Novel High-Throughput Assay for Polysorbate Quantification in Biopharmaceutical Products by Using the Fluorescent Dye DiI

CASSS AT Europe 2020

Tim Menzen

04 Nov. 2020

Coriolis Pharma

Provide 5 answers about the DiI assay

01 What is the purpose?

02 How does it work?

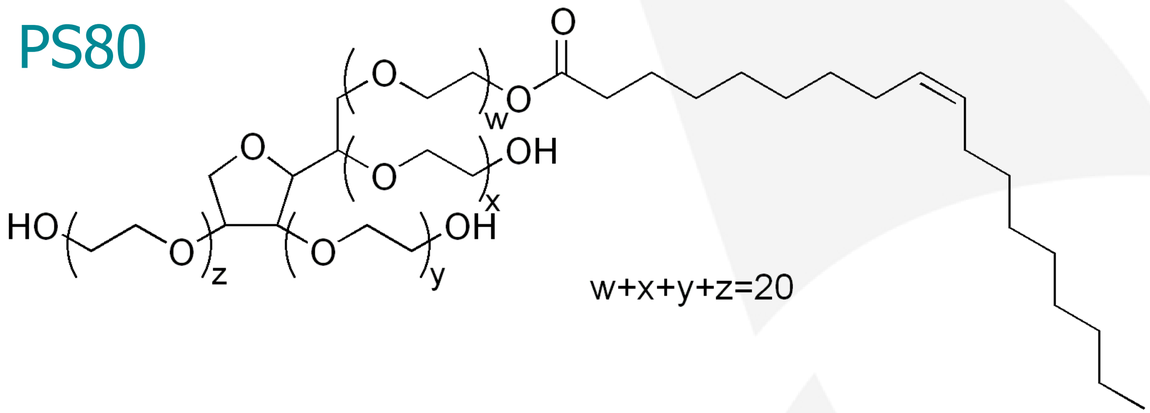
03 Why does it work?

04 How does it perform?

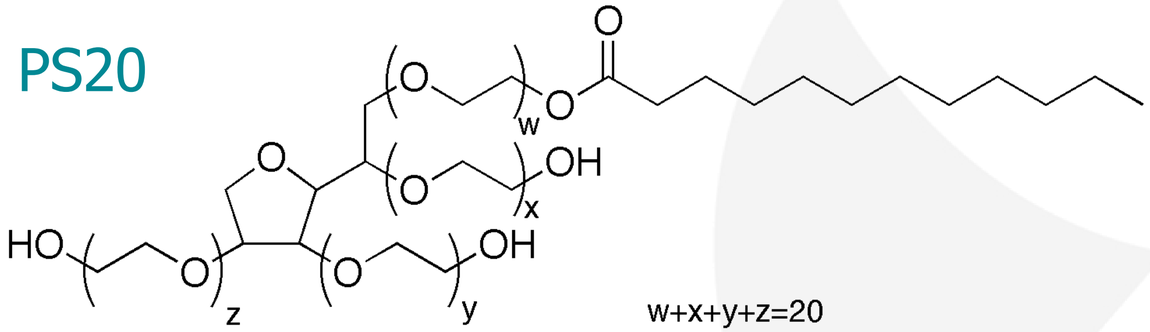
05 Why do we like it?

Polysorbates (PS) as excipient and their degradation products

PS80



PS20



Hydrolysis

- Non-esterified species
- Free fatty acids

Oxidation

- Peroxides
- Oxidized fatty acids
- Alkanes
- Short chain organic acids
- Aldehydes, ketones, …

- Common excipients to stabilize biopharmaceutical drug products
- Prevent proteins from adsorption to surfaces and interfaces

