Controlled release of proteins from self-assembling hydrogels based on oppositely charged dextran microspheres

Sophie Van Tomme[a]
Rene van Nostrum[a], Stefaan De Smedt[b], Wim Hennink[a]

[a]Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences
Utrecht University, The Netherlands
[b] Laboratory of General Biochemistry and Physical Pharmacy,
Department of Pharmaceutics, Ghent University, Belgium

GPEN 2006, 25th October 2006

University of Kansas, Lawrence

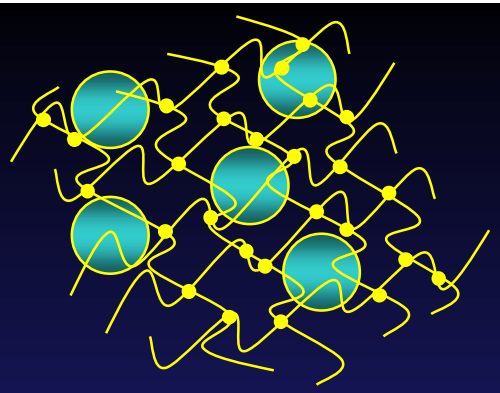
Universiteit Utrecht

UIPS Utrecht Institute for Pharmaceutical Sciences

Outline

- Hydrogels
  - ✓ general features
  - ✓ crosslinking methods
- Aim of the project
- Approach
- Results:
  - ✓ network characterization
  - ✓ protein release
  - ✓ degradation
- Conclusions

Introduction

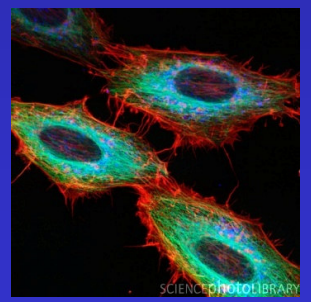


Hydrogel

- 3-dimensional hydrophilic network
- Good biocompatibility
  - ✓ minimal tissue irritation
  - ✓ low tendency for proteins and cells to adhere

Possible applications

- Drug delivery system
  Release can be tailored



- Scaffold for tissue engineering
  Degradation rate can be tailored
  Growth factors can be incorporated
  RGD peptides can be coupled to promote cell adhesion

Introduction

1. Chemical crosslinking ↔ physical crosslinking

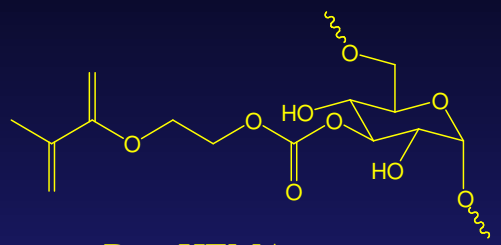
Chemical crosslinking	physical crosslinking
• Radical polymerization • Reaction of complementary groups (e.g. NH_2/COOH) • Enzymes (e.g. proteases) • …	• Crystallization • Hydrogen bonding • Ionic interactions • …
Covalent bonds	Non-permanent bonds
☺ high mechanical strength ☹ toxicity/denaturation	☺ self-assembly ☹ weaker network

2. Implants ↔ injectable matrix

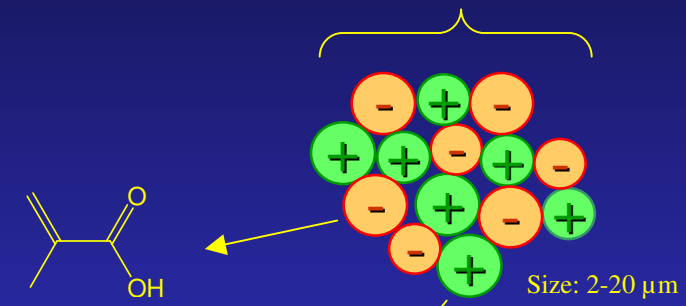
Aim of the Project

Synthesis, characterization and evaluation of an injectable, self-assembling hydrogel for *pharmaceutical* and *tissue engineering applications*

