HESylation® – Innovation in Drug Delivery

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BU HESylation® Technology

25 February 2010

HESylation® –
Overview

HESylation® –
Polymer Coupling to Pharmaceutical Targets

FRESENIUS KABI

Polymer

HES (Hydroxy Ethyl Starch)

Linker

Pharmaceutical target (e.g. proteins, peptides, oligonucleotides etc.)

- ✓ **Extended circulating life**
- ✓ **Improved efficacy**
- ✓ **Lowered immunogenicity**
- ✓ **Reduced toxicity**
- ✓ **Improved stability**
- ✓ **Improved solubility**

HESylation® – General Characteristics of HES

HESylation®

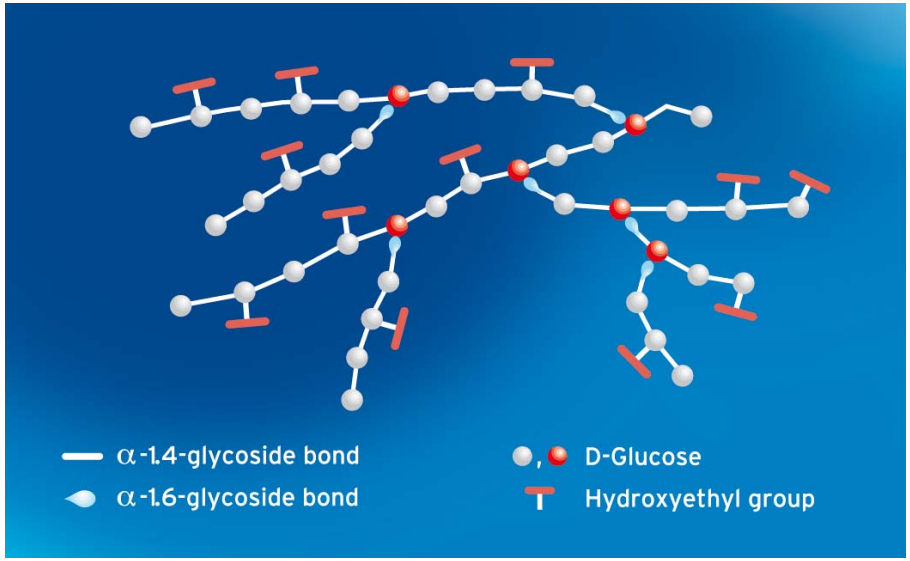
General Characteristics of HES

Modified natural polymer
manufactured from waxy maize starch (Amylopectin)

Hydroxyethylation increases water binding capacity

Branched polymer,
one reducing end group

Cost effective manufacturing
process in large quantities established

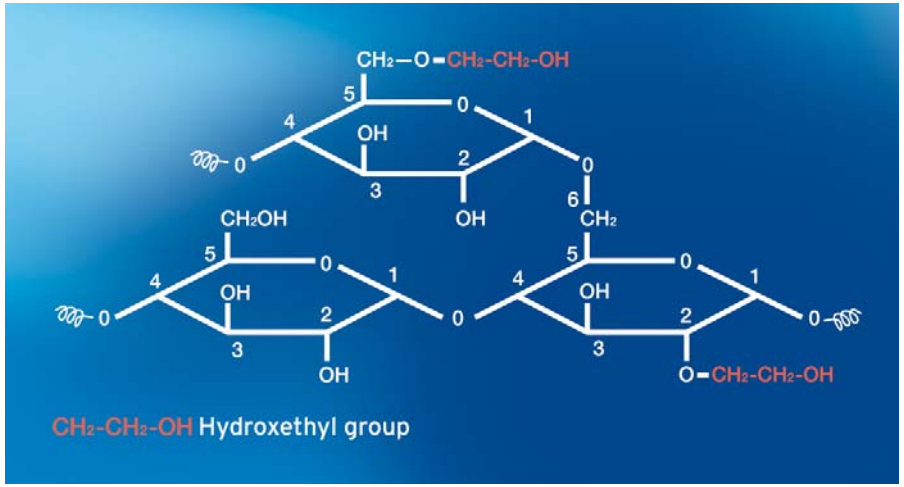


HESylation®

General Characteristics of HES

HES is characterized by:

- **Molecular weight (M_w, M_n)**
  - ➔ Polydispersity (M_w/M_n)
- **Molar substitution (MS),**
  i.e. mean number of hydroxyethyl groups per glucose unit



Fine tuning allows tailor made properties

HESylation®

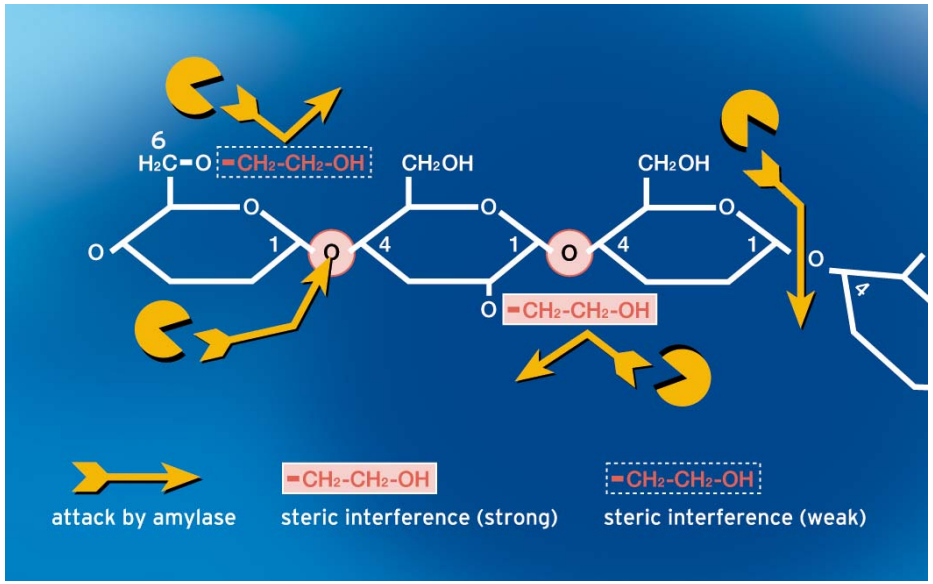
In Vivo Degradation Mechanism

Cleavage of alpha-1,4 glycosidic bonds by plasma α-amylase

Degradation depends on:

1. Molecular weight
   (high: slower clearence)

2. Degree of substitution
   (high: slower degradation)



HESylation® – HES Safety

HESylation®

HES – The Safe Alternative to Blood and Albumin

Therapeutic indications:

Volume deficiency and shock

- Surgery (haemorrhagic shock)
- Injuries (traumatic shock)
- Infections (septic shock)
- Burns (burn shock)

Haemodilution

- Saving of donor blood during surgery
- Therapeutic dilution of blood

VOLUVEN®
Class of its own

Further applications:

Cold storage solution (organ transplants)

HESylation®

HAES-steril®
The classic HES

HyperHAES®
Small-Volume-Resuscitation

HESylation®

HES Safety – Preclinical Toxicology

DEPARTMENT OF HEALTH & HUMAN SERVICES

PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
ROCKVILLE, MD 20852

NDA REVIEW MEMO (MID-CYCLE)

VOLUVEN®
Class of its own

13 weeks repeated dose study in dogs:

- Daily administration of 9 g/kg body weight (10% Voluven solution)
- Cumulative administered mass ≈7,5 kg/dog
- Corresponds to 6,300 ml HES solution daily for 13 weeks in a 70 kg adult

➔ Dog trials showed signs of increased workload, without specific mortality, toxicity, or organ damage.

➔ The observed changes were recovered partially or completely at the end of 4-week follow-up period.

NOEL is higher than 9g HES/kg/day (90 mL/kg/day).

HESylation® –

HES Safety – Approved Pharmaceutical Product

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VOLUVEN®
Class of its own

- **HES is an FDA approved drug**
- **High maximum daily dosage**
  - 50 ml/kg body weight/day, i.e. 210 g HES/day

    {repetitive (16 days), i.e. > 3.3 kg HES, i.v.}

HyperHAES®
Small-Volume-Resuscitation

- **Approved for use in children & infants < 24 months**
- **Low antigenicity**
  - No evidence for HES-reactive antibody formation