Martos et al. (2017), J Pharm Sci, 106: 1722-1735

Provide 5 answers about the DiI assay

01 What is the purpose?

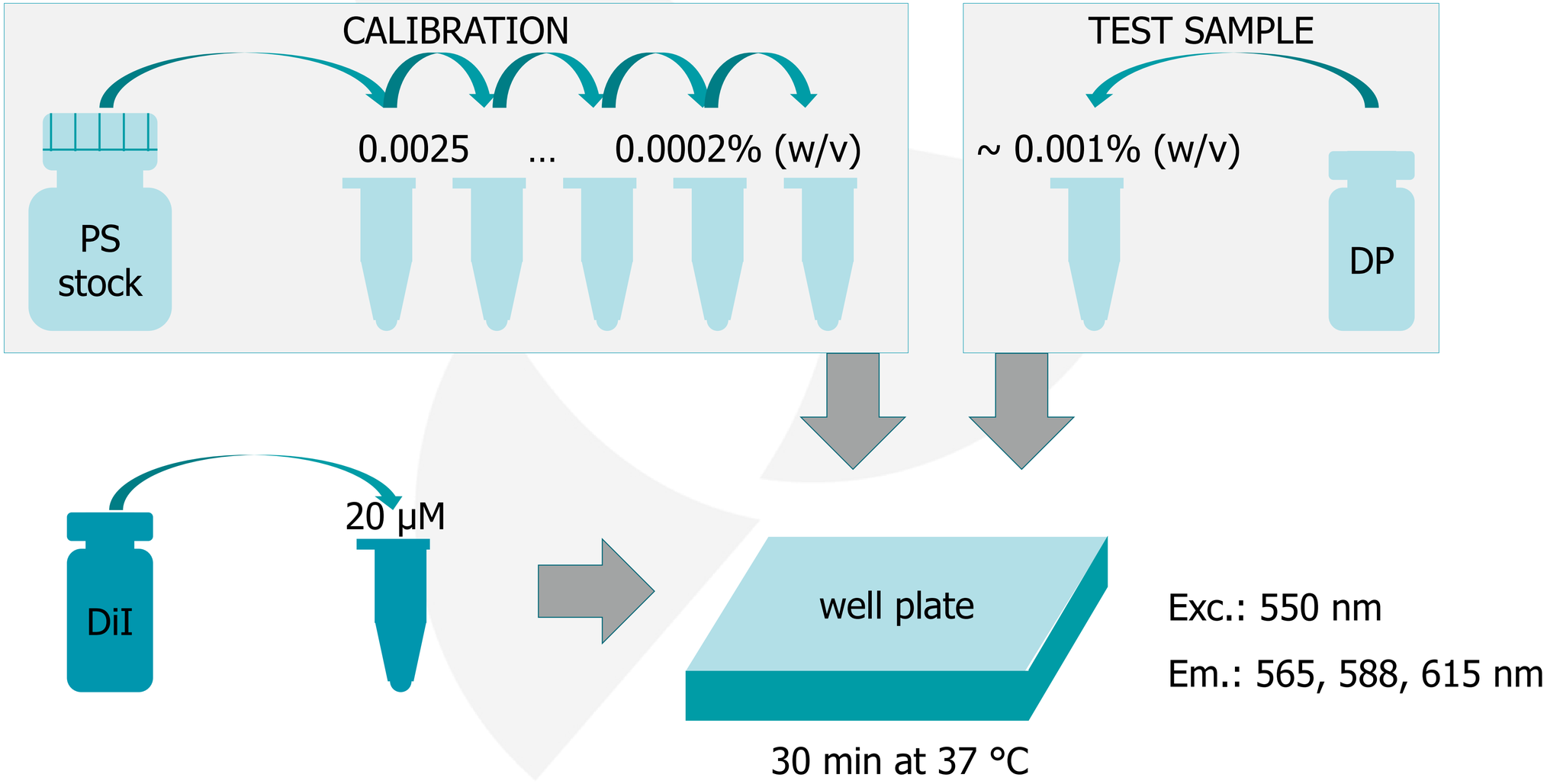
02 How does it work?