Design

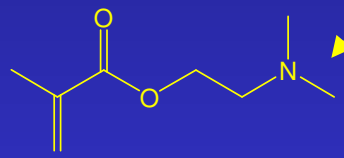


Dex-HEMA



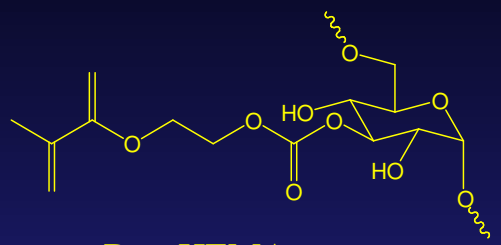
methacrylic acid

Size: 2-20 µm

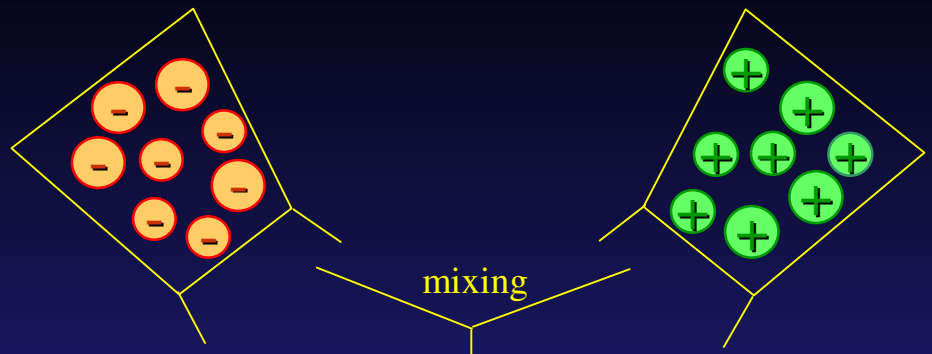


dimethylaminoethyl methacrylate

Design

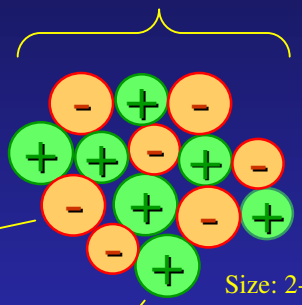


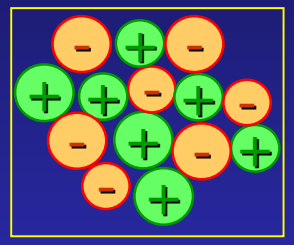
Dex-HEMA



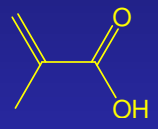
dex-HEMA-MAA microspheres

dex-HEMA-DMAEMA microspheres

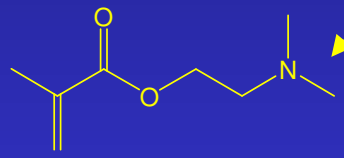




network formation

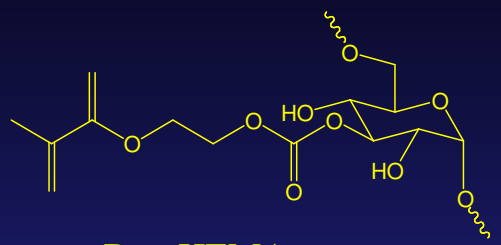


methacrylic acid

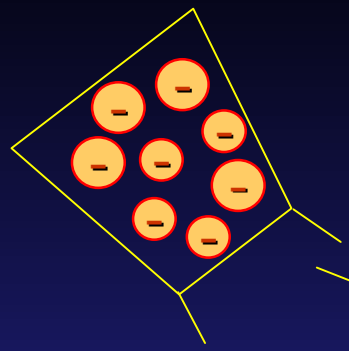


dimethylaminoethyl methacrylate

Design

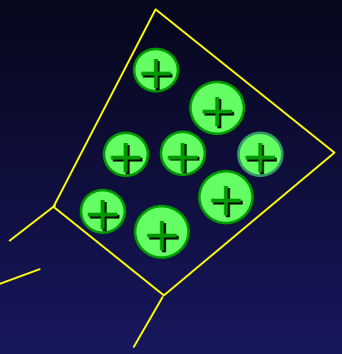


Dex-HEMA

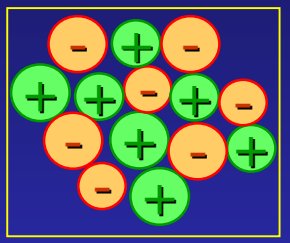


dex-HEMA-MAA microspheres

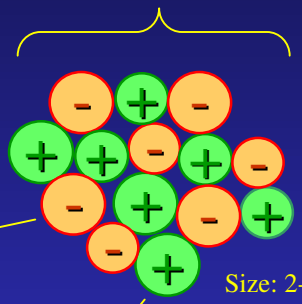
mixing



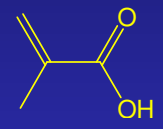
dex-HEMA-DMAEMA microspheres



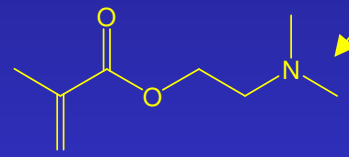
network formation



Size: 2-20 µm

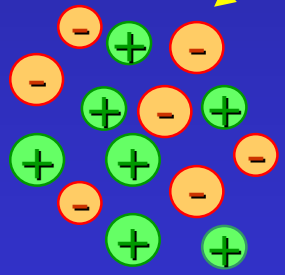


methacrylic acid

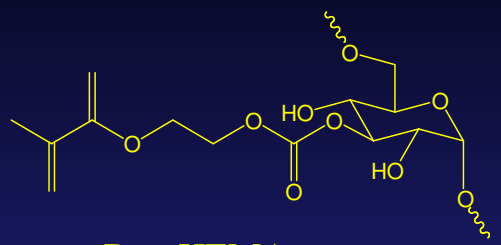


dimethylaminoethyl methacrylate

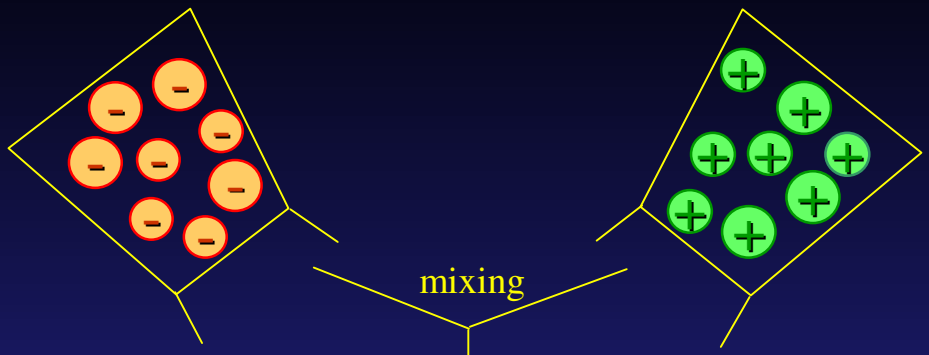
shear



Design



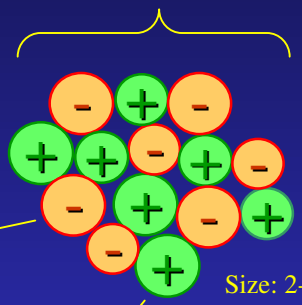
Dex-HEMA



dex-HEMA-MAA microspheres

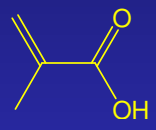
mixing

dex-HEMA-DMAEMA microspheres

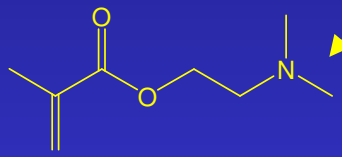


Size: 2-20 µm

network formation

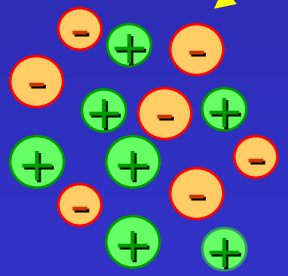


methacrylic acid

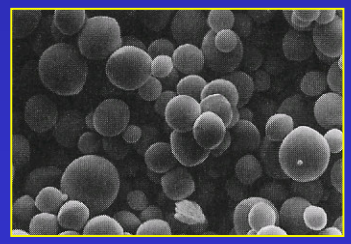


dimethylaminoethyl methacrylate

shear

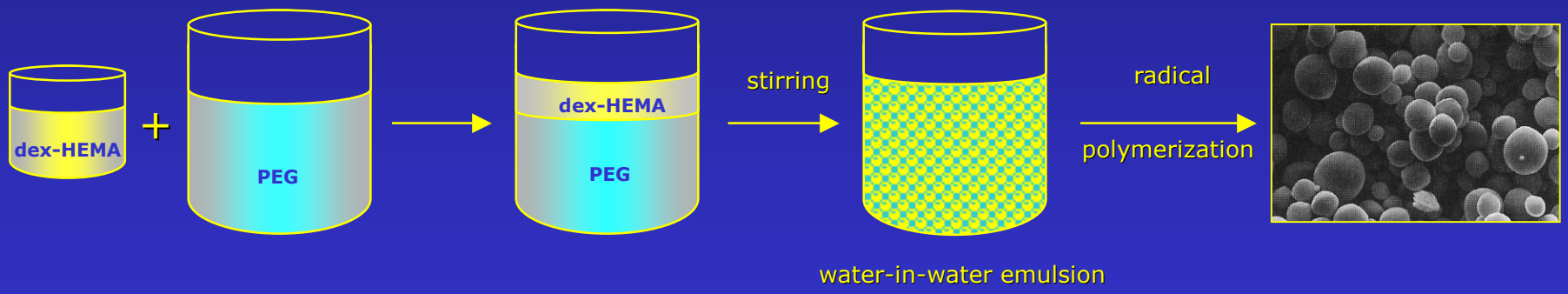


in situ gelling at site of injection



Microsphere preparation

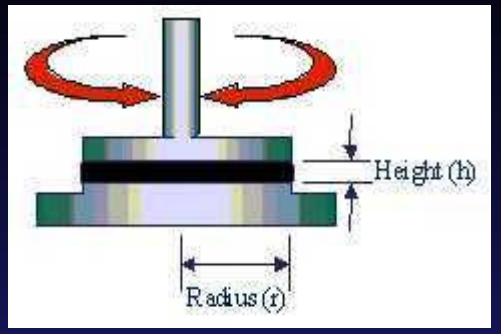
- Radical polymerization of dexHEMA, emulsified in an aqueous PEG solution
- initiation by KPS (=potassium peroxodisulphate) and TEMED (=tetramethyl ethylene diamine)



Network formation

Rheology

'Study of flow and deformation'



Geometry: 20mm flat plate
Gap: 500 μm

- G'= Storage modulus (elasticity)
- G"= Loss modulus (viscosity)

