Nano-structure Development of Oral Pharmaceutical Formulations in Simulated Intestine – D-contrast SANS and DLS

Therapeutic Nanoparticles as Drug carriers:
Mixed nanoparticles bearing material domains

Combination: DLS and SAXS and D-contrast Neutron scattering SANS

Therapy material development

I) Parenteral (injection):
study of formulations and interactions

II) Oral (tablet, capsule, solution):
study in a Gastro-Intestinal simulator models (GI-Sim)

Therapy method development
Immu-no-therapy (mRNA)
Vaccination
(I) Parenteral drugs in nanoparticles

Drug nanoparticles for parenteral application (injection): The drug (pharma agent, mRNA) in lipid, liposomes or polymer nanoparticles release the drug, if the structure is sufficient.

A) Conventional parenteral drugs: release the drug (pharma agent) from solution or nanoparticles non-specific.

B) mRNA nano-drugs: code for the cellular synthesis of a specific protein, which later induces antibody generation. This implies a double amplification (> 1000 x) by cell elements (ribosomes, T-Cells)

mRNA is:
- not toxic, as it is a native cellular intermediate (short-life work-copy of a gene)
- sensitive, as cells and tissue switch it off quickly by enzymatic degradation (RNase)
- temperature and hydrolysis sensitive, need sterility and cooling, if pure
- Nanoparticle structure embedding can stabilize, and facilitate cell uptake (activity)

Analogy in a computer – CNC system:
1 Harddisk (DNA) ⇔ 20 file-copies on USB-Sticks (mRNA) ⇔ 1000 products by 3D-printer set (ribosomes)
mRNA-Nanoparticles for immuno-therapy: two forms

1. mRNA polymer nanoparticles

SANS with Deuterium-contrast variation \( \Rightarrow \) component domains in drug nanoparticles

mRNA in AminoDextrane NP's

mRNA in DEAE-Dextrane NP's

SANS @ MLZ KWS2

Improvements
a) charged polymer
b) basic lipid+ polymer, protein

Core-shell-NP's

DLS

embolic risk limit

DLS as med. security test

mRNA immuno Nanoparticles:
Medical use for:
a) vaccination, e.g. Covid-19
b) cancer immuno therapy

Structure based development:
variant coding for luciferase
SANS, SAXS, DLS, animal tests


MLZ FRM-II, KWS2 DESY-Petra III, P12

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**MLZ**

**PETRA III**

**SAS**

**SANS**

**DLS**

**SAXS**
**mRNA-Nanoparticles for immuno-therapy: two forms**

(2) **mRNA lipid-layer nanoparticles**

RNA is assembled with lipids: charged, neutral and extended hydrophilic headgroups. Sequential mixing, shearing in a pH-regime results in spherical solid lipid nucleo particles (LNP), or multilayer nanoparticles (below), depending on the charge-lipid-head ratio.

For **structure based nanoparticle development** a mRNA variant is choosen, which codes for luciferase (the light generator of fire fly). Thus the medical success of a mRNA nanodrug treatment (cell uptake and induced protein synthesis) can be proven in an animal test (mice) by light generation: the active regions get luminescent.
Nanoparticle & Therapy – Licencing and Patents
no medical use without patent and licence possible

Pharma-product development and licensing steps: 8 -10 years, costs ~ 20 mio €

- Fast licencensing, emergency licence ~ individual healing trial ; e.g. in case of Covid-19
- Development and licensing can be accelerated by fast bioequivalent lab methods,
  e.g. a drug application simulator device ➞ second part of the presentation
Pharma-Nanoparticles II: Drug application simulation

Transient nanoparticles from oral forms in the human digestion system (GI)

FaSSIF-C: GI-model fluid with cholesterol nanoparticles

Drug (tablets, capsules):

- Stomach

Particle size scale: 5 nm – 200 µm!

⇒ TR-SANS + DLS

The micelle to liposome structure development in simulated intestinal fluid (FaSSIF) was detected by time resolved SANS + DLS. (ILL-D11, and @ FRM-II KWS2)


At FRM-II KWS2 a set of cholesterol based bioequivalent model fluids “FaSSIF-C” for the in vitro study of intestinal drug resolution and uptake in the duodenum was developed. The drug solubilization capacity was related to the nanostructure estimated by DLS and neutron scattering SANS; bio-equivalent location: 2nd half of duodenum


FaSSIF-C with Cholesterol ⇒ Study with 8 drugs (2015-2020: R. Johnson, P. Thumarati, V. Stahl)