03 Why does it work?

04 How does it perform?

05 Why do we like it?

How DiI assay works



Adapted from Martos et al. (2020), J Pharm Sci, 109: 646-655

Provide 5 answers about the DiI assay

01 What is the purpose?

02 How does it work?

03 Why does it work?

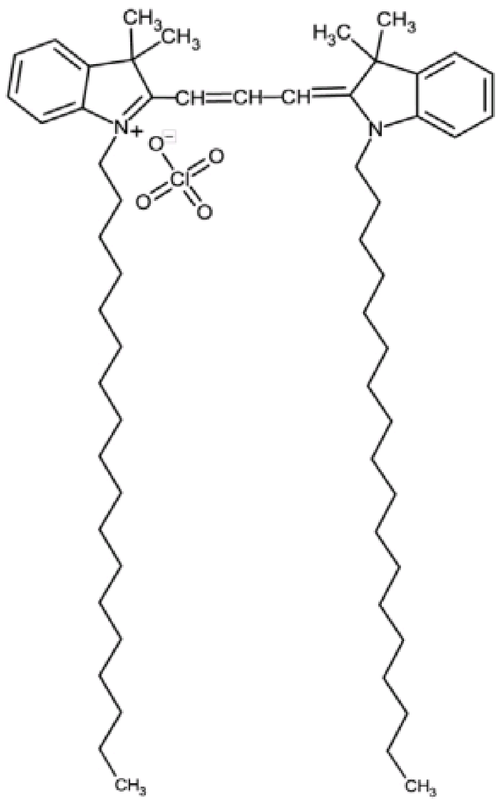
04 How does it perform?

05 Why do we like it?

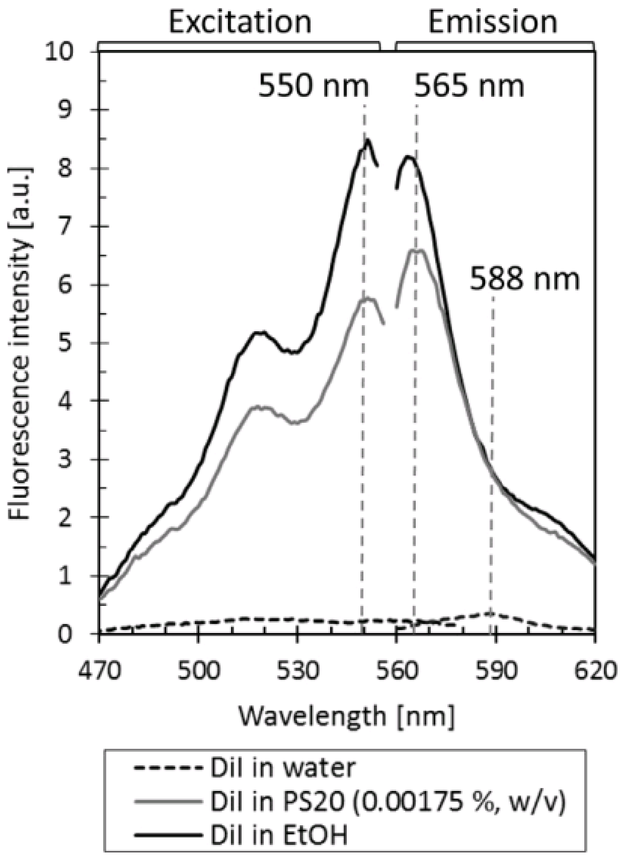
Fluorescent dye: DiI to quantify polysorbate

- 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI)
- Sensitive to the polarity of the environment: weakly fluorescent in water but highly fluorescent when incorporated into non-pola environments