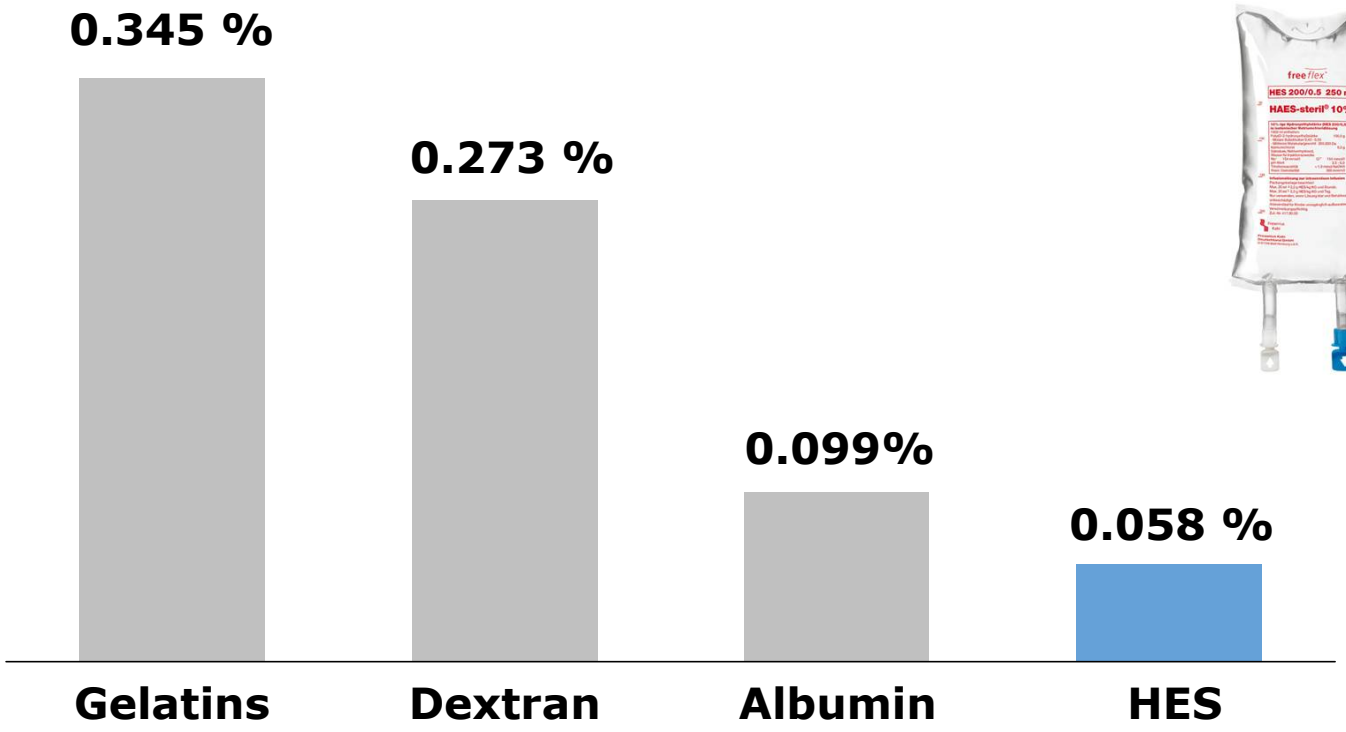
    (prospective study in >1000 patients)

HAES-steril®
The classic HES

HESylation® –

HES Safety – No/Low Immunogenicity

Allergic reactions after colloid application



Prospective multi-center trial (~ 20,000 patients)

Laxenaire et al. (1994)

HESylation®

From indigestible Polyethers…

…to biodegradable Polysaccharides…

HESylation® Technology –

Polyethylenglycol (PEG) Safety Margin

FRESENIUS KABI

PEG is generally accepted as safe …

- Peg is permitted as food additive (EU Directive No. 95/2/EC)
- Estimated acceptable daily intake (orally):≤ 10 mg/kg body weight (WHO)*
- **Therapeutic index** of PEGylated biologics (PEG-Intron or Pegasys) **> 600****

… but chronic administration of PEGylated drugs open up a new dimension !!!

- Administration of PEG in higher amounts over a longer time period
  - Dosage Pegasys® (180 µg/week ≈ 0,5 mg peg/month)
  - Dosage Mircera® (360 µg/month ≈ 0,2 mg peg/month)
  - Dosage Cimzia® (AB fragment) (400 **mg**/month ≈ 175 mg peg/month)

    ≈600 fold increase !!! Limited experience with higher cumulative amounts

➔ HES is the preferred polymer for high dosage or chronically administration

* World Health Organ Tech. Rep. Ser. No. 648

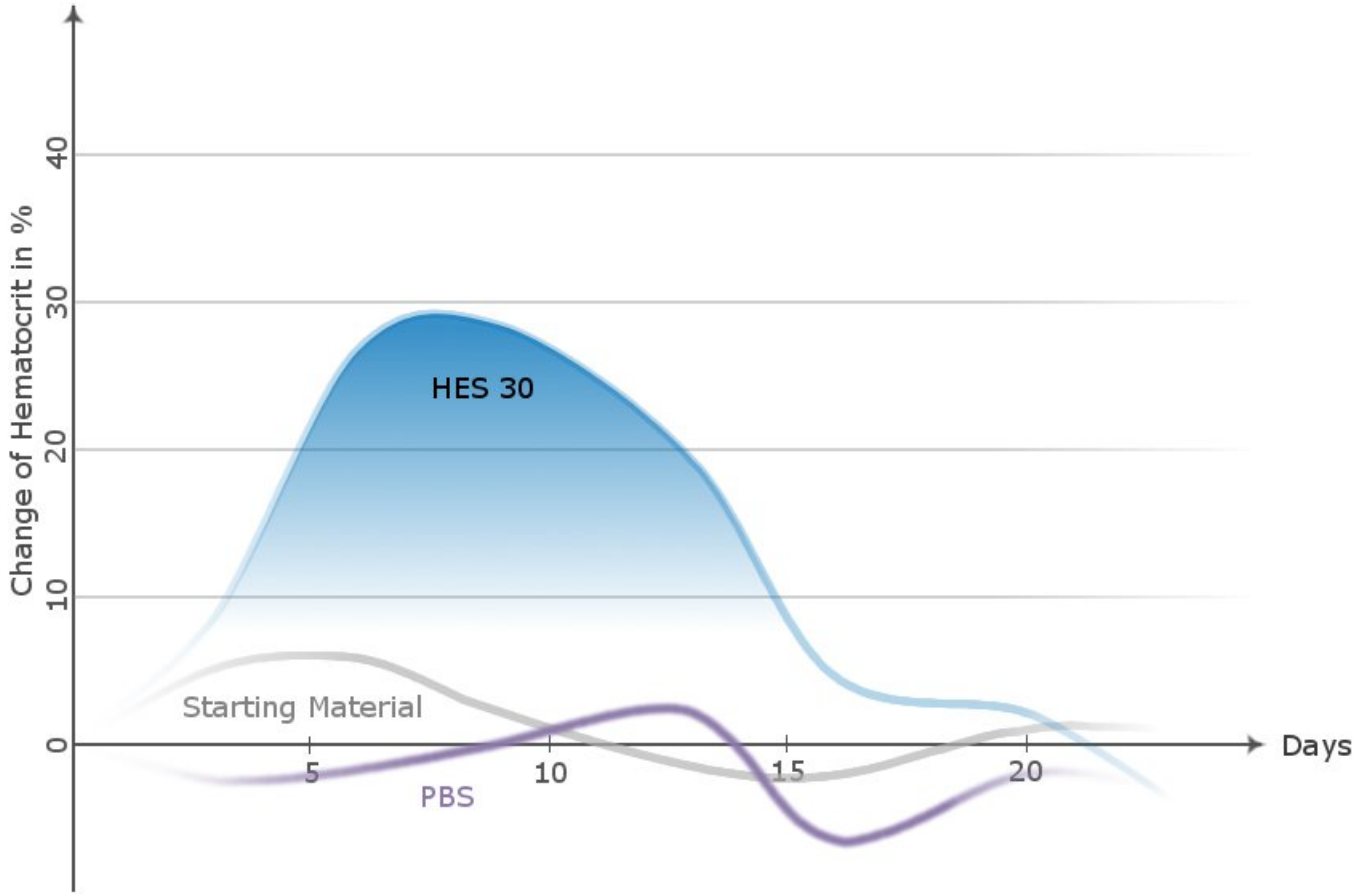
** Webster et al. Drug Metab Dispos. 2007 Jan;35(1):9-16.

HESylation® – Results

HESylation® –

Modulation of PD Profile with HES (I)

