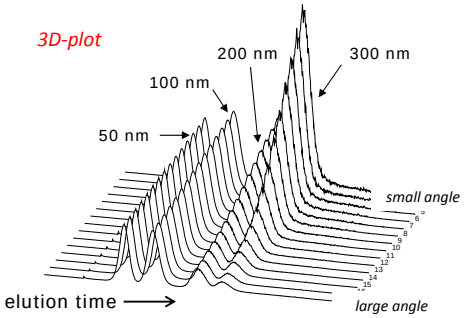
Promises of AF4 for the characterization of nano-sized drug carriers

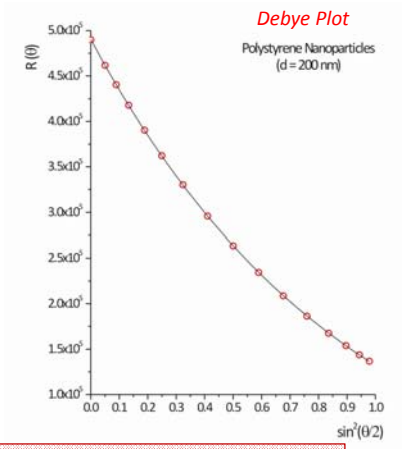
- broad separation range (~ 5/10 kDa - ~ 1 µm)
- large variability in flow conditions, channel geometries, solvents, …
- accurate size determinations due to separation prior size determination
- overall gentle separation and no stationary phase
- semi-preparative fractionation
- connection with various detectors:
  - multi-angle light scattering (MALLS) (size)
  - refractive index (concentration of dissolved compounds)
  - absorbance (scattering effects needs to be taken into account when measuring particulate formulations)
  - others: fluorescence, MS, …

Size determination by MALLS

Multi-angle laser light scattering (MALLS)

Polystyrene nanoparticle mixture
(d = 50, 100, 200 and 300 nm)

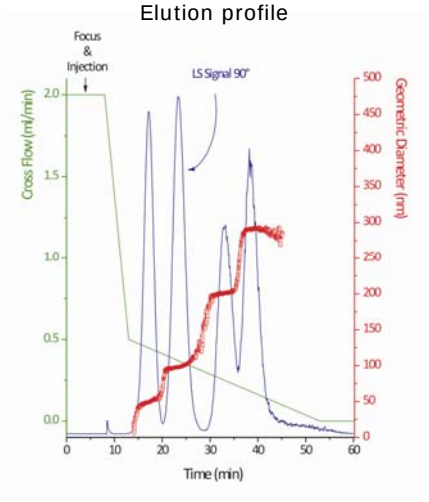




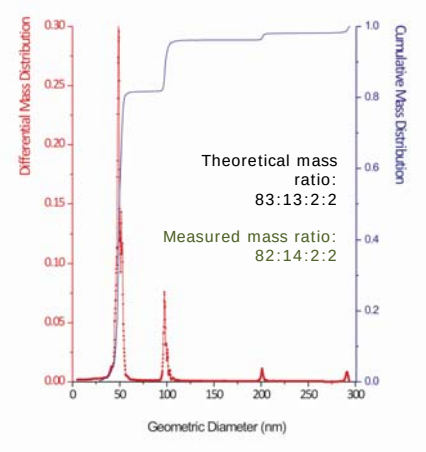
Due to sample fractionation prior size determination (MALLS), accurate information about size distributions is obtained.

Size determination (mixture of PS nanospheres)

Elution profile



Size distribution



Kuntsche et al., J. Biomed. Nanotechnol. 5 (2009).

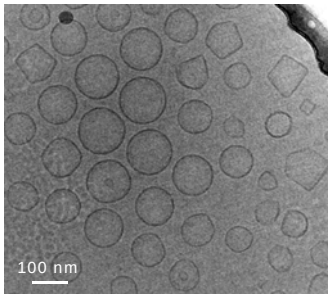
Example 1: Studies on drug release and transfer

After intravenous injection of a drug carrier formulation (liposomes, lipid nanoparticles), the drug may (depending on its physicochemical properties on carrier properties):

- be release into the aqueous phase of blood (molecularly dissolved)
- transfer to other colloidal components of the blood (e.g. lipoproteins, albumin)
- transfer to membranes (e.g. erythrocytes)

↓

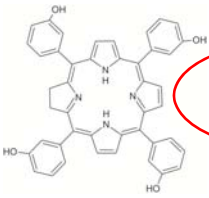
challenge in the development of liposomes & lipid nanoparticles for lipophilic drugs