→ DiI is typically used for staining of cells/membranes



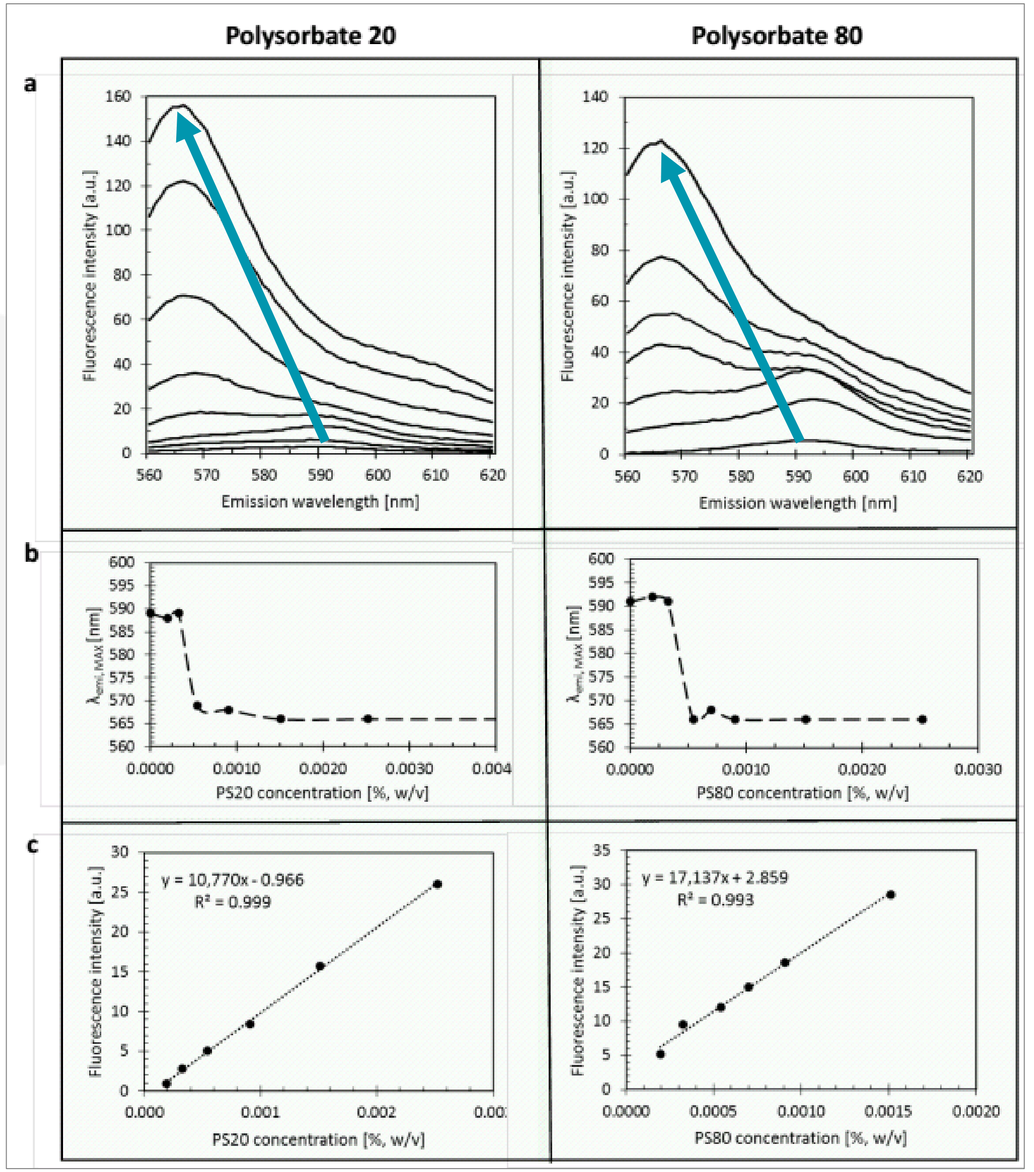
DiI



Martos et al. (2020), J Pharm Sci, 109: 646-655

DiI assay: principle

- Quantification of PS below the CMC in in liquid pharmaceutical formulations
  → dilution of sample to linear range
- Exc.: 550 nm
  Em.: 565, 588, and <u>615 nm</u>