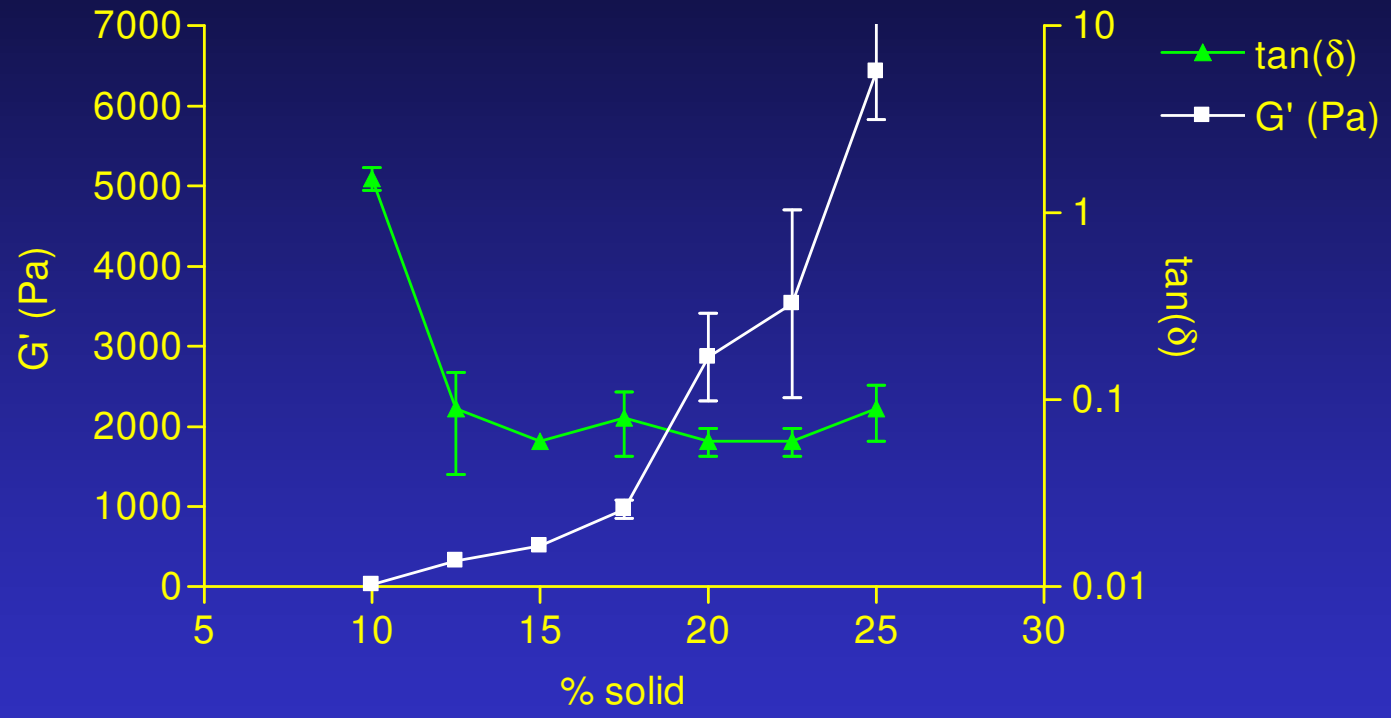
$$\tan \delta = G''/G'$$

$\tan \delta = 0$ → fully elastic

$\tan \delta = \infty$ → Newtonian

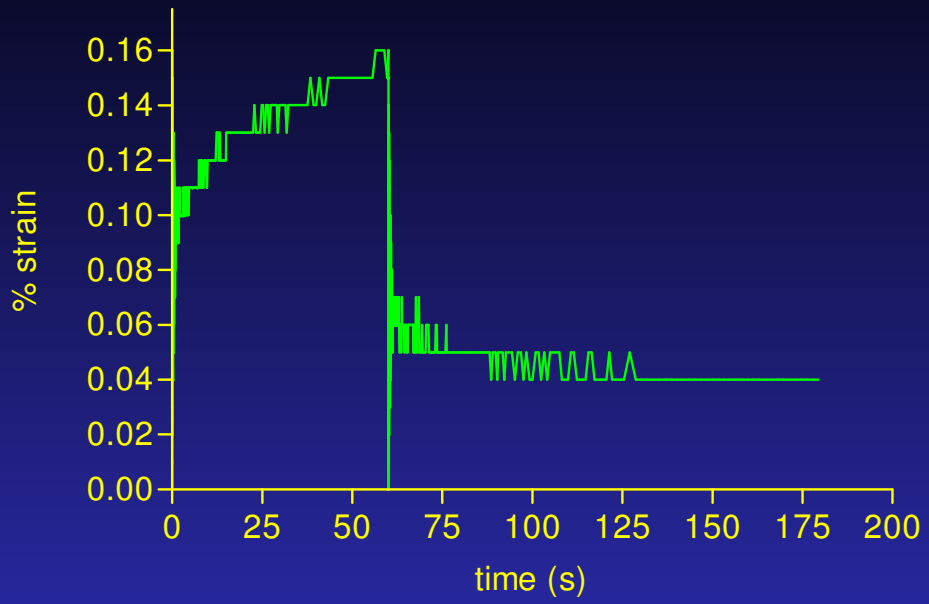
$0 < \tan \delta < 1$ → visco-elastic

Rheology: controlled strain



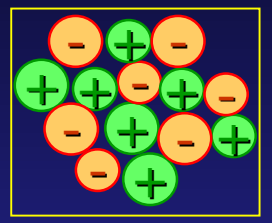
Strength (modulus) of the network can be tailored by the solid content of the hydrogel

Rheology: creep

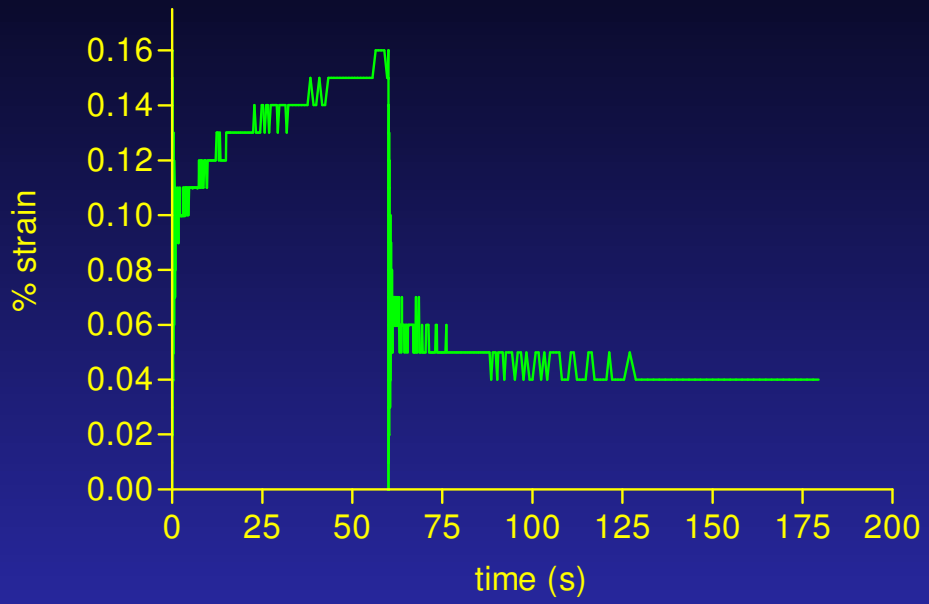


dex-HEMA-MAA/dex-HEMA-DMAEMA mixture

→ Elastic network

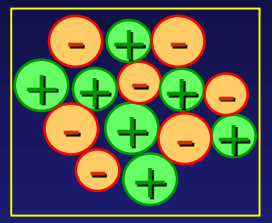


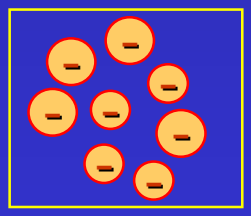
Rheology: creep



dex-HEMA-MAA/dex-HEMA-DMAEMA mixture

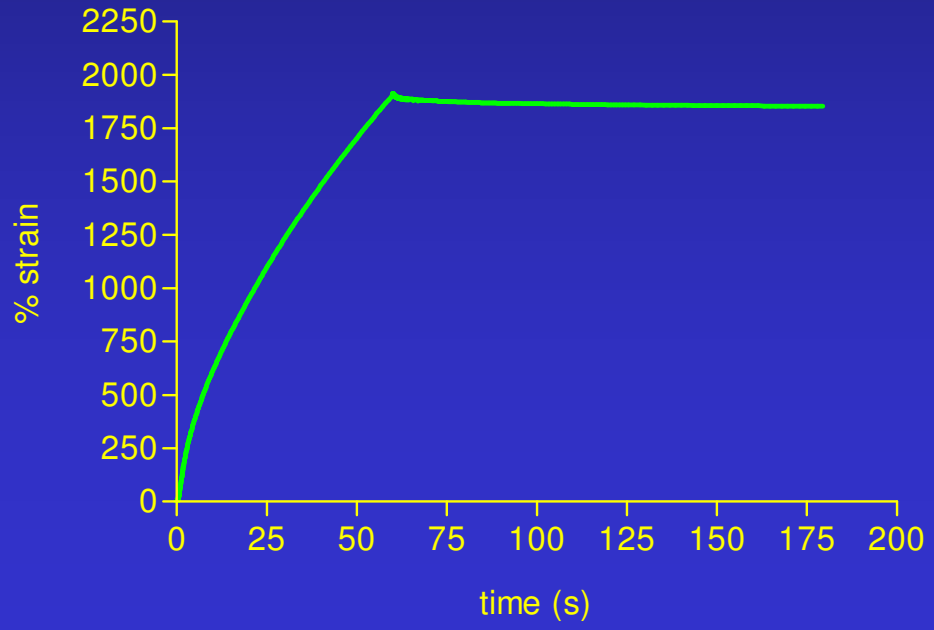
→ Elastic network





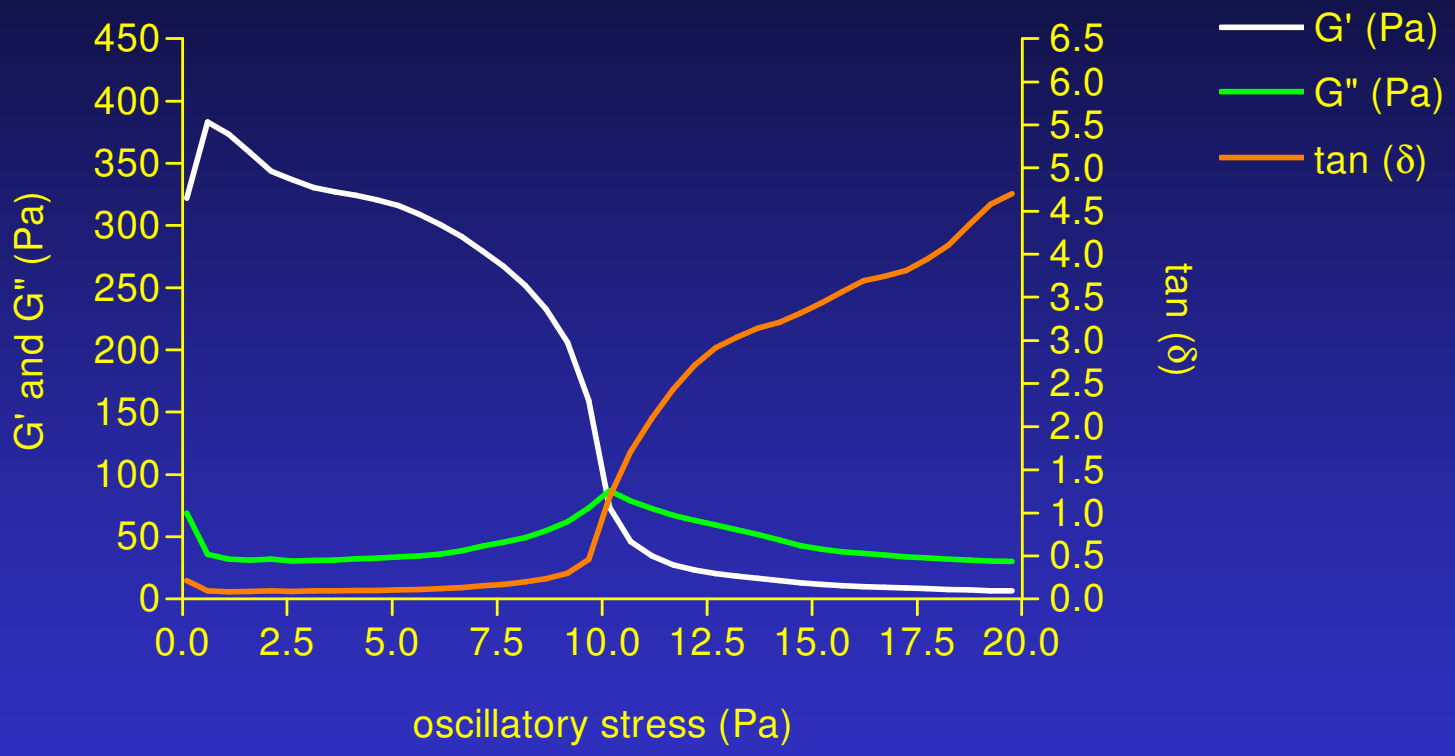
Newtonian liquid ←

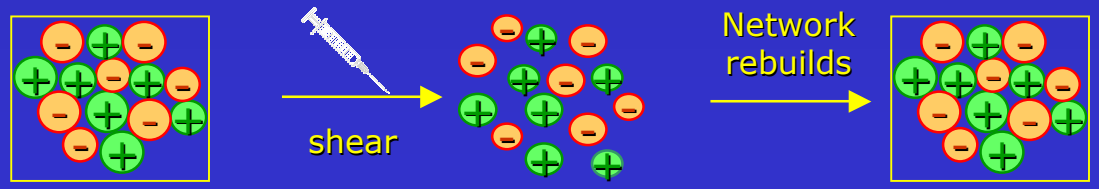
dex-HEMA-MAA dispersion



Injectability

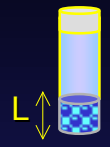
↳Reversibility of the network?