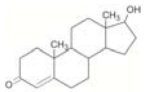
cryo-TEM of mTHPC-loaded liposomes

Lipid and drug recovery after AF4

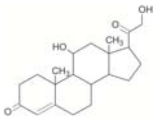
Radioactively labeled liposomes (lipid and drug, ^{3}H and ^{14}C)
(20 mg/ml DPPC/DPPG loaded with 8 mol% drug, diameter ~ 110-120 nm)



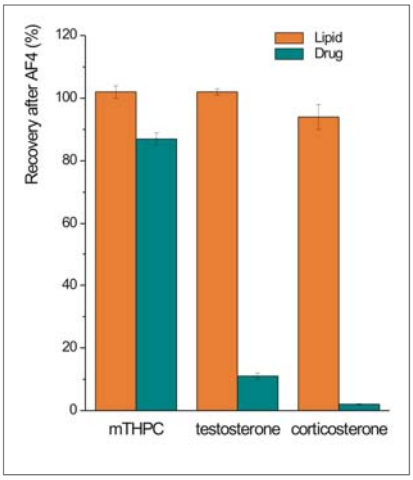
temoporfin (mTHPC)
(log P ~ 9)



testosterone
(log P ~ 3)



corticosterone
(logP ~ 2)



Kuntsche et al., J. Sep. Sci. 35 (2012).

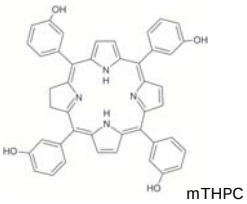
Transfer studies

donor
pTHPP-loaded liposomes

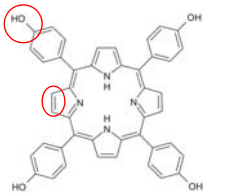
acceptor
liposomes
nanoemulsion
albumin
serum

INKUBATION
37 °C, stirring

fractionation (**AF4**) and quantification of pTHPP in the different fractions (online absorbance after correction of scattering effects)



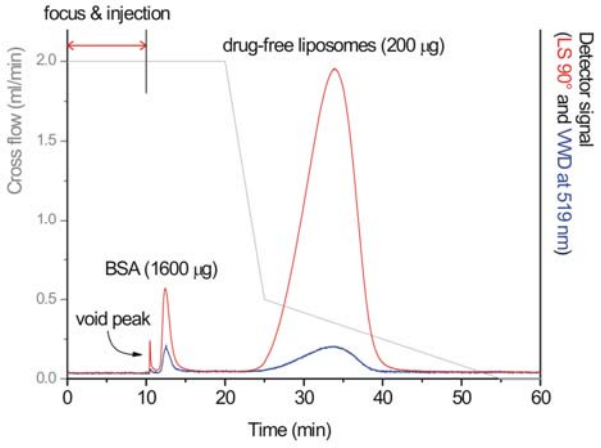
mTHPC



pTHPP

Prerequisite: No (or only very minor) overlap of size distributions of the donor and acceptor particles!

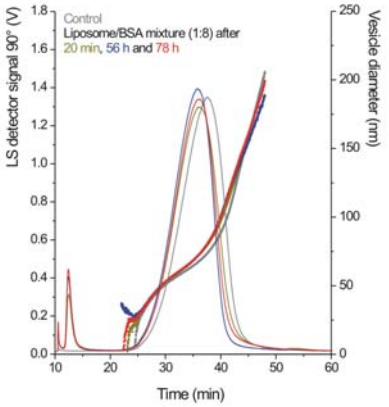
Separation of liposomes and BSA



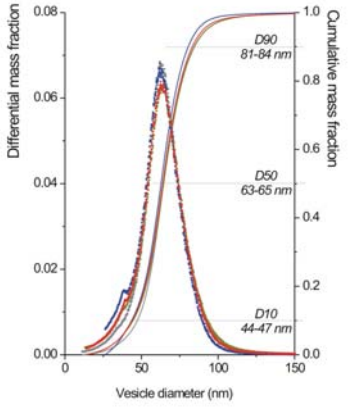
Mixture of liposomes (d ~ 65 nm) and bovine serum albumin (d ~ 6 nm)