Interaction of BCS2/4-drugs in nanoparticles, with intestinal model fluids and transient nanoparticles therein (intermediates)

- 8 Drugs:
  - BCS2: Fenofibrate, Danazol, Griseofulvin, Carbamazepin, Curcumin; in nanoparticles
  - BCS4: Amphotericin B, Amphotericin B - Cholesterol (SL Dr. R. Johnson, Ghana)

Methods combined (time resolved):

- Drug solubility (in FaSSIF, FaSSIF-C)
- DLS (time resolved): micelles, liposomes, large NP’s
- SANS (time resolved) at FRM-II, Muni-Garching

Intestinal model fluids, physiological relevant:

- FaSSIFmod:B: pH-stabilized (BiCarbonate+HEPES)
- FaSSIF-C, pH-stabilized, containing Cholesterol (as bile)
Pharma-Nanoparticles II: Nano-drug-structure in intestine

Gastro-Intestinal Simulator devices (GI Sim) with SANS + DLS

**Duties of a GI-Simulator:**
- Interaction of BCS2/4-drugs in nanoparticles, with intestinal model fluids and **transient nanoparticles** therein (intermediates)
- **Licensing process** of drug formulations: kinetics
- **Food effect**, viscosity
- **Comparison** of pharmaceutical products, generic products
- **Methods** combined in GI-Sim:
  - Drug solubility and uptake (time resolved, in FaSSIF-C)
  - DLS (time resolved): **size and kinetics** of micelles, liposomes, large NP's
  - SANS (time resolved) **structure and dynamics** of nanoparticles

**Solid form:**
- Disintegration
- Dissolution
- Solution
- uptake

**Particle size scale:** 5 nm – 200 µm!
GISim 1: one shot mix

GISim 2: flow-through

GISim 3, 4: batch flow (n x 50ml) multi-vessel

GISim 5: coated solid form transport

GISim 1 setup for SANS+DLS with a stopped-flow mixer at ILL D11

GISim 2 setup for SANS+DLS with flow-through pumps at MLZ/JCNS KWS2

DLS as q-range extension for large drug-particles
SANS for nanoparticles
Stopped-flow Simulator GI\textit{Sim}1 with combined SANS+DLS

Nanoparticle development in Intestinal model fluid

- \textit{FaSSIF}_{\text{mod,5}}: pH-stabilized intestinal model fluid
  - HEPES as pH stabilizer
  - physiological osmolality
- Nanostructures develop in time after dilution (FeSSIF to FaSSIF, bile influx in gut)
  - time resolved DLS (@university-lab and with SANS): large structures <50µm, but limited precision
  - time resolved neutron scattering SANS: high precision, component resolution by partial deuteration, 71% D\textsubscript{2}O

- SANS-Facilities:
  1. ILL, Grenoble (D11)
  2. FRM2, Munich-Garching (KWS2)

- Results:
  - Intermediate nanostructures detected at ILL (Grenoble) and FRM-II (Munich-Garching)
  - Components resolution by deuterium matching with D\textsubscript{2}O-solvent possible (see <2> HST)
  - Drug contribution: Fenofibrate, Danazol, Griseofulvin, Curcumin (FRM-II KWS2, GI-simulator)

Cooperation: Ralf Schweins
ILL Grenoble, LSS group
Flow-through GI-Simulator GISim 2 with Nano-analysis
combination of structure estimation with uptake/ dissolving

- Parallel tracing of:
  - drug solubilization
  - nanoparticle structure
  - model uptake/ transport

- Time- and local resolved drug & nanostructure profiles (2D)
  - $C_{drug}(x,t)$ for gut and blood side

- SF: Stopped flow (duodenum-2)
  - for fast events (<20 cm, <20 min after bile influx into the Duodenum)

- CF: Continuous flow (flow through)
  - model device for full simulation
  - module concept (flexible)
  - two compartment/ channels
  - long modules: uptake-dialysis
  - short modules: cell layer, gut

- Drug x-t-profiles: time-local resolved sampling

- Nanostructure estimation:
  - x-t profiles
  - DLS as overview (±20%), in lab
  - neutron scattering SANS for precision and deuterium contrast

- Resolution of nanocarrier and drug:
  - for neutrons by deuteration (lipid, solvent contrast $D_2O$-matching)
  - option for improved tracing: FCPS (DLS with fluorescence)

Drug analysis:
- gut & blood side (layers)
- membrane separator - gut
- drug nano-dissolution

Detection:
- drug-transfer (dialysis cells)
- DLS (x,t resolved)
- SANS (x,t resolved)