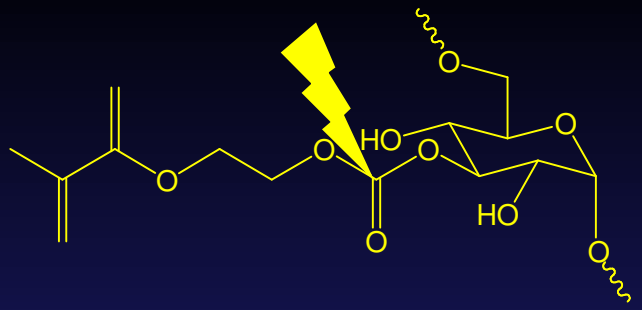


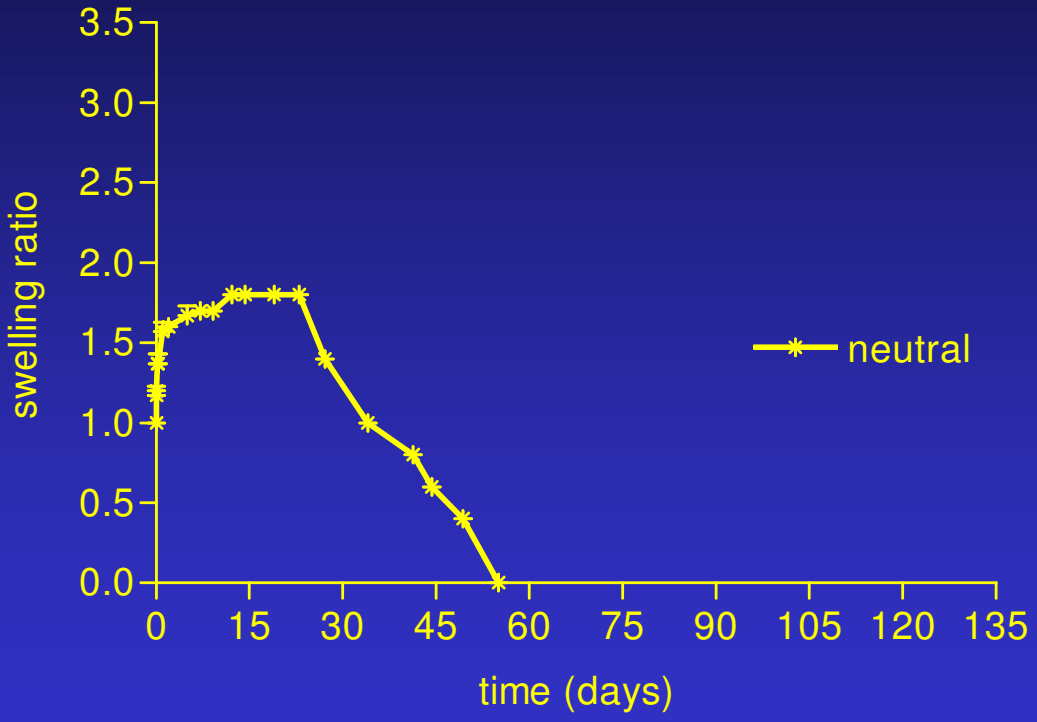


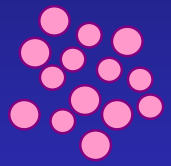
Degradation: influence of *charge*

Swelling ratio $S=L_t/L_0$



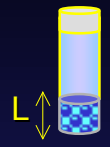


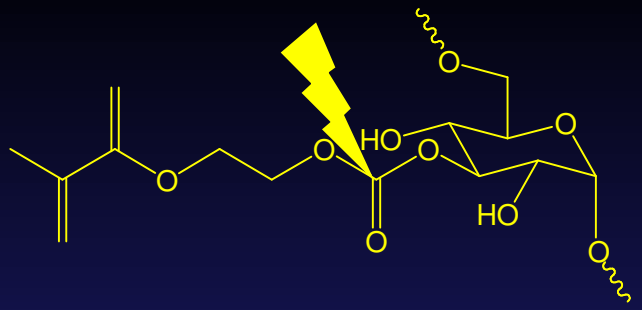


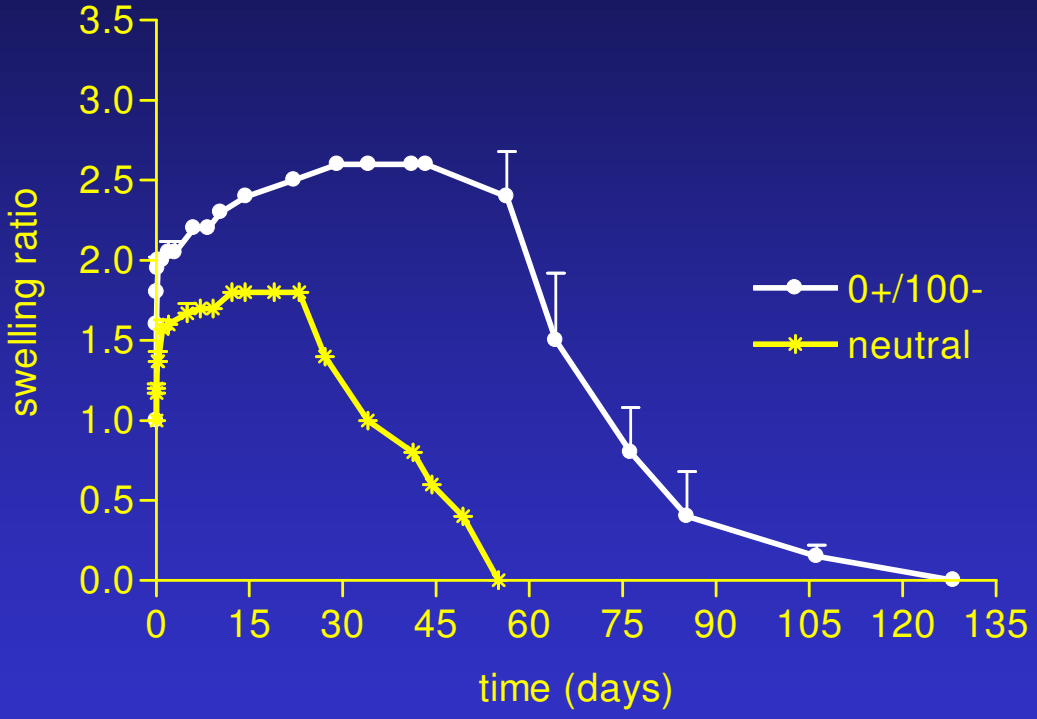


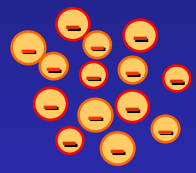
Degradation: influence of *charge*

Swelling ratio $S=L_t/L_0$



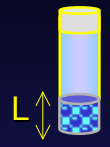


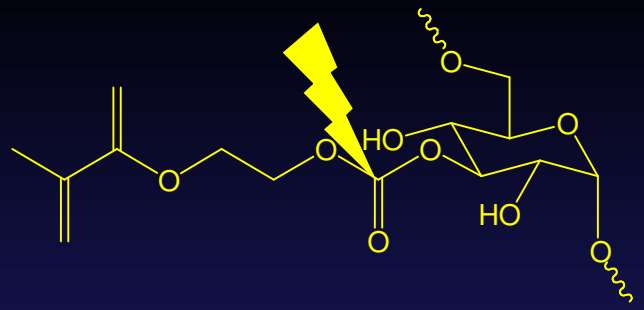


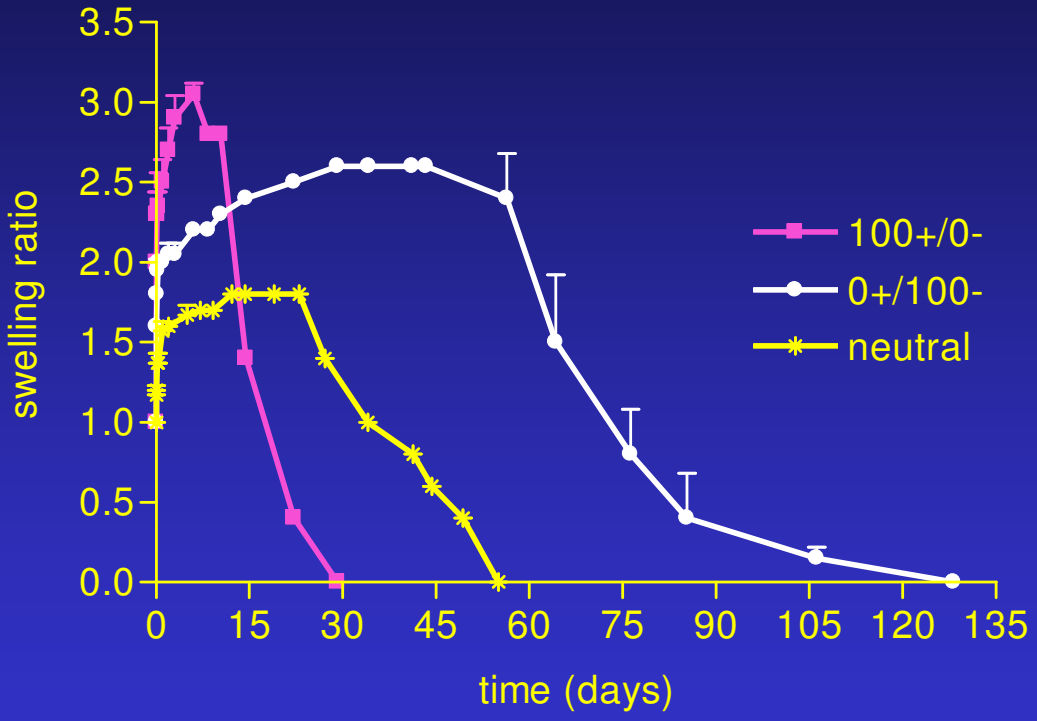


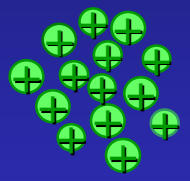
Degradation: influence of *charge*