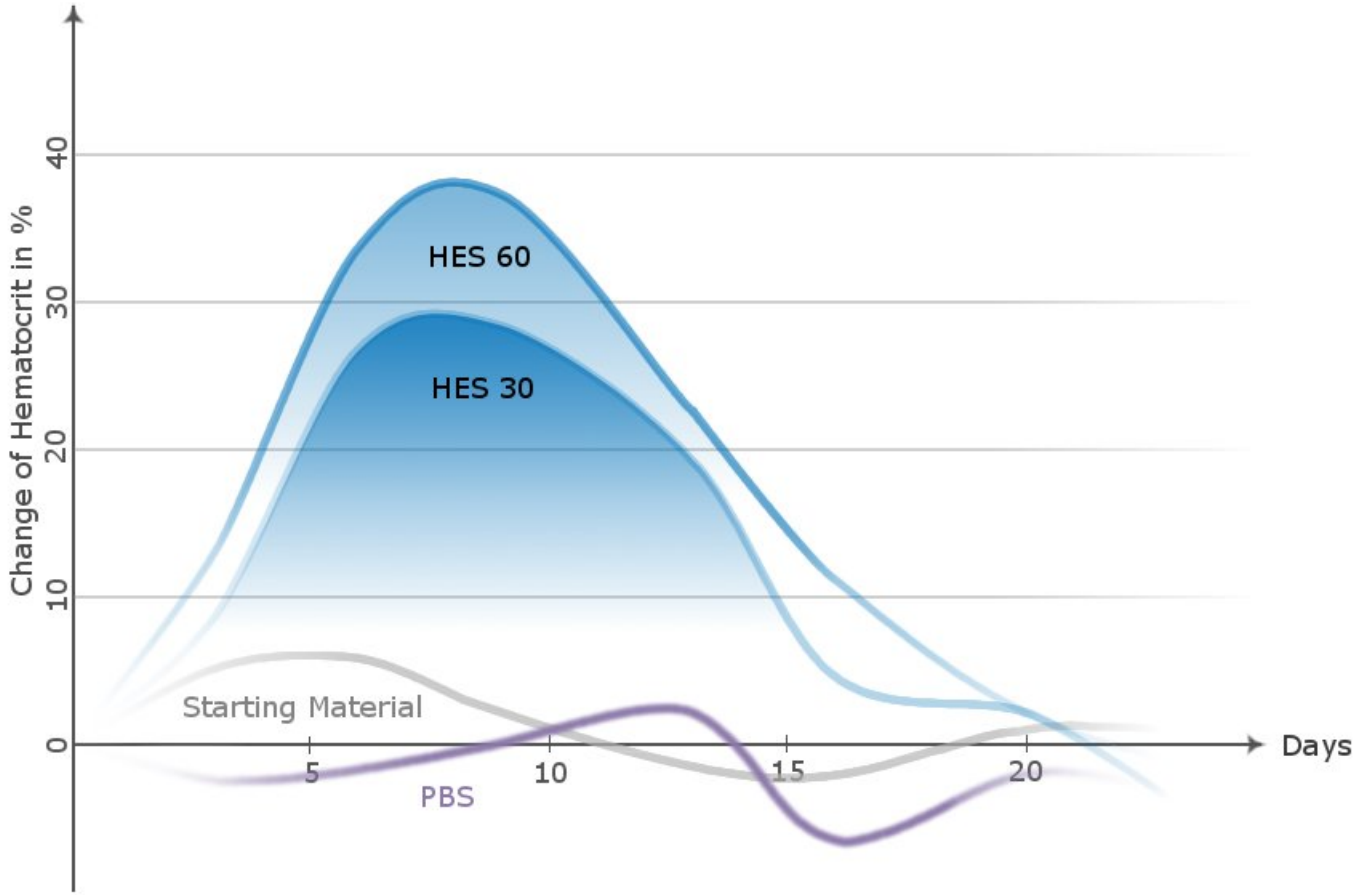
EPO PD Study Results in Mice



HESylation® –

Modulation of PD Profile with HES (II)

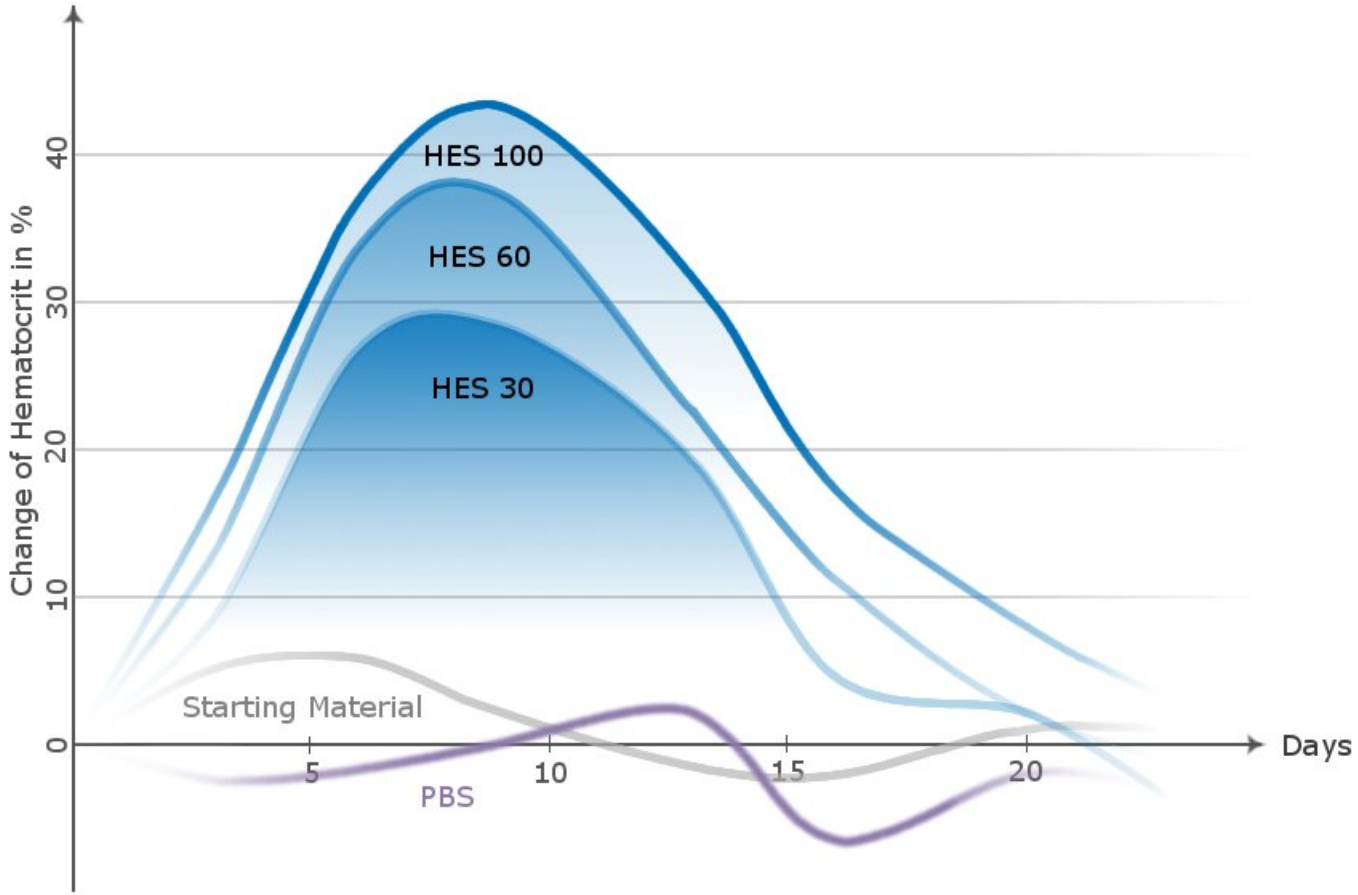
EPO PD Study Results in Mice



HESylation® –

Modulation of PD Profile with HES (III)

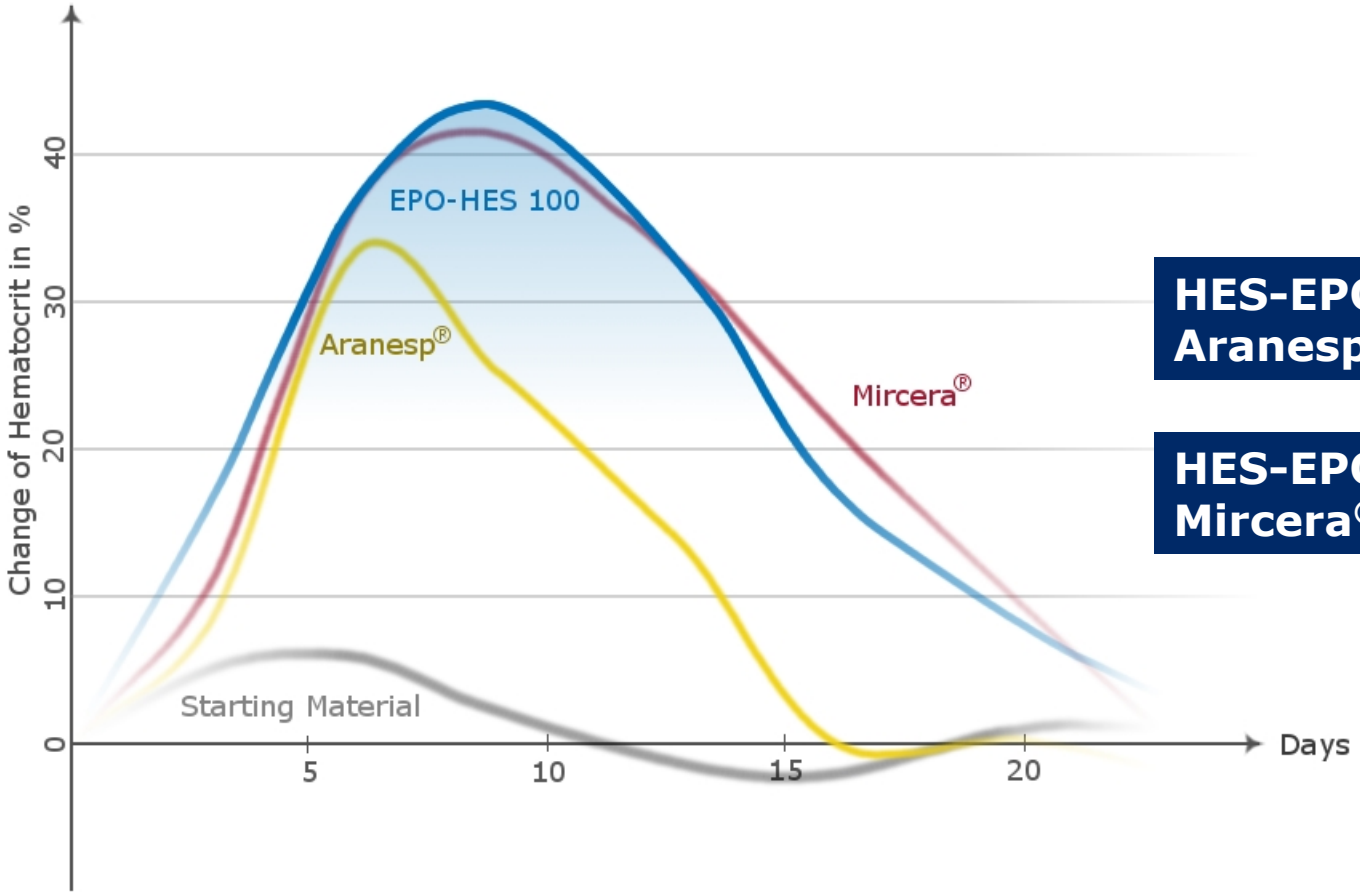
EPO PD Study Results in Mice



HESylation® –

Comparison HES & Marketed Products (I)

EPO PD Study Results in Mice



HES-EPO 100 superior to Aranesp® !