Liposome size stability during incubation

Elution profile



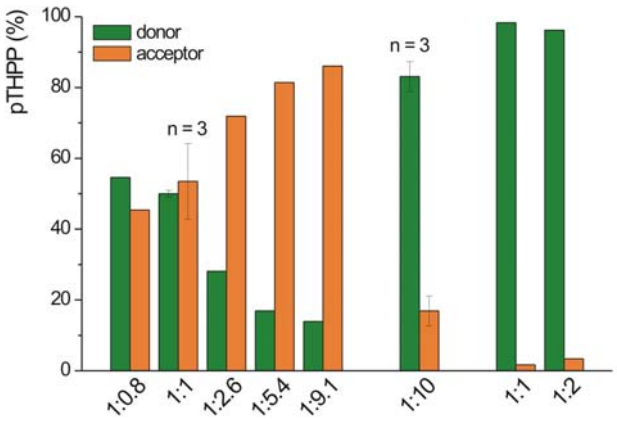
Size distributions



Changes (i.e. in size) in the fractions can be monitored, but (minor) alterations in composition may not be detected

Extent of pTHPP transfer

pTHPP amount in the **donor** and **acceptor** phase (*in equilibrium*)

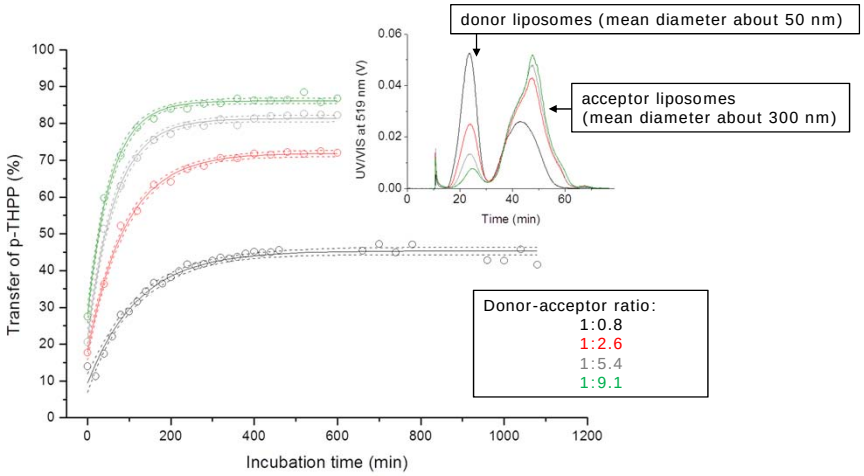


pTHPP recovery usually > 90%

Acceptor phase:

liposomes | BSA | Intralipid (triglyceride nanoemulsion)

Transfer kinetics of pTHPP between liposomes



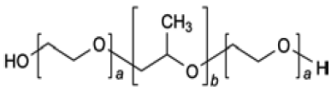
Hinna et al., Analy. Bioanal. Chem. 406 (2014);
Hinna et al., J. Pharm. Biomed. Anal. 124 (2016); Hinna et al., J. Control. Rel., submitted.

Example 2: Effect of autoclaving on poloxamer-stabilized trimyristin dispersions

Composition

- 10% w/w lipid phase (trimyristin)
- 5 or 12% w/w stabilizer (poloxamer 188)
- water phase with 2.25% w/w glycerol and 0.05% w/w sodium azide

poloxamer 188 (Ph.Eur.)

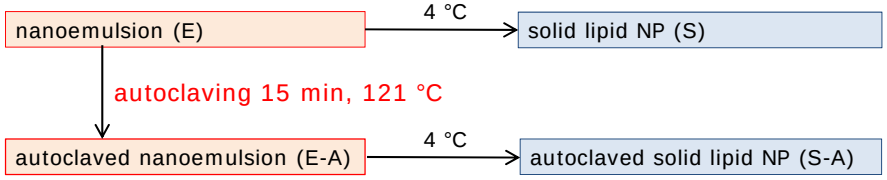


a = 75-85, b = 25-30

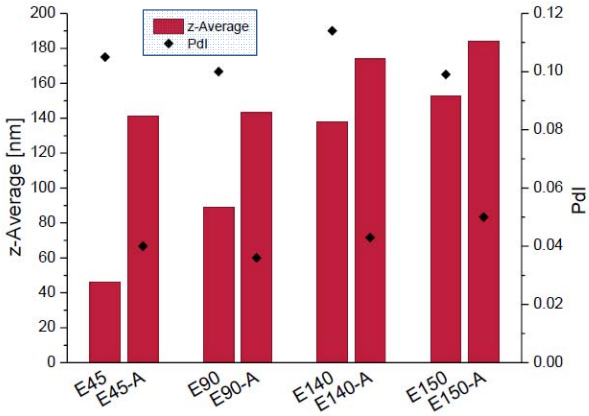
average Mw = 7.68-9.51 kDa

cloud point > 100 °C

High-pressure melt homogenization (75 °C, different pressures)