GISim 2 simulator,
drug uptake and digestion
simulator concept,
NP structure analysis:
continous flow CF

xtr-SANS + DLS
flow-trough pumps
⇒ steady-state
Quarz-channel
200x12x2 mm

GI-drive
sample pump set:
 continuously flow
stopped flow

T. Nawroth, K. Buch, P. Langguth, R. Schweins; (2011) Molecular Pharmaceutics / 8, 2162-72
**Continous Flow (GSIM 2): time-space Resolution xtr**

long Quartz-cell with continous sample-mix supply

**V1 @ FRM-II_KWS2**: SANS + DLS

**V2 @ Pharma-Lab**: DLS+sampling

**Continuous flow (CF) study of simulated intestinal colloids:**
- Nano-development in *time & distance*: x, t
- Long Quartz flow-through channel (cuvette)
- Mixer in front (fluids + bile; by pumps)
- SANS + DLS investigation (online or offline)
- Large structure investigation *in situ* by projecting DLS: 50 nm – 200 µm (ProSpecD: Nanovel)

**CF results at FRM-II_KWS2:**
- Modules with melt-drawn Quartz ok.
- Duodenum-II : segment after bile influx, 71% D$_2$O
- Shortest time (first frame @ 2mm) ~337 µl after mixing of model bile with fluid from stomach (TM)
- Inspection by: a) DLS, and b) *neutrons*, SANS, CF-module on a CNC-drive) : well statistics
- novel bio-equivalent model intestinal medium FaSSIF-7C, containing 7% cholesterol
- Excipients (detergents+ targeting lipids)
- Core-shell drug polymer nanoparticles and lipid particles

**Duodenum-II**: a) CF-distance evolution during 30min.

b) shortest frame – distance (2 mm = 337 µl = 73 s)

CF SANS at FRM-II_KWS2: The development of diluted bile in a long flow-through cuvette (160x18x2 mm) from micelles to liposomes and large particles, with cholesterol containing model fluid FaSSIF-C (7% cholesterol)


Cooperation:
Aurel Radulescu, Noemi Szekely
FRM-II, JCNS, KWS2
Cancer problem: in 95% of the cases, the disease is individual different at cell level

=> Concept of individual therapeutics by case selective surface modification
  – transport of anti-cancer drug by nanoparticles (10,000,000 drug molecules / particle of 100 nm size); for radiotherapy: Lanthanides, Isotopes (Er, Gd, Lu, ^{10}B, Pt, Bi); or/and chemo-therapeutics
  – Individual modification of pre-manufactured Nano-carriers (coated) as very last step (1 day, „click-link“)
  – The surface ligand(protein) is recognized by over-expressed receptors of the cancer cell => endocytosis

Drug carrier and linker development:
  – Domain structure and linker conformation (ligand exposition) SANS + DLS : Deuterium-contrast SANS

Product Quality control: DLS + SANS for medical security (embolic risk exclosure)
Target Polymer Nanoparticles as drug carriers
PLGA triple component NP for modification by Ligands (Proteins)

PLGA w/o/w nanoparticles (10% heavy metal) ⇒ cell target nanoparticles

Cooperations:
Ralf Schweins
ILL Grenoble, LSS group
FRM-II, MEDAPP

PLGA w/o/w triple NP’s
double emulsion + protein
prepared from activated
target-polymer: 2% PLA,
98% PLGA (Resomer 502H)

- D-contrast variation SANS
SANS + DLS

- Ligand-protein loading on
D-matched target-PLGA NP
upon time-resolved SANS of
D-contrast matched polymer
nanoparticles (PLGA w/o/w)

- Cell targeting nanoparticles
with surface-protein ligand
as final product: non-toxic,
bio-degradable; for RT & CT
Target Nanoparticles Polymer for Cancer Therapy

PLGA triple component NP: modification by Ligands (Proteins)

PLGA w/o/w nanoparticles loading by SH-protein \( \implies \) cell targeting nanoparticles

**ILL-D11: TR-SANS + DLS** of PLGA (w/o/w) NP with activated targeting polymer + protein

Cooperation: **Ralf Schweins, ILL Grenoble, LSS group**

- D-contrast variation SANS
- TR-SANS + DLS
- Ligand-protein loading on D-matched target-PLGA NP upon time-resolved SANS of D-contrast matched polymer nanoparticles (PLGA w/o/w)
- noise reduction by q-rastering
- current work: improvements for stabilization by surface coatings