HES-EPO 100 similar to Mircera® !

HESylation® –

Comparison HES & Marketed Products (II)

EPO in vivo Study in Dogs – Results PK

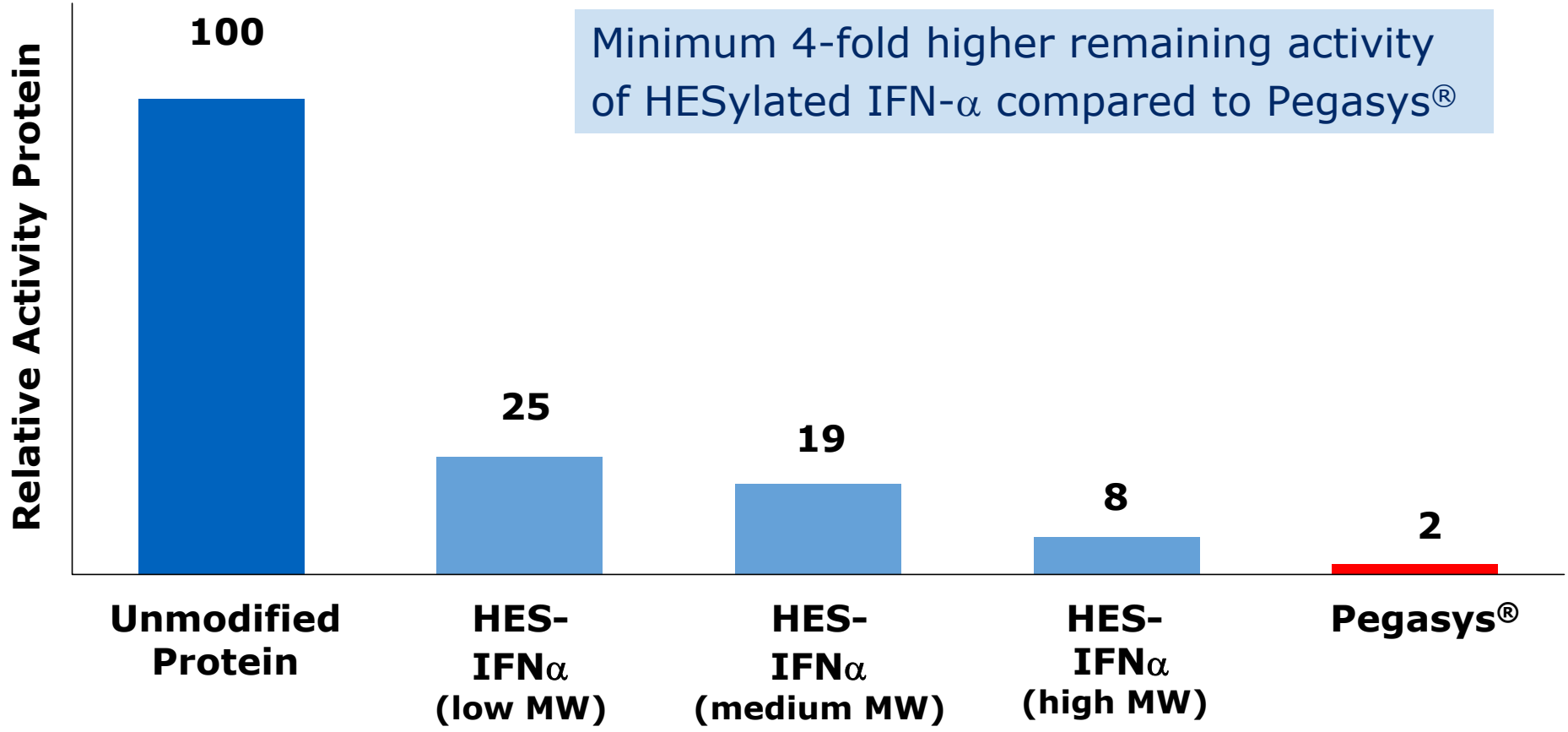
Treatment	AUC	$t_{1/2}$ [h]
HES-EPO (low MW/low MS)	**5,846**	**46**
HES-EPO (low MW/high MS)	**8,722**	**57**
HES-EPO (high MW/low MS)	**10,095**	**61**
Aranesp®	**5,323**	**22**

HES-EPO (high MW/low MS) shows a 3 times longer half-life compared to Aranesp®

HESylation® –

IFNα – In vitro CPE Assay

Remaining activity of HESylated IFNα compared to unmodified IFNα & Pegasys®

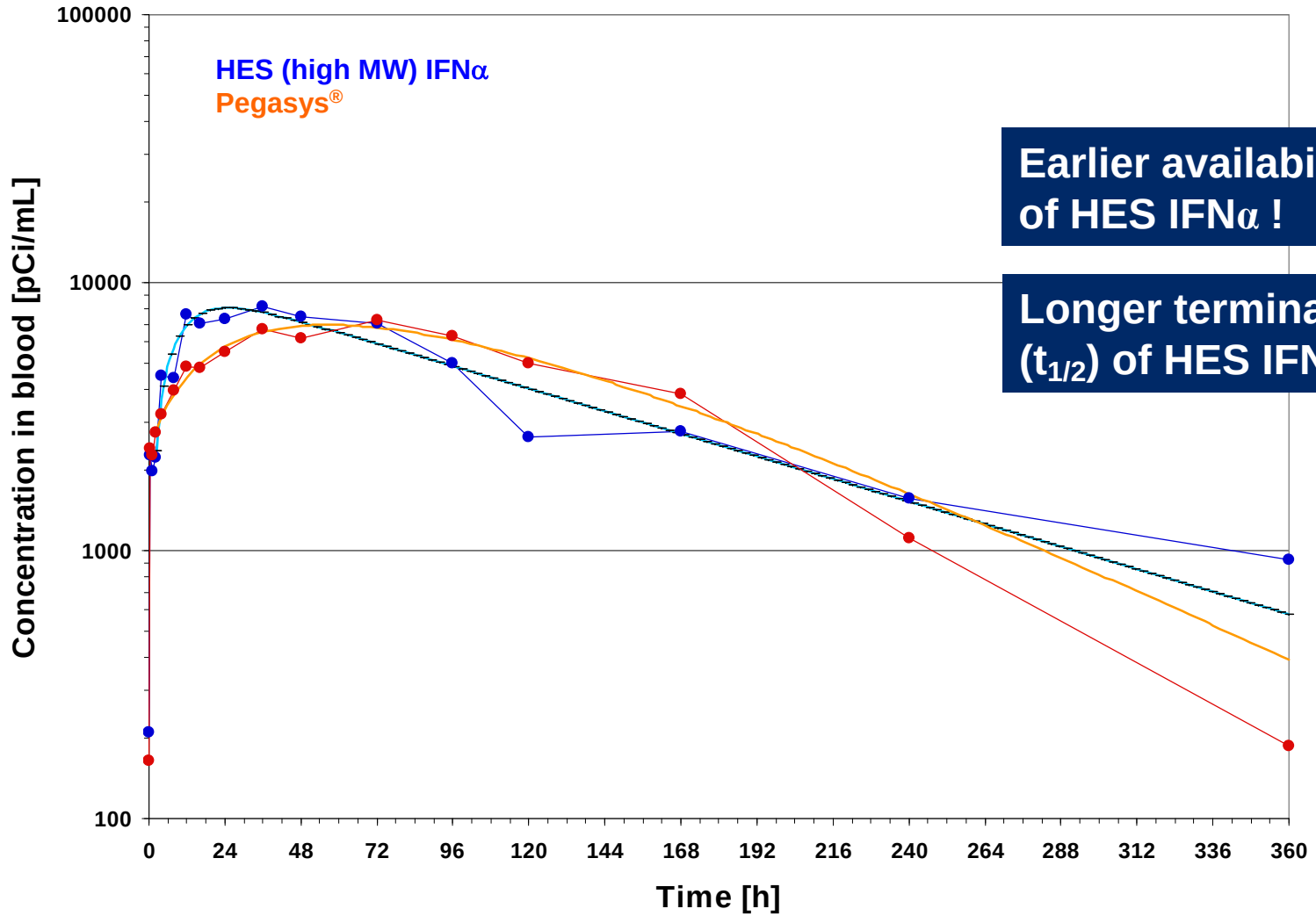


Minimum 4-fold higher remaining activity of HESylated IFN-α compared to Pegasys®

HESylation®

Comparison HES & Marketed Products (III)

IFNα in vivo Study in Rabbits – Results PK



Earlier availability (t_{max}) of HES IFNα !

Longer terminal half life ($t_{1/2}$) of HES IFNα !