Effect of autoclaving on poloxamer-stabilized trimyristin dispersions



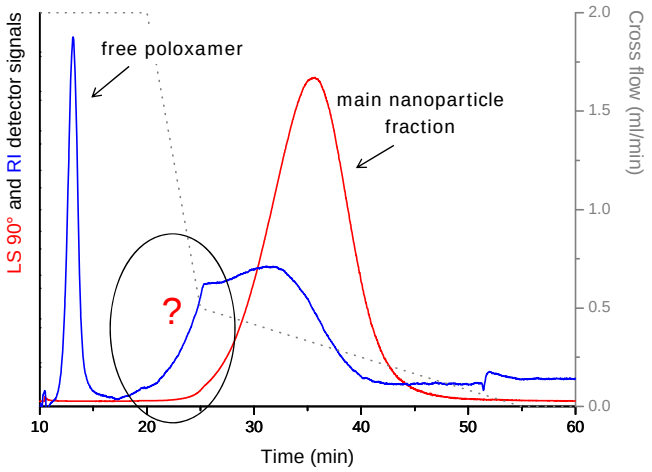
increase in mean size, but decrease of the PDI (polydispersity index) after autoclaving

similar trends for the corresponding solid lipid nanoparticles

➡ asymmetrical flow field-flow fractionation (AF4)

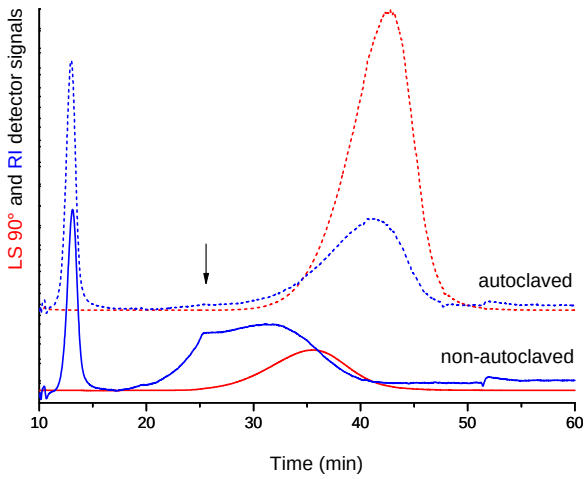
Rose et al., Poster presentation, 9th PBP World Meeting, Lisbon, 2014.

Elution profile: Non-autoclaved nanoemulsion



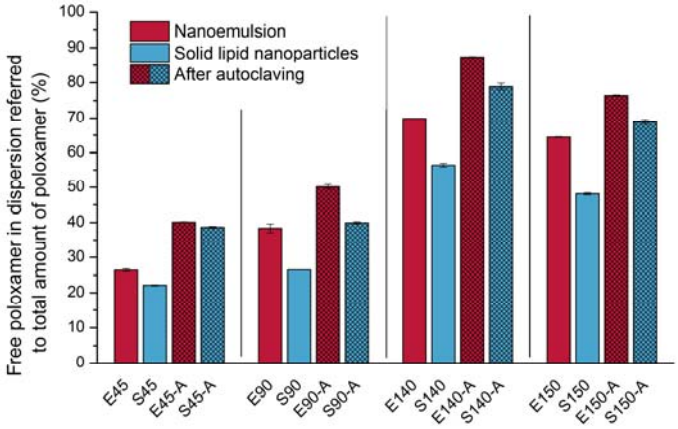
E90 (nanoemulsion prior autoclaving)

Elution profile: Effect of autoclaving



E90 (nanoemulsion prior and after autoclaving)

Amount of free poloxamer in the dispersions



Lower amount of free poloxamer in the solid lipid nanoparticle dispersions and increased amount of free poloxamer after autoclaving.

Poster 01

Arnold et al., Poster presentation, 9th PBP World Meeting, Lisbon, 2014.

Summary and outlook

- AF4 presents a valuable tool for the characterization of colloidal formulations as it facilitates separation of even complex samples.

- *Advantages:*
  - broad separation range over the whole colloidal size range
  - robust and reproducible separations
  - no stationary phase and overall gentle separation conditions
  - monitoring of changes in size during in drug transfer studies

- *Disadvantages/challenges:*
  - requirements on sample (size in the nm-range, physical stability)
  - relatively time-consuming (normally ~ between 45 and 70 min)
  - potential sample-membrane interactions and artifacts due to sample dilution
  - method development may be challenging and time-consuming

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