Swelling ratio $S=L_t/L_0$



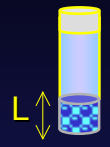


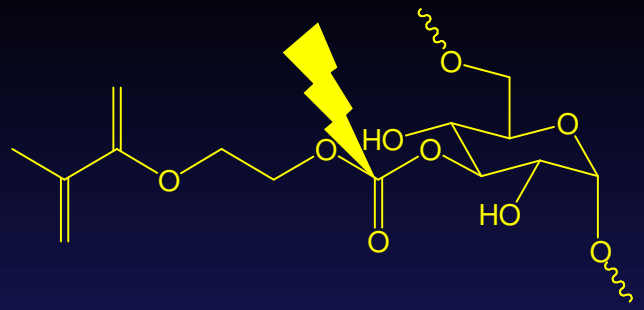


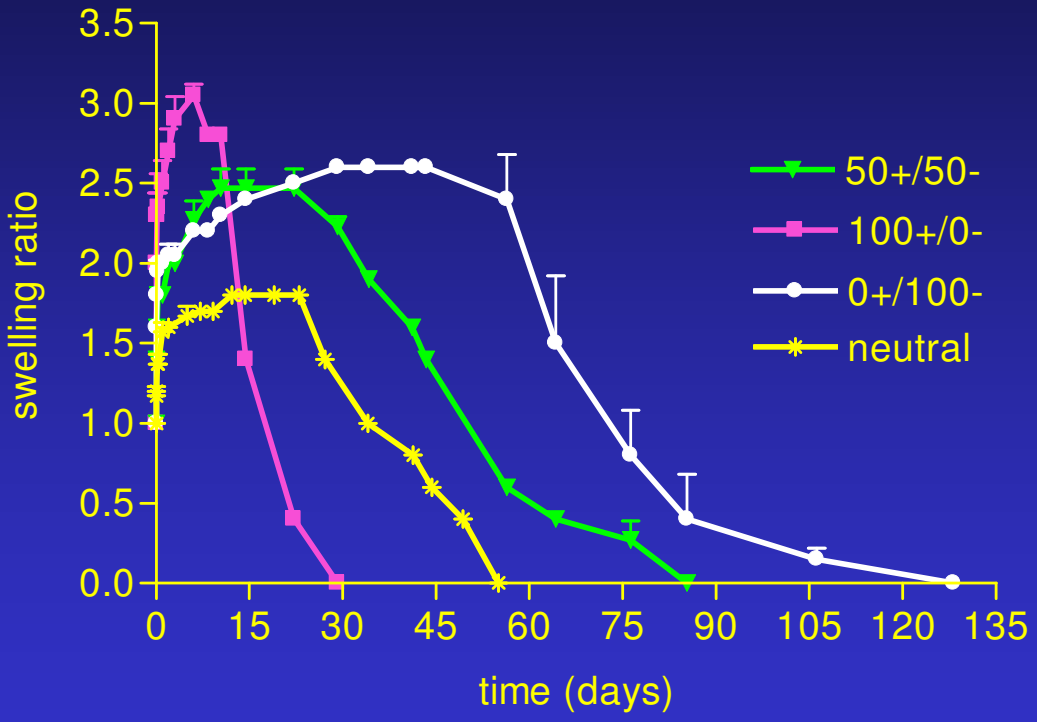


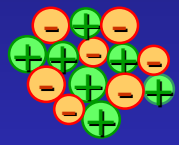
Degradation: influence of *charge*

Swelling ratio $S=L_t/L_0$



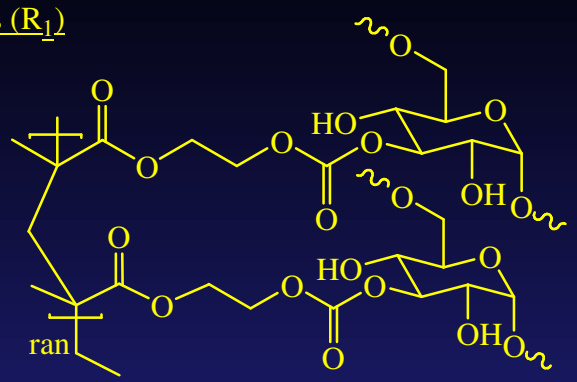






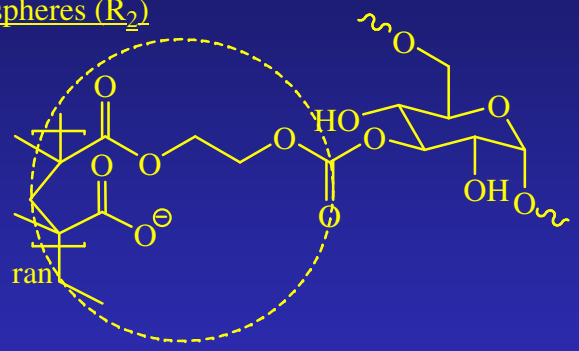
Ratio +/- ↑ ⟶ degradation time ↓

dex-HEMA microspheres (R_1)



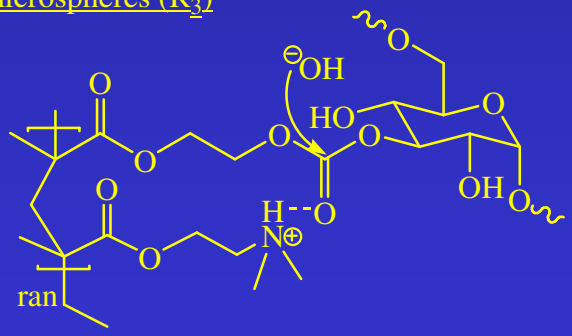
normal situation

dex-HEMA-MAA microspheres (R_2)



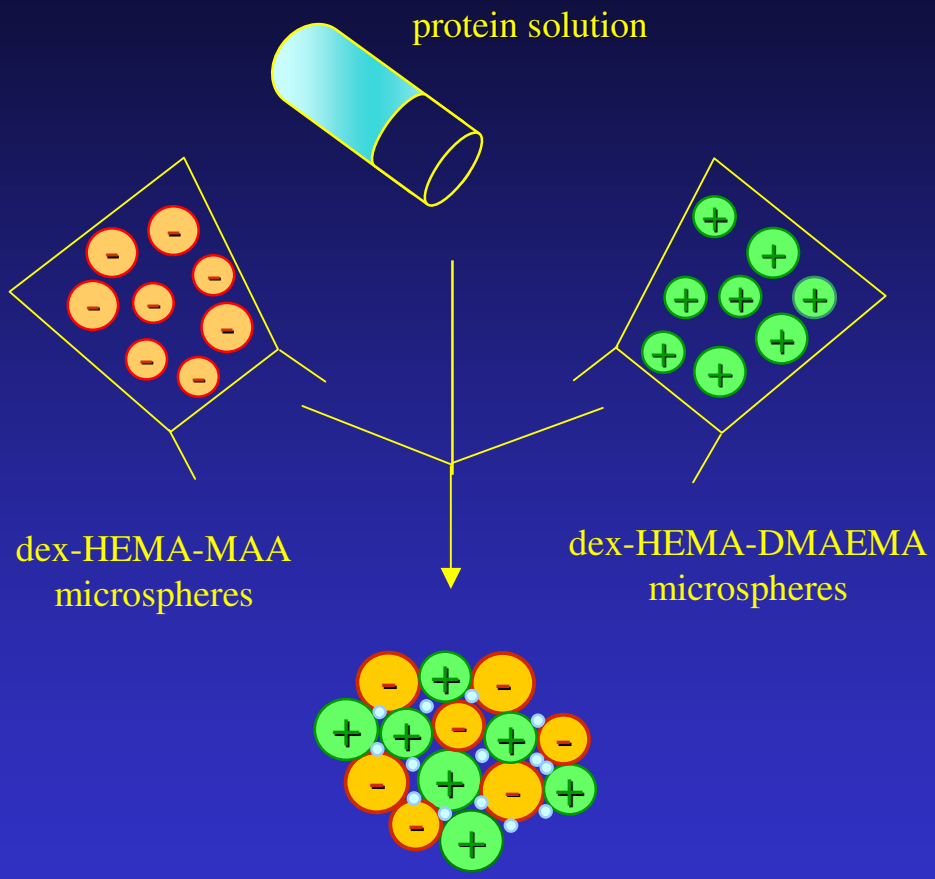
repulsion

dex-HEMA-DMAEMA microspheres (R_3)