**SANS of protein-loaded polymer nanoparticles (PLGA w/o/w) in polymer-matching D\textsubscript{2}O buffer:** logarithmic q-rastering for noise reduction

**Protein coupling of D-contrast matched Polymer nanoparticles for individual therapy**

**Difference SANS** empty PLGA nanoparticles before loading

**q-raster B-spline merge**

**ILL D11**
Target Nanoparticles: Stabilization by Coating
mixed shell of Polymer, Lipids and Ligands (target-Proteins)

1) Hydrophobic drug nanoparticles coating by lipid+gelatin => stable nanoparticles

Cooperation: Aurel Radulescu, Noemi Szekely, FRM-II, JCNS, KWS2

AmB-entraping core shell nanoparticles:
- estimation of the shell span by a Kratky-Porod plot evaluation of SANS yields a span radius $R_d = 1.63 \pm 0.052$ nm; i.e. a shell span of $d = 5.64 \pm 0.18$ nm, similar to biomembranes (lecithin bilayers ~ 5 nm).
- b) Improvement: AmB in Cholesterol-DOPC (manuscript)

2) Polymer nanoparticles coating by SH-protein + polymer => stable cell targeting nanoparticles

Cooperation: Ralf Schweins ILL Grenoble, LSS group

ILL-D11: TR-SANS + DLS of PLGA NP with activated targeting polymer + protein + lipid-coat

- D-contrast variation SANS
  - TR-SANS + DLS
- D-contrast matched polymer nanoparticles (PLGA w/o/w) with protein ltargeting load
  - current work: improvements for stabilization by surface coatings
    - Lipid based coats, domains
    - Cholesterol-stealth coat
    - C-C-chain polymer coat (PVA)
    - Charge coupled ionic coating (polyplexes with dextranes, mRNA / DEAE-Dextrane)
    - Hydrophobic drugs in NP
- Bio-Medical and Pharmaceutical Nanoparticles are **multicomponent systems** consisting of therapeutic drugs (10%), polymers, lipids, metal oxides (filler) and proteins, which can be distinguished by **neutron scattering with D-contrast variation**

- The **wide size scale** varies from 1 nm (drugs), over ~5 nm (proteins) to the upper 100 nm scale, in several cases up to 200µm. This requires a combination of complementary methods: **SANS + DLS (projecting dual optics, long focal length)**

- The **SANS + DLS combination** can be applied **online** (dual beam) or **offline** (DLS immediately after SANS)

- Metal drug domains can be distinguished by **ASAXS at the L-edge**, eg. Erbium+Gd for cancer radiotherapy.

- For systems developing in **structure in a process**, e.g. digestion, binding, uptake, SANS and DLS have to be applied as **time resolved methods : TR-SANS + TR-DLS**

**Conclusion**

**HZB BESSY**

**SANS Deuterium-contrast variation**

**ASAXS energy contrast variation**

**SANS + DLS / FCPS**

**SANS / SAXS & DLS (online)**

**DLS (offline)**

**TR-SANS / -SAXS + TR-DLS**

**Thanks**
Beam-Target Nanoparticles for Cancer Radio-Therapy

Liposomes and PLGA NP for Radiotherapy by Photons and Neutrons

**Liposome** nanoparticles with entrapped Erbium

- **HJB / BESSY**

- **ESRF**

  - Liposomes from lecithin (DOPC) with entrapped metal salt (0.5 M)
  - L-edge contrast variation SAXS = ASAXS (5 energies)
  - Bio-targeting by protein ligand surface modification

**PLGA** w/o/w nanoparticles (foam-like) with Erbium in domains

- 2x ultrasound treatment,
- wash

- **PLGA in CH₂Cl₂**

  - w/o

  - emulsion in CH₂Cl₂

  - Er-Acetate in H₂O

  - 1% PVA in H₂O

- **ASAXS** @ HZB BESSY

- **PLGA NP’s 10% metal**
  - prepared as w/o/w double emulsion from polymer (PLGA) and metal-salt solution (Erbium-Acetate)

- L-edge contrast variation SAXS = ASAXS (5 energies)

**SANS** : @ ILL-D11, Ralf Schweins

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**Cooperation:**
- **Guenther Goerigk**
  - HZB / BESSY
  - M. Ballauf group

- **Peter Boesecke**
  - ESRF
  - ID01

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**JGU Mainz / inventors:** Buch K, Nawroth T, Langguth P, Schmidberger H:

- (2013/12) „Diminishing Cell Growth with Metal-Polymer Nanoparticles upon Radiotherapy“ … Metal-PLGA as example

- **BMBF project 05KS7UMA**