Martos et al. (2020), J Pharm Sci, 109: 646-655

Provide 5 answers about the DiI assay

01 What is the purpose?

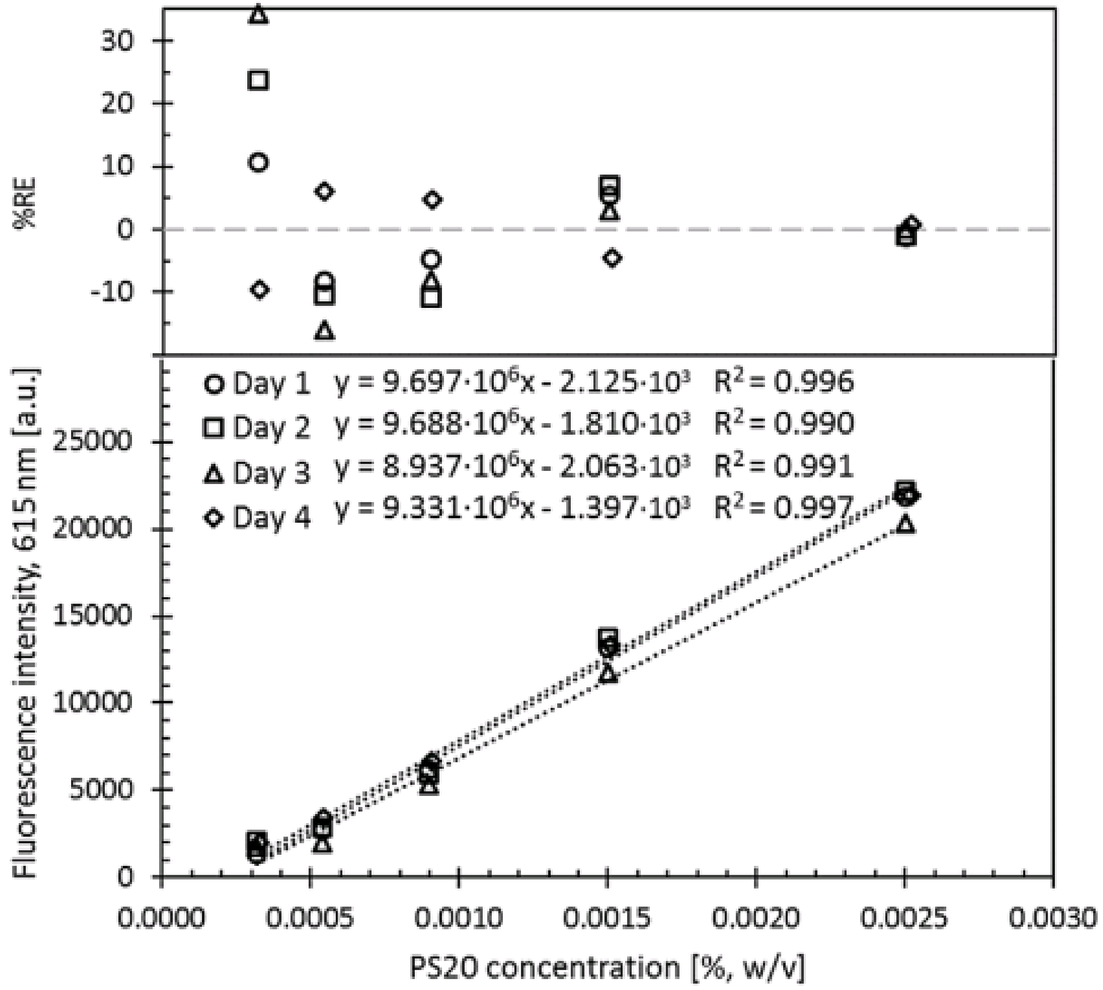
02 How does it work?