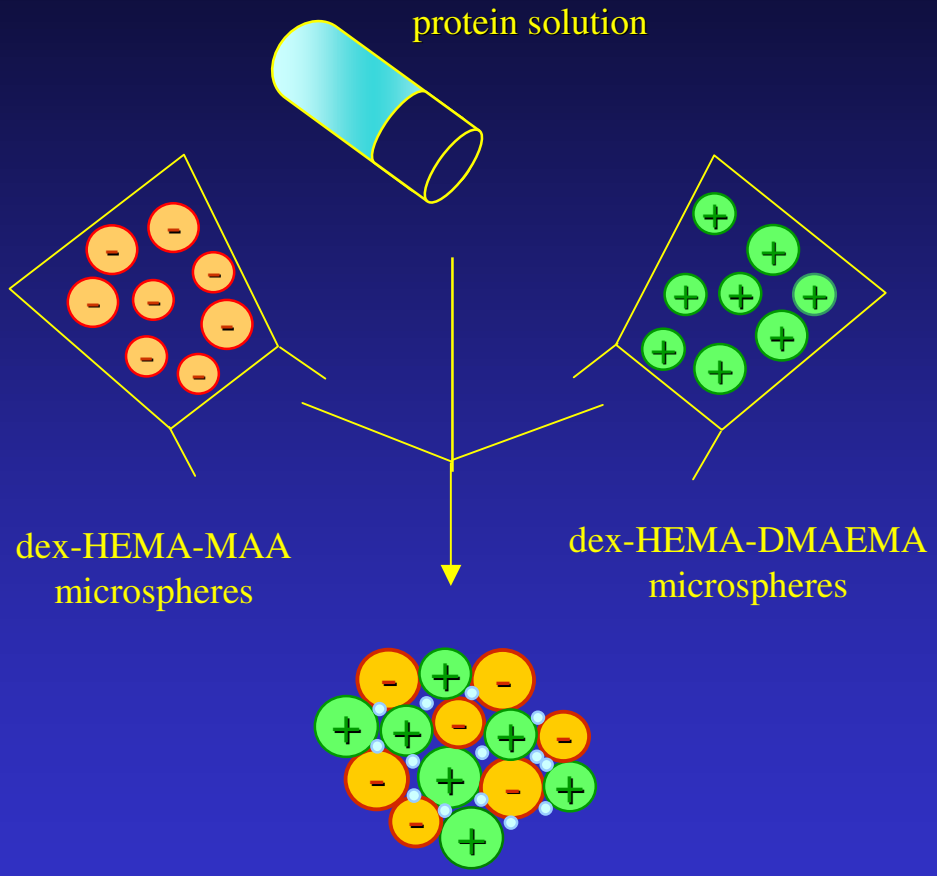


attraction
+
stabilization transition state

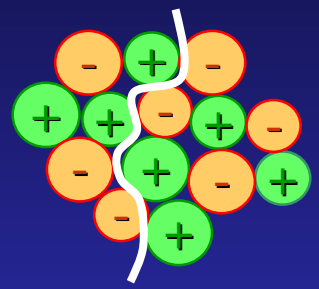
In vitro protein release



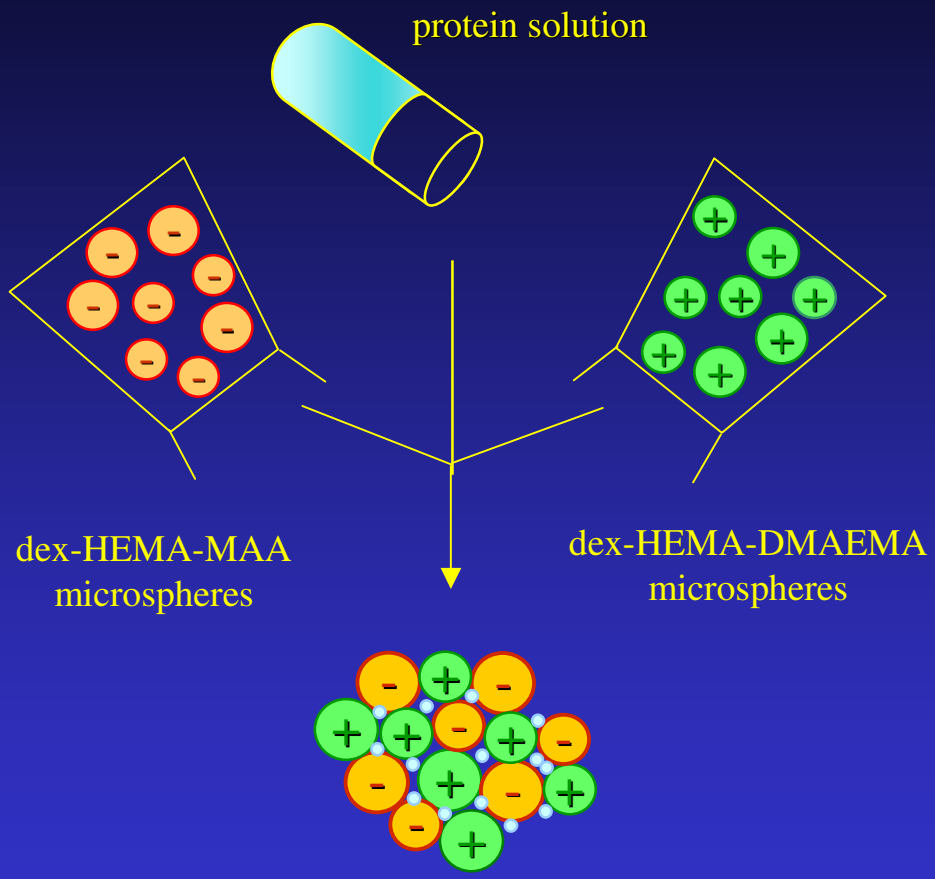
In vitro protein release



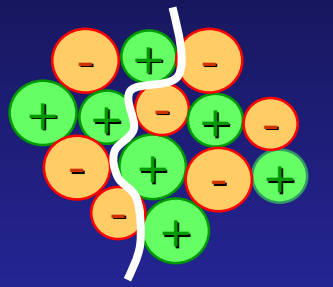
Possible release mechanisms

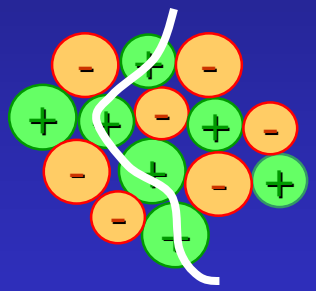


In vitro protein release

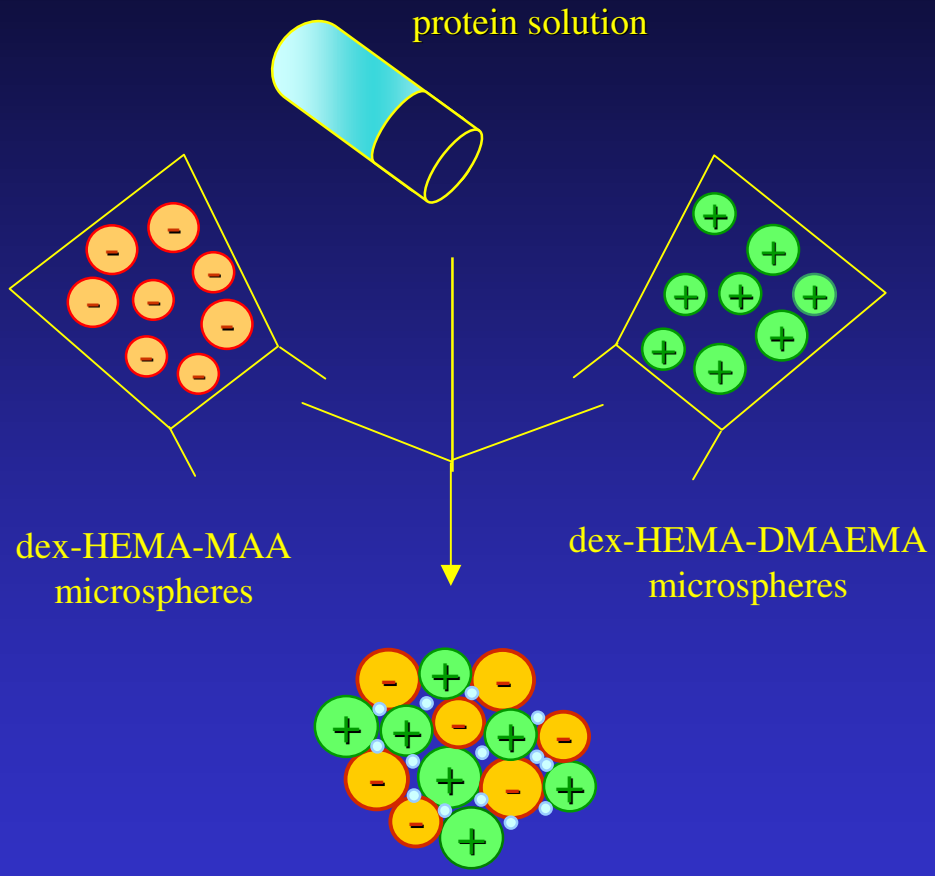


Possible release mechanisms

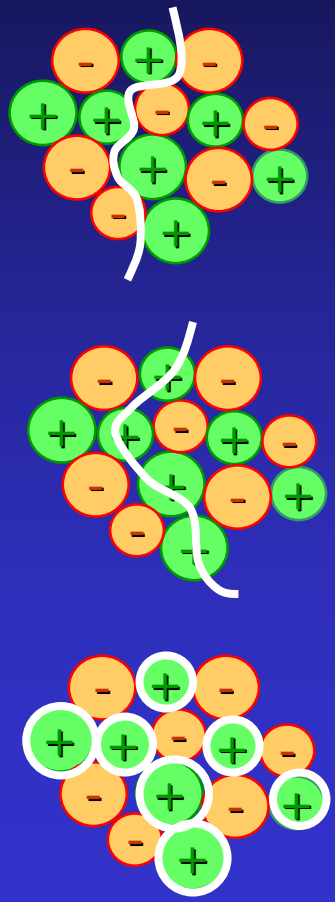