HESylation®

Comparison HES & Marketed Products (IV)

Parameter (non-compartment model)			**Pegasys®**	**HES (high MW) IFNα**
$t_{½}$	[h]		51	102
C_{max}	[pCi/ml]		7246	8132
t_{max}	[h]		72	36
AUC	[pCih/ml]	0→ t	1182566	1170880
		0→∞	1197805	1286342

HES IFNα conjugate superior to PEG IFNα

- **Higher in vitro activity**
- **Longer half-life**
- **Earlier onset of antiviral effect**

HESylation® of Post-translational Modifications

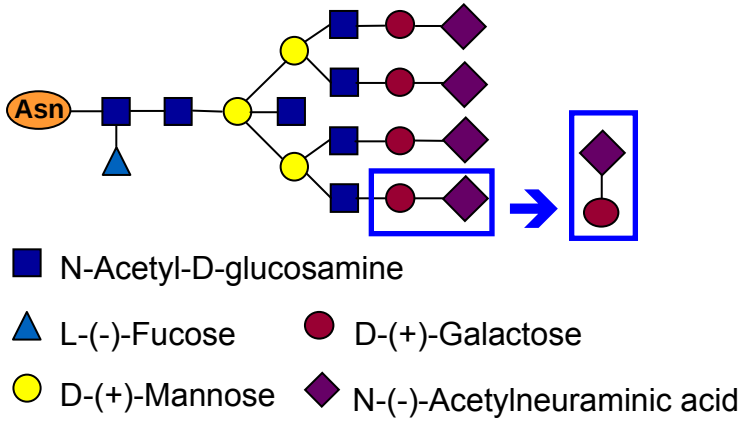
HESylation® –

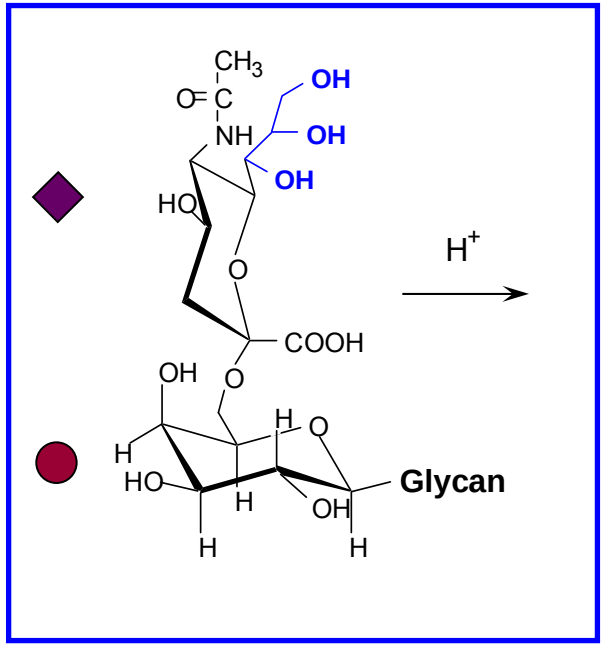
Improvement of undersialylated Glycoprotein's

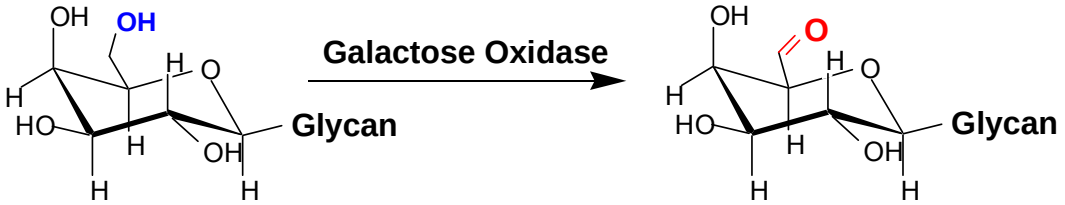
Introduction of aldehyde groups into glycans :

Model substance: erythropoietin (14 sialic acids)

- ➔ Time dependent acid hydrolysis
- ➔ Galactose oxidase treatment



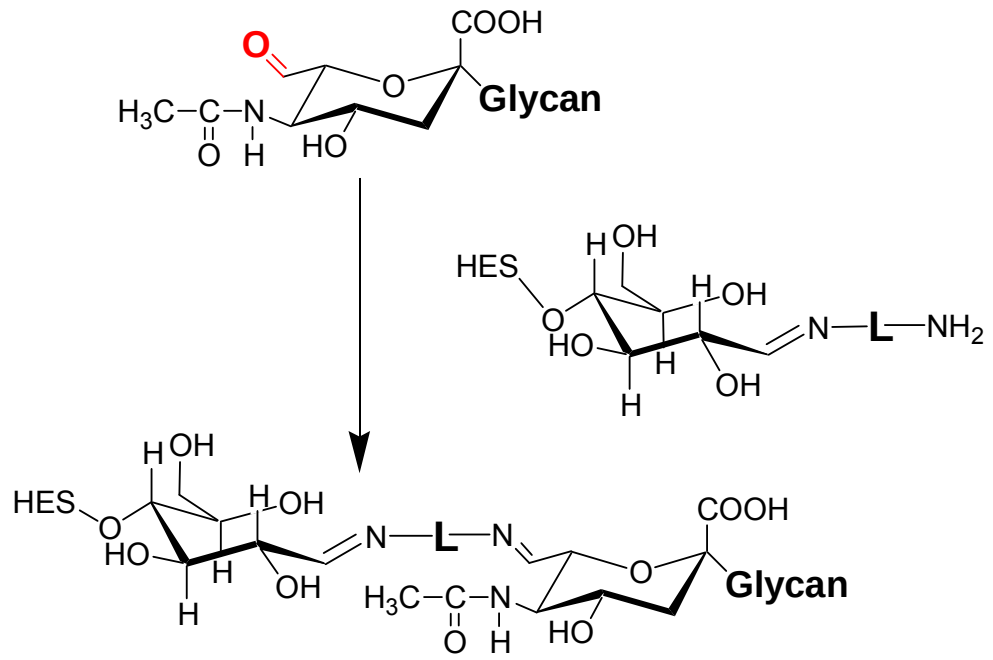




HESylation® –

Improvement of undersialylated Glycoprotein's

Coupling of modified HES to glycan



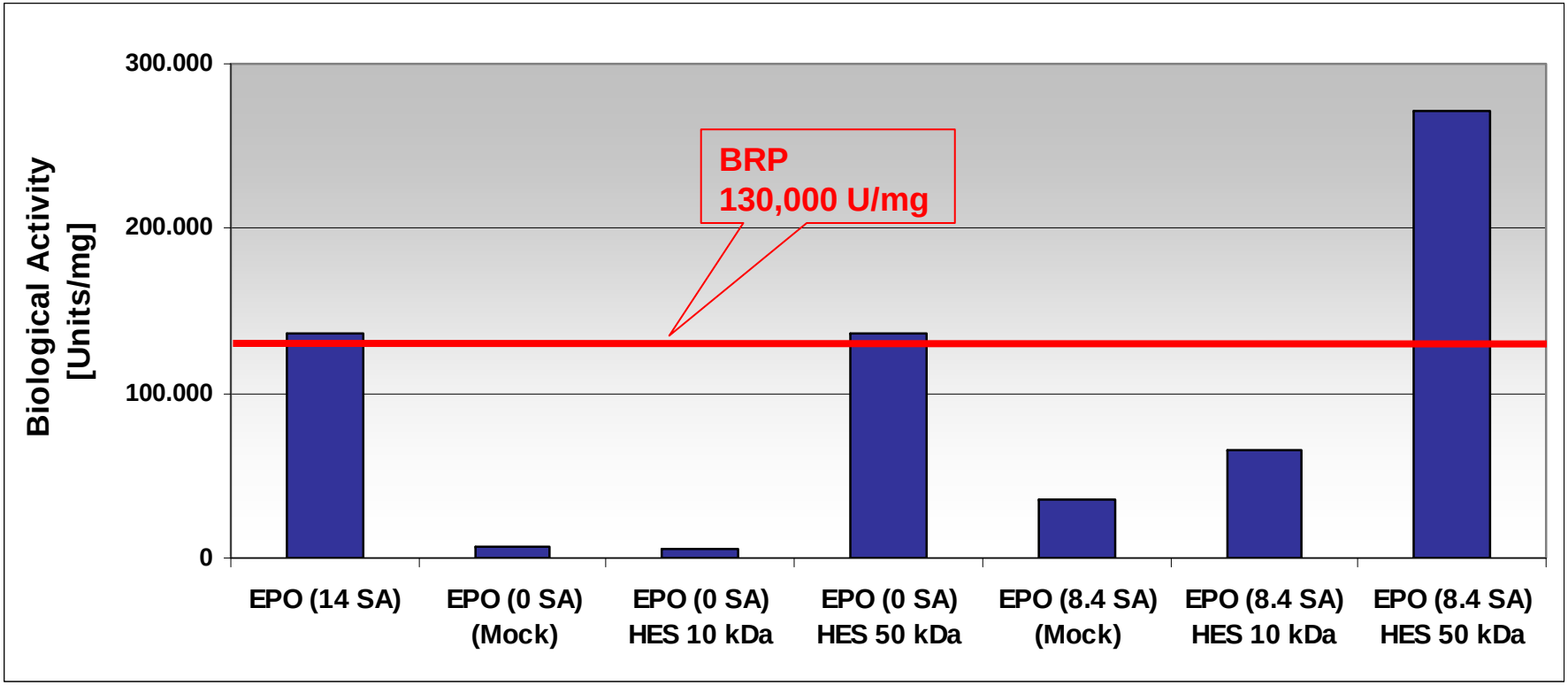
L = Linker developed by FK for glycan strategy

Site specific HESylation® of glycan side chain

HESylation® –

Improvement of undersialylated Glycoprotein's

EPO in vivo bioassay in normocythaemic mouse (Pharm. Eur.)



HESylation® is capable to increase the bioactivity of undersialylated glycoprotein's dramatically!

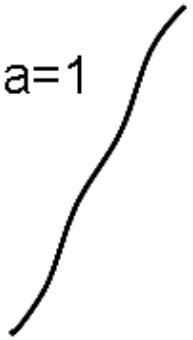
HESylation® – Viscosity

HESylation® –

Polymer Conformation

linear rod

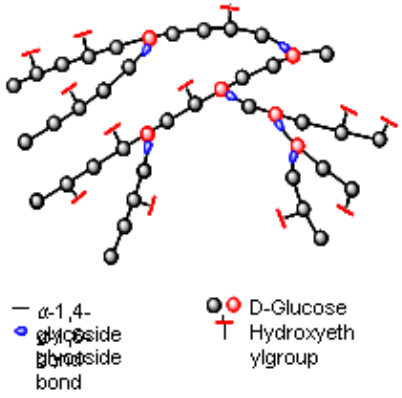
a=1



extended coil (PEG)
a=0.76

random coil (Dextran)
a=0.5

branched polymer (HES)
a=0.35



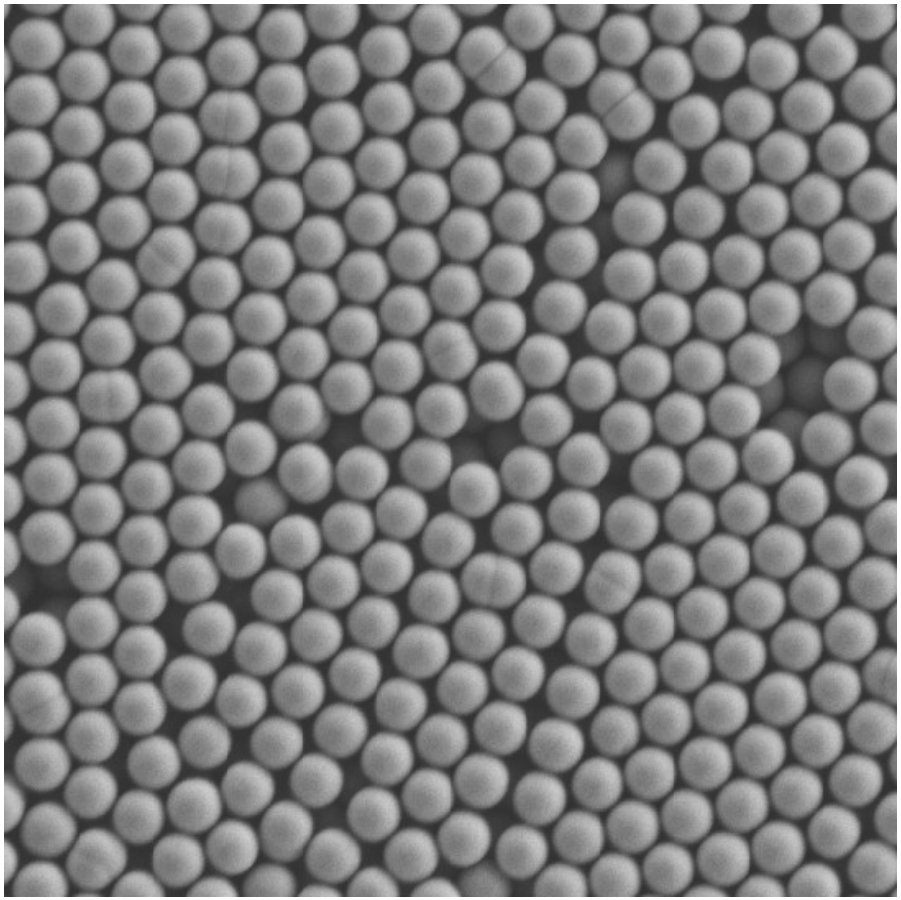
Sphere (protein)
a=0

Mark-Houwink-equation: $[\eta]=K \cdot M^a$

HESylation® –

Viscosity and Polymer Conformation

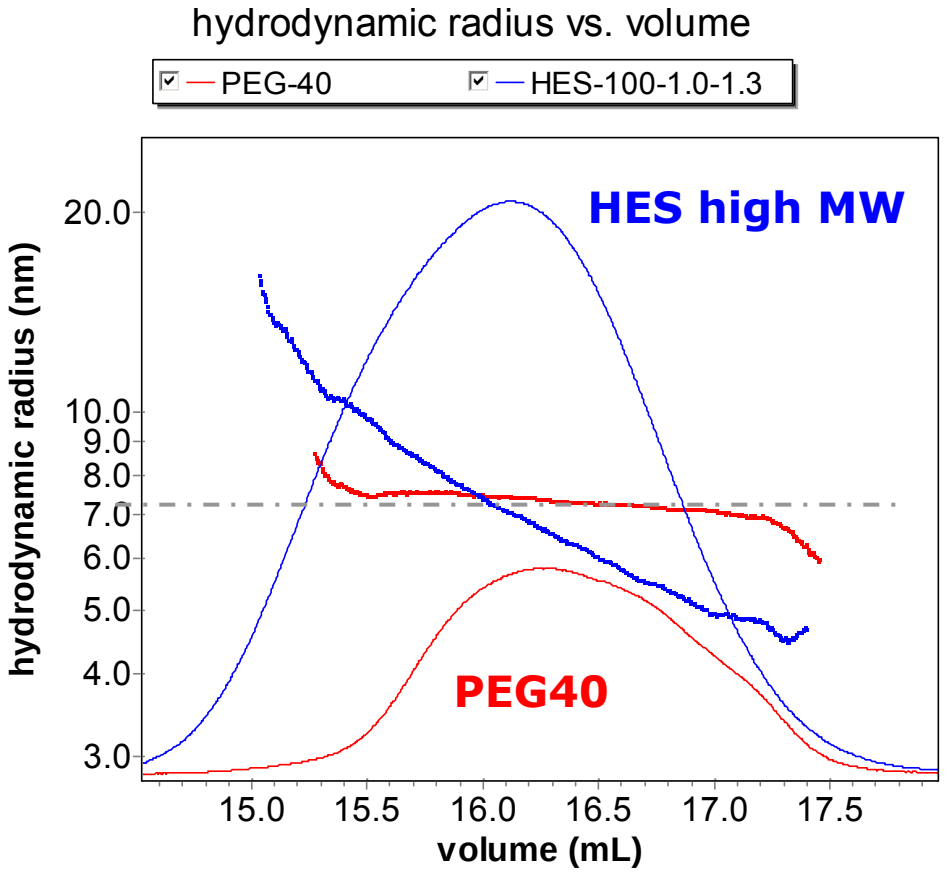
Stiff rods – high viscosity



Hard spheres – low viscosity

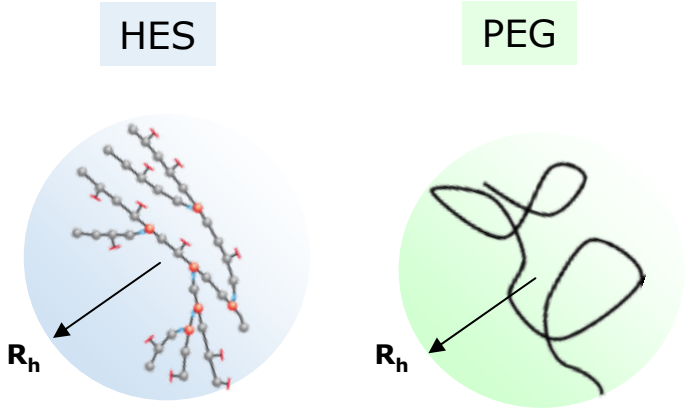
HESylation® –

Hydrodynamic Radius



HES high MW (branched) and PEG40 (linear):
comparable hydrodynamic radius in solution (approx. 7 nm *)