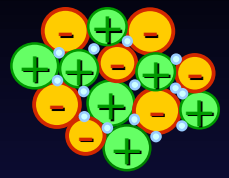
In vitro protein release



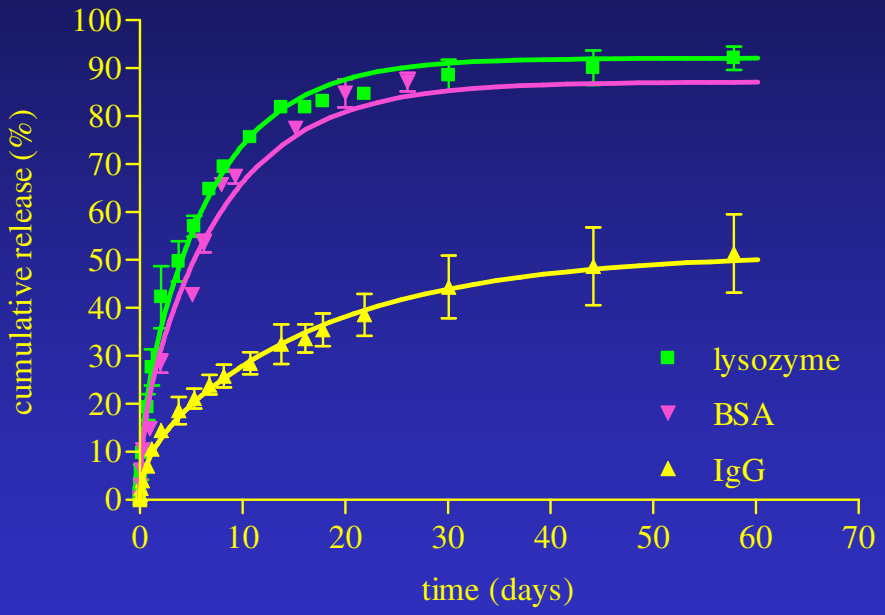
Possible release mechanisms

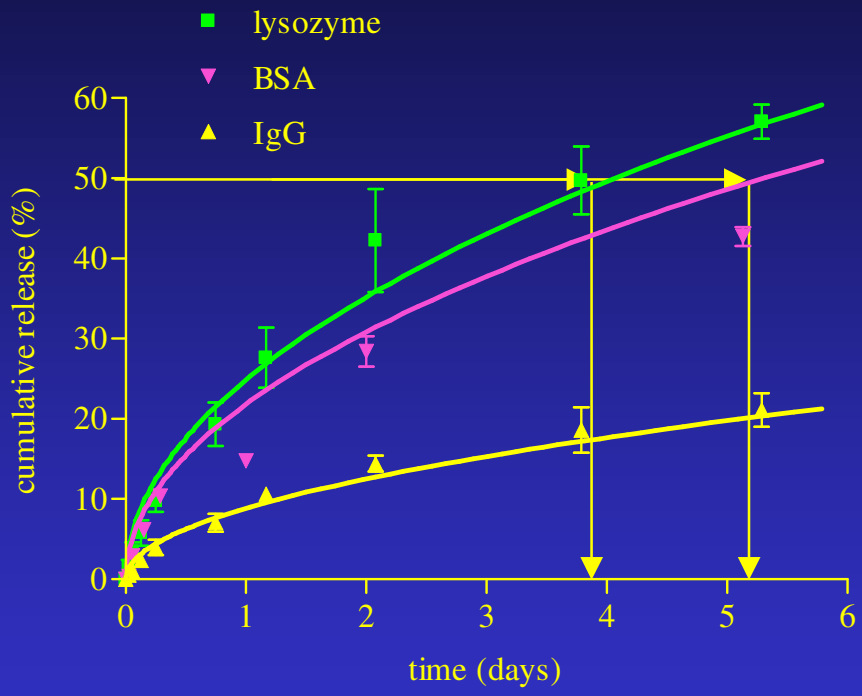


In vitro protein release



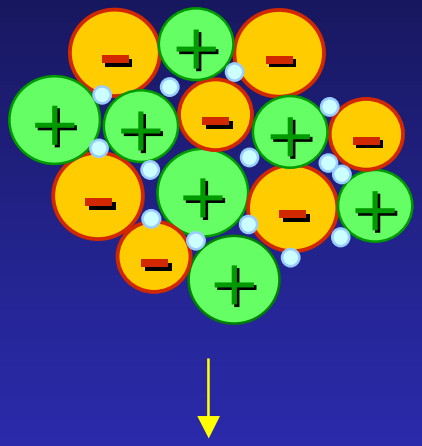
Hydrogels composed of 15% microspheres and 85% buffer





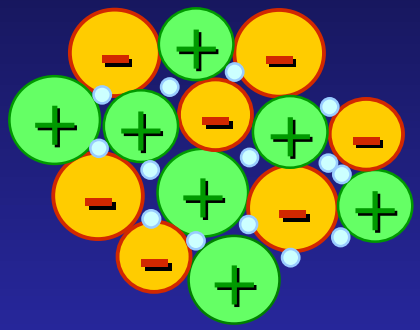
- Quantitative release of lysozyme and BSA
- Full preservation of enzymatic activity of released lysozyme