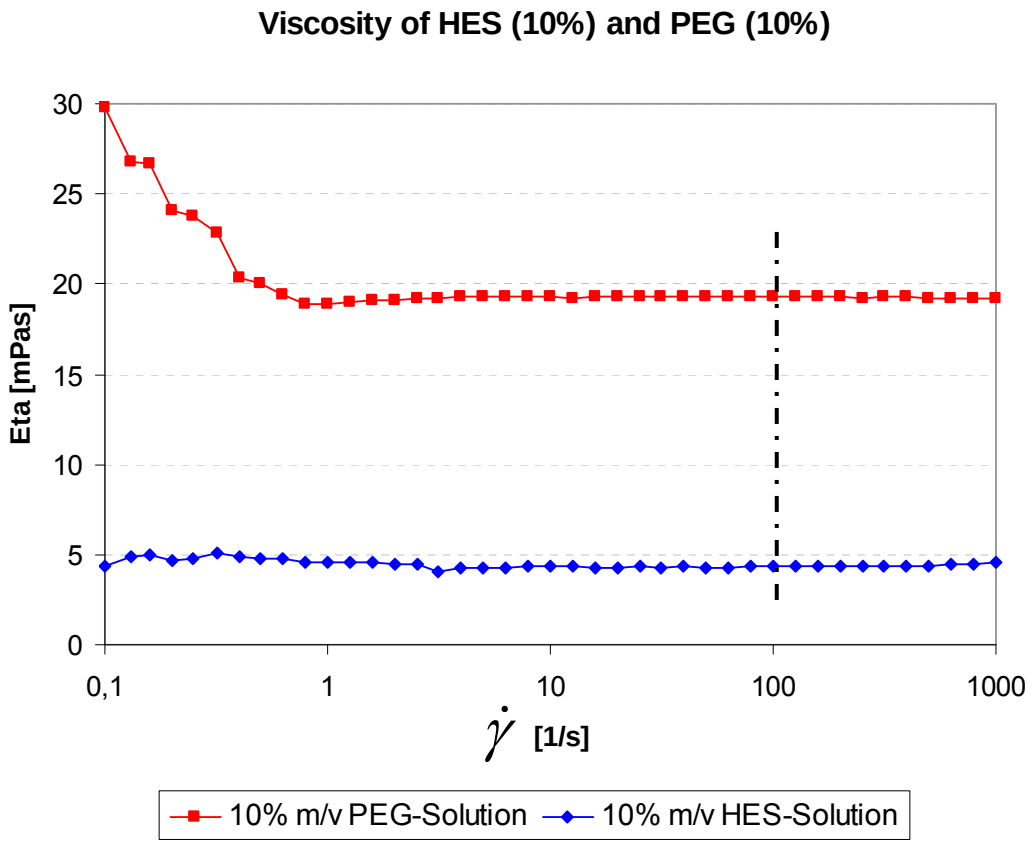
* as measured by SEC with Multi angle light-scattering, refractive index and viscosimetry detection (Wyatt system)



Dimensions in solution of PEG40 and HES high MW are comparable

HESylation® –

HES vs. PEG 10% (m/v) Solution



PEG40 (M_w=36 kD) and HES (high MW)
Haake Mars II Rheometer with double slit geometry

$\eta =$ **19.3 mPas**

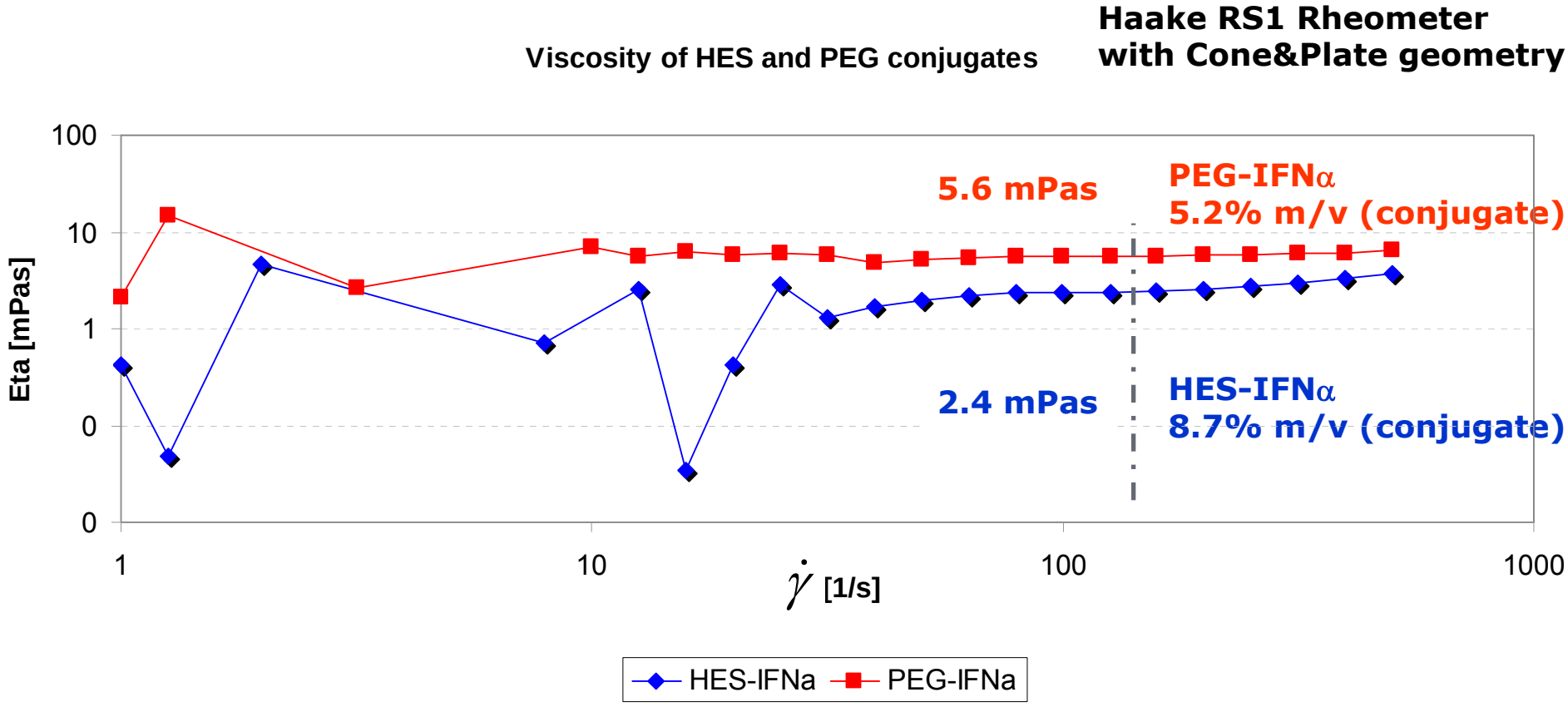
$\eta =$ **4.33 mPas**

Solution Viscosity of HES << PEG

HESylation® –

HES vs. PEG – Conjugate Viscosity

Haake RS1 Rheometer with Cone&Plate geometry



HES conjugate shows a > 2 fold lower solution viscosity

HESylation® –

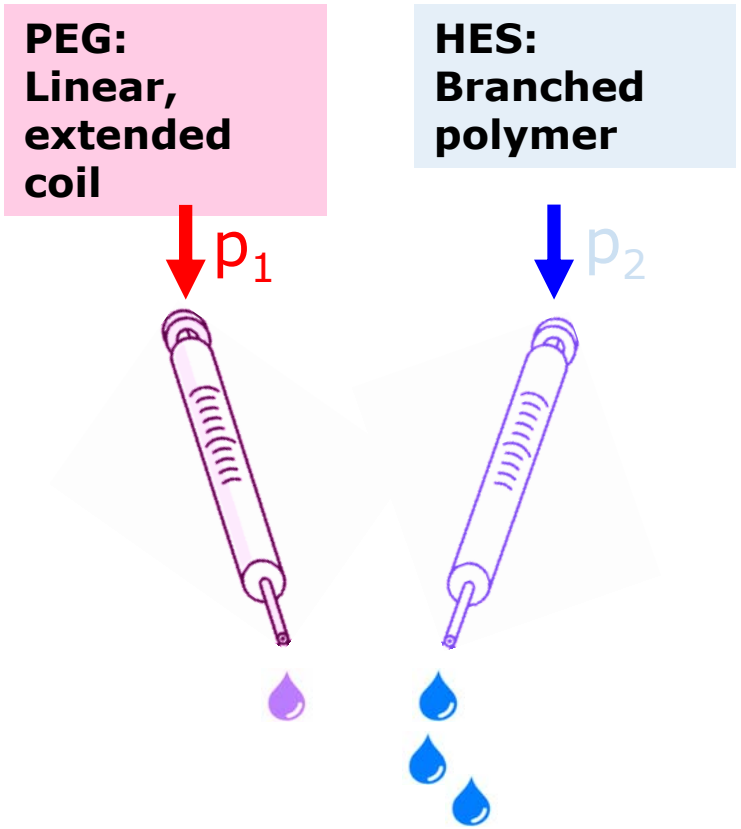
HES vs. PEG – Conjugate Viscosity Conclusion

Lower viscosity opens potential for:

- Easier processing of solutions
- Higher concentrations (smaller volumes)
- Better patient compliance by use of thinner cannula

$$\frac{d\dot{V}}{dt} = \frac{\pi\, r^4 \Delta p}{8\eta l}$$

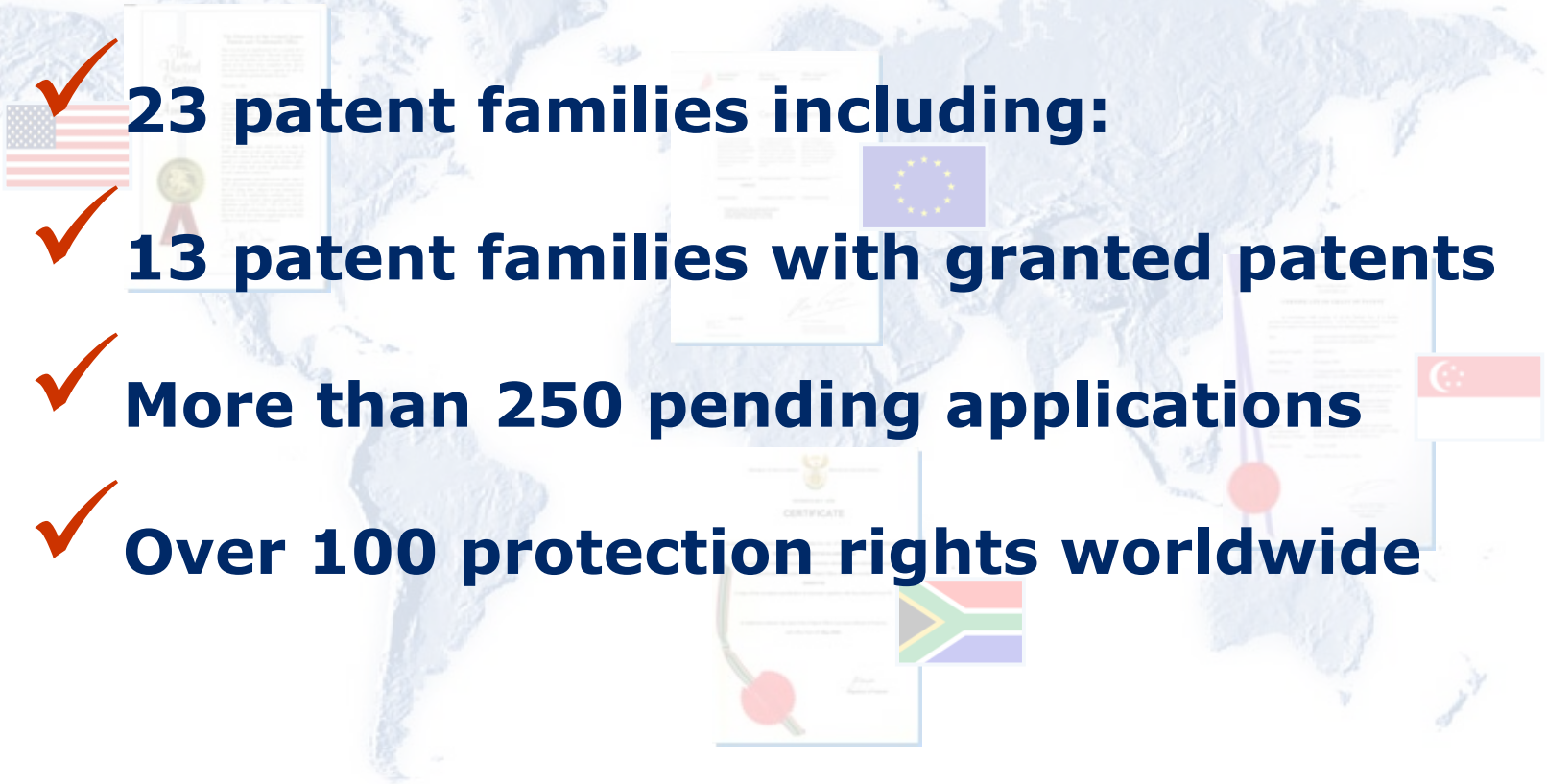
Equation of Hagen-Poiseuille



➔ HES is the preferred polymer for high dosage or chronically administration

HESylation® –

Comprehensive Protection through Intellectual Property

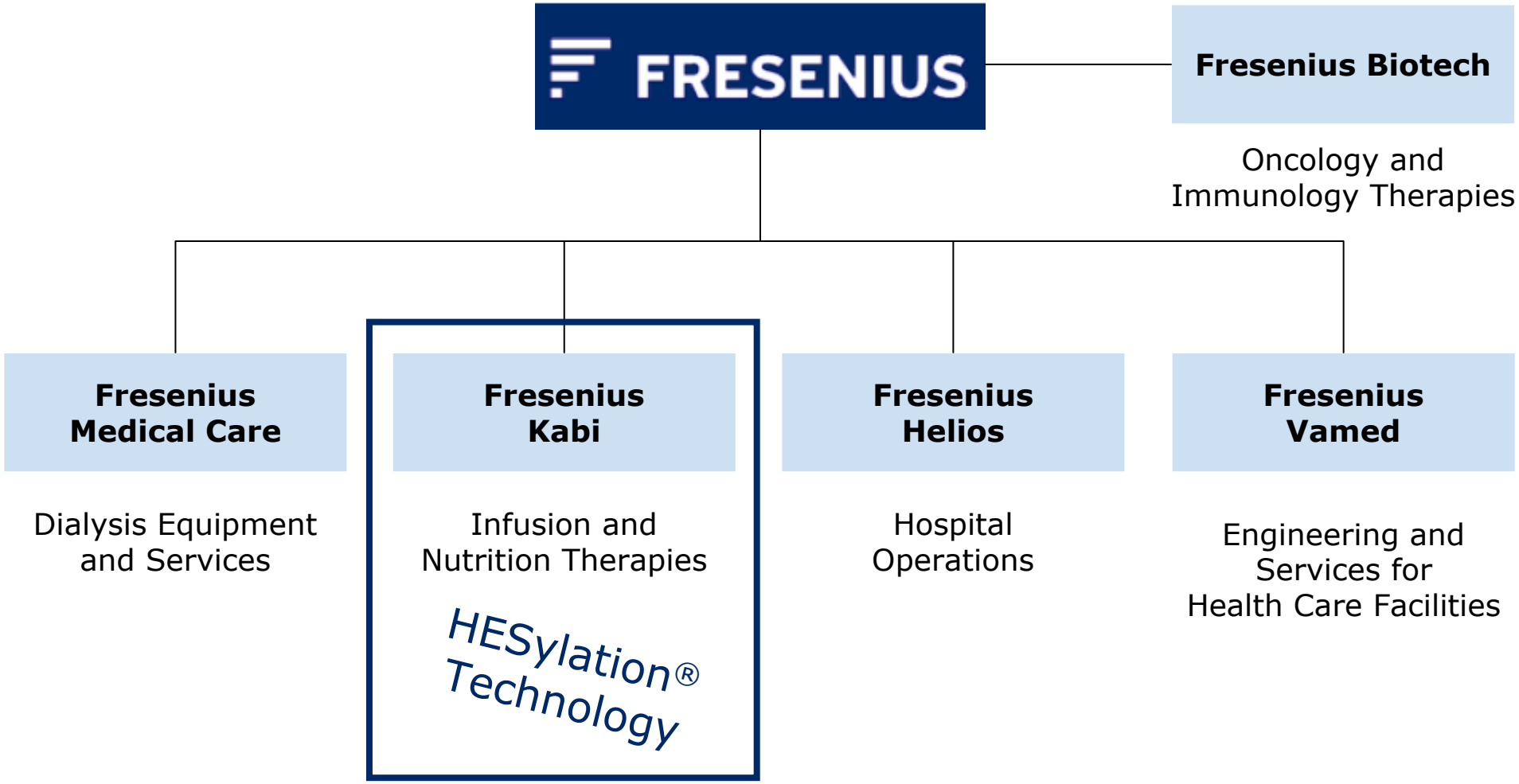


- **23 patent families including:**
- **13 patent families with granted patents**
- **More than 250 pending applications**
- **Over 100 protection rights worldwide**

HESylation® Technology secured by know how & worldwide IP!
Trademark registered in all relevant countries of the world!

HESylation® –

Fresenius Group – a strong Partner with Commitment

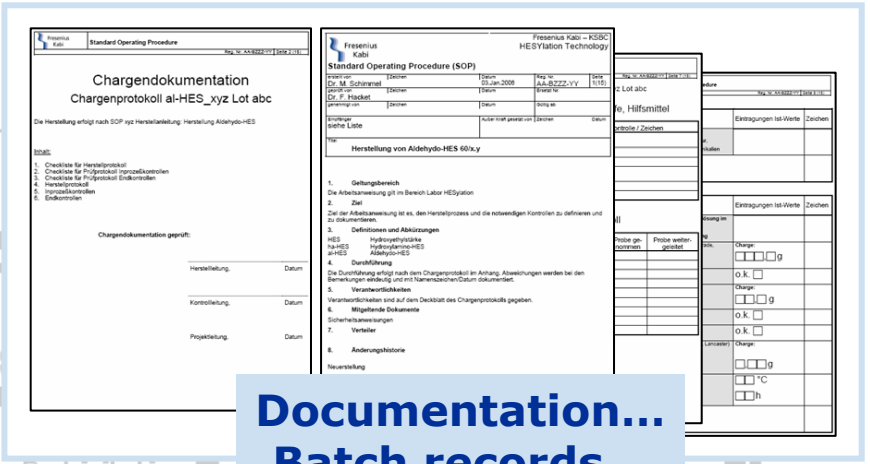


HESylation® –

Dedicated towards Quality

Training…
Clothing…

Cleanrooms…



Documentation…
Batch records…

Equipment…
Qualification…

Quality System…
SOPs, Audits

HESylation® –

Summary & Conclusions

- World´s largest producer of pharmaceutical grade HES
- Linkers for selective HES coupling established
- HESylation® successfully applied to several molecules
- In vitro / in vivo studies indicate similar or longer half life extension compared to marketed benchmarks
- Broad IP portfolio covering HES species, HESylation® chemistries, HESylated targets, linker classes, etc.
- HESylation® with significant advantages over competing technologies
  - safety
  - flexibility

Two published license agreements in 2009 with major pharma partners

HESylation® – Transmitting Efficiency

Dr. Helmut Knoller
Head of Scientific Management
HESylation Technology
Kabi Innovation Centre