In vitro protein release

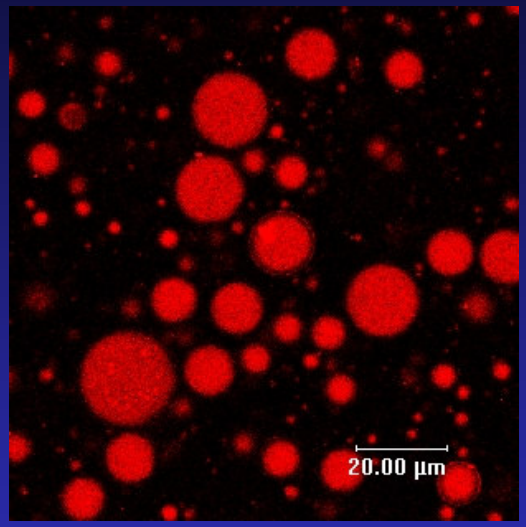


diffusion-controlled release

In vitro protein release



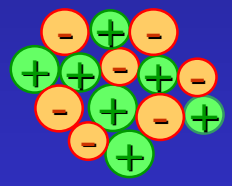
≠

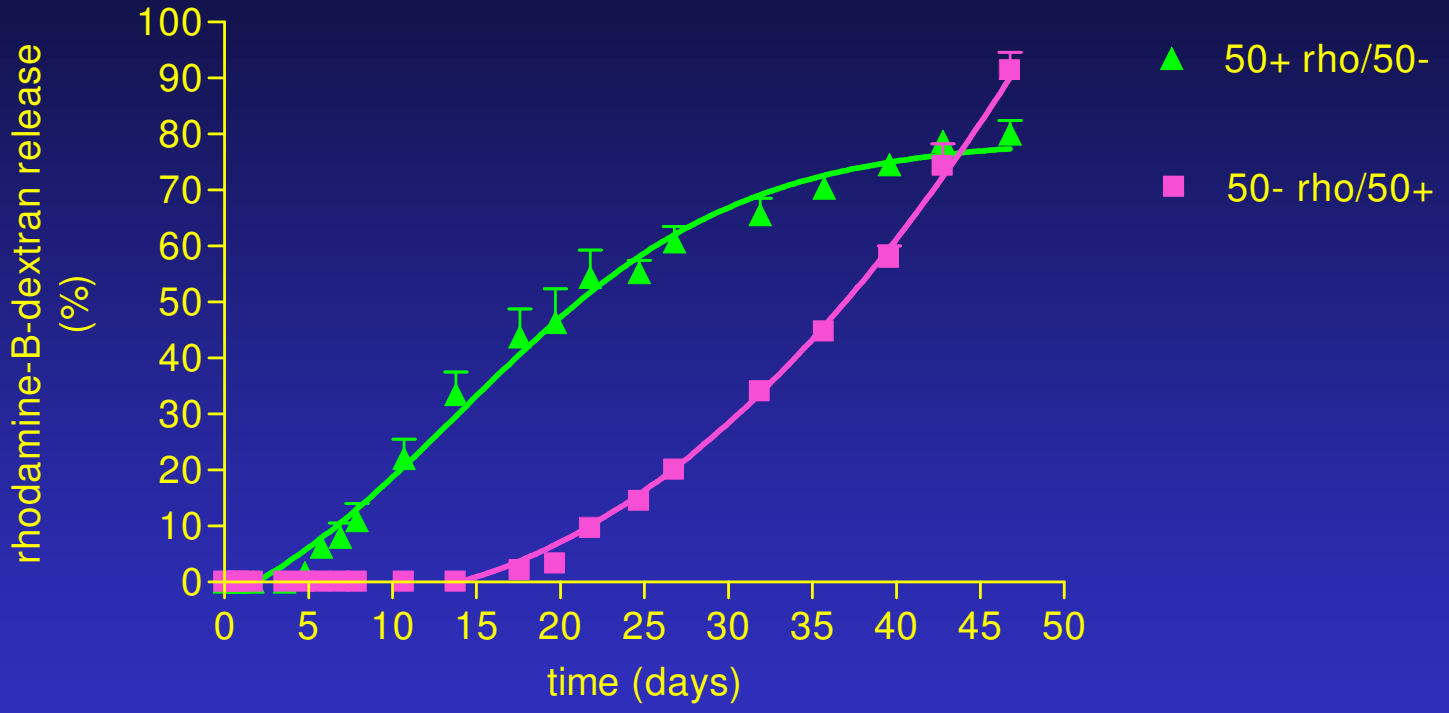


diffusion-controlled release

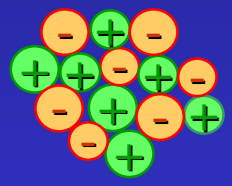
degradation-controlled release

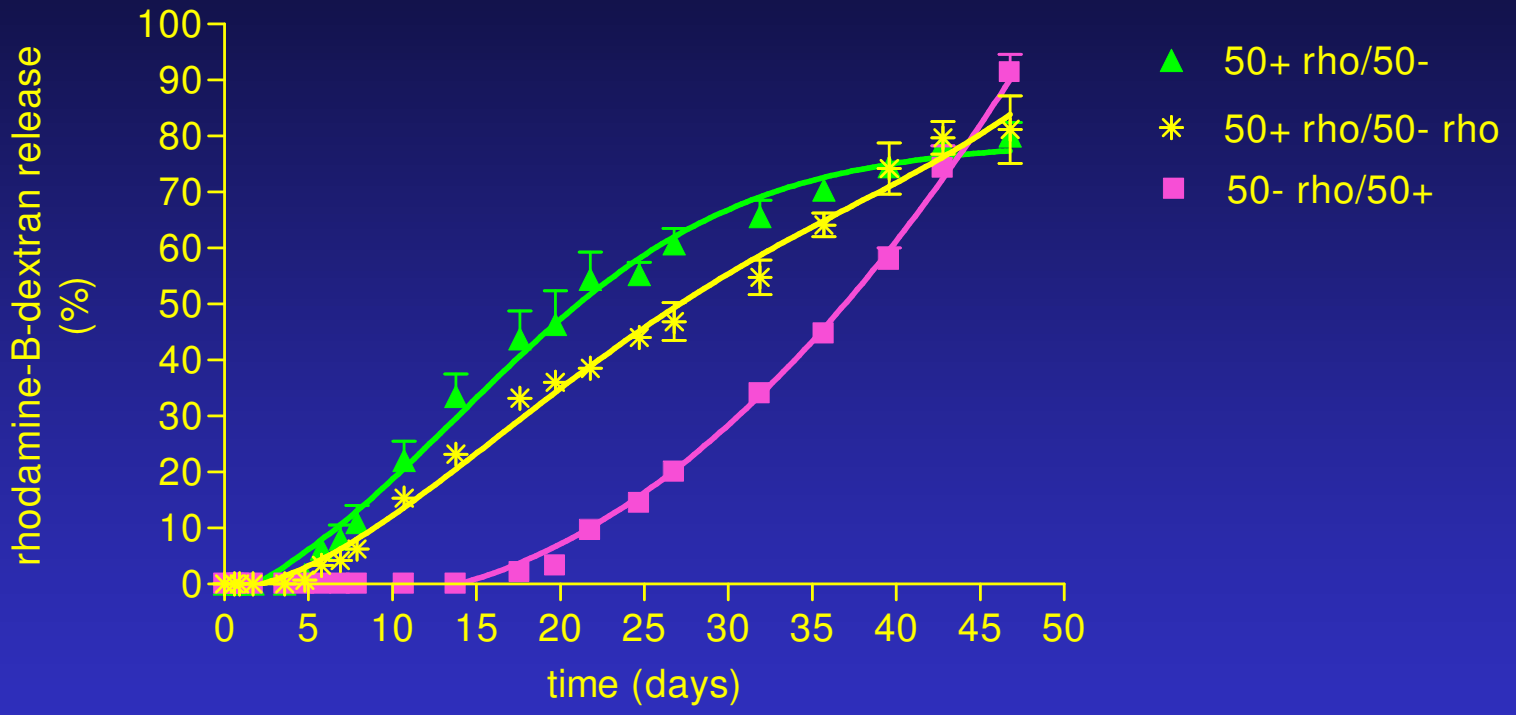
In vitro rhodamine-B-dextran release





In vitro rhodamine-B-dextran release



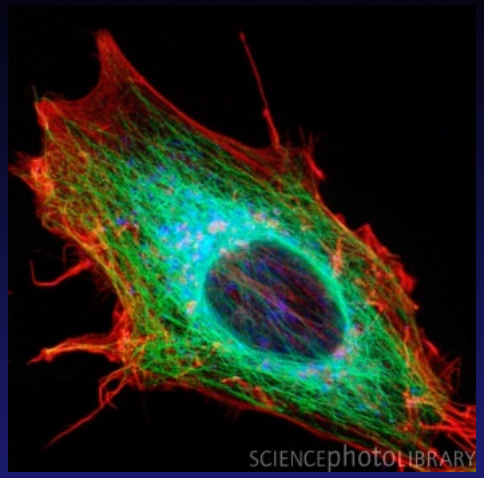


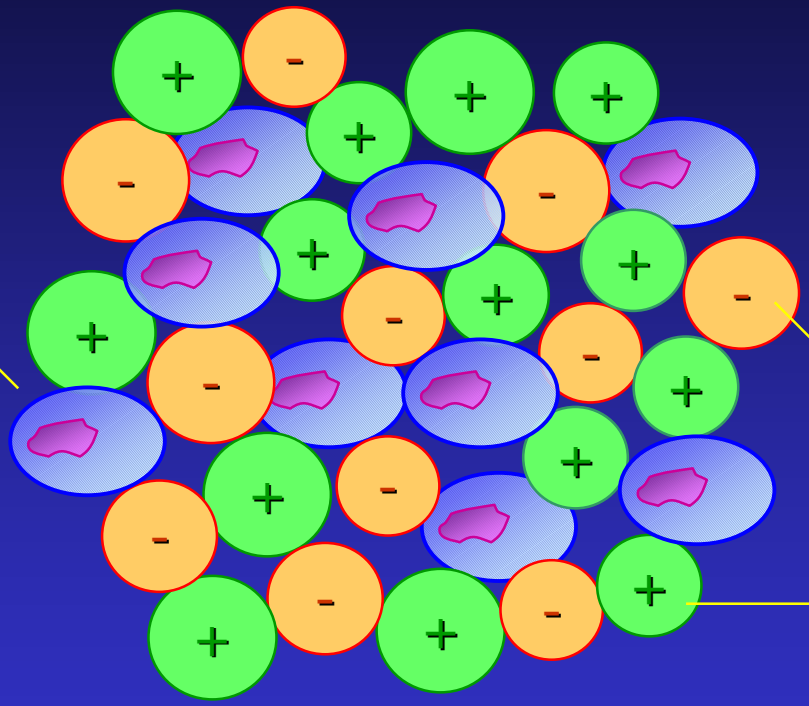
Combination of release from positive and negative microspheres gives a zero order release

Conclusions

- ✓ Mainly elastic hydrogels are formed by mixing oppositely charged dextran microspheres
- ✓ Reversible gelation occurs
- ✓ Continuous diffusion-controlled release of proteins
- ✓ Degradation behavior can be tailored

→ *Hydrogel properties can be tailored for various applications in drug delivery and tissue engineering*


SCIENCEphotoLIBRARY




SCIENCEphotoLIBRARY

www.fip.org/pswc

Abstract submission deadline 15th December 2006

Travel grant requests: see website

Abstract submission deadline
15th December 2006

www.fip.org/pswc