03 Why does it work?

04 How does it perform?

05 Why do we like it?

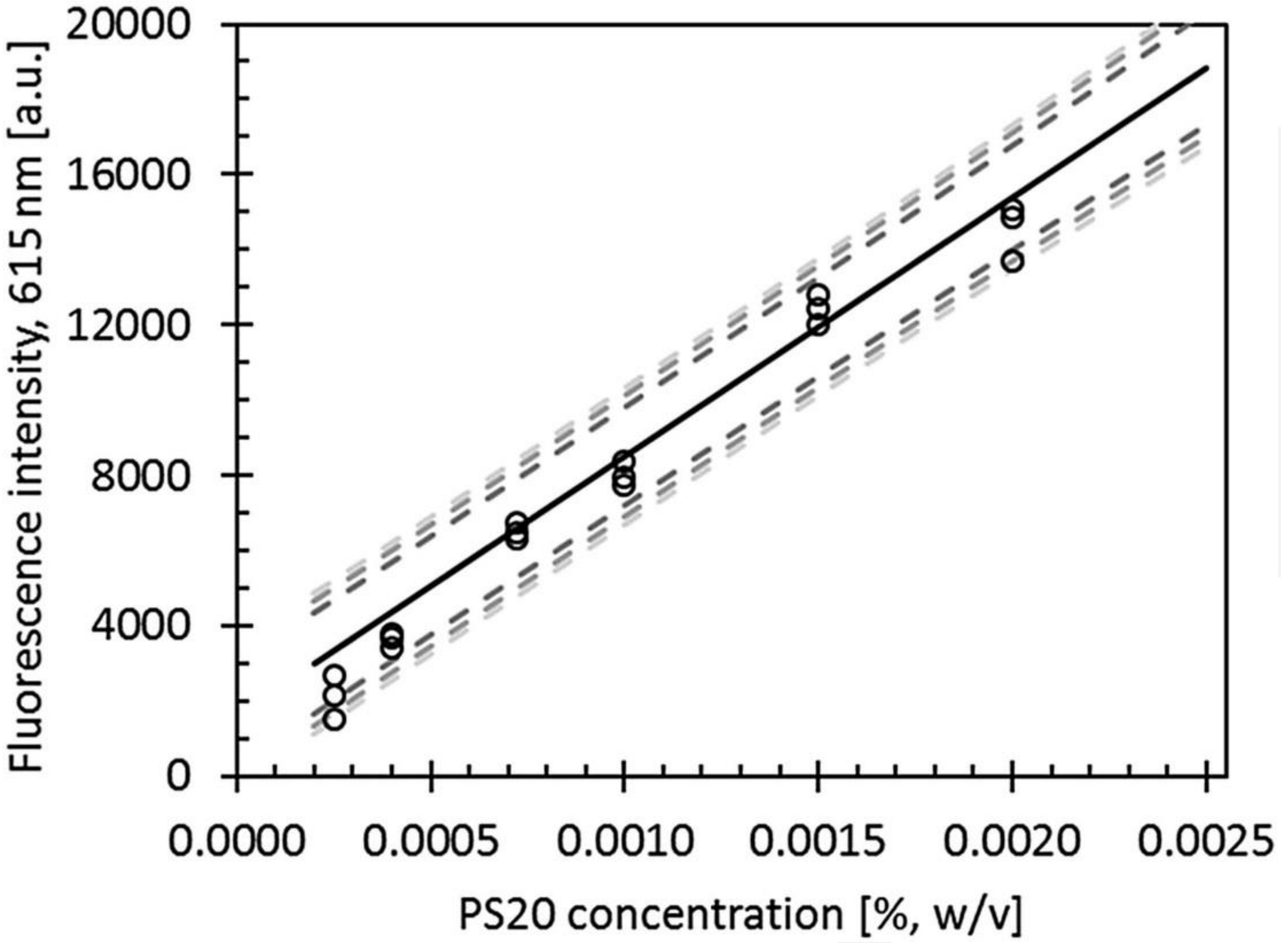
DiI assay performance (96-well plates) (i)



- PS20 linear range: 0.0002 - 0.0025% (w/v)
- LOD: 0.00020% (w/v)
- LOQ: 0.00055% (w/v)

Martos et al. (2020), J Pharm Sci, 109: 646-655

DiI assay performance (96-well plates) (ii)



- Recommendation: Ideally use same PS material for calibration
- But two different batches of PS20 used for calibration led to similar results

Martos et al. (2020), J Pharm Sci, 109: 646-655

Comparison of DiI with NPN and LC-CAD

Case Study

Sample	DiI Assay	
	Calculated [PS20]% (W/V)	%Recovery
PS20	0.039	99 ± 4
IgGA-PS20	0.036	91 ± 4
IgGB-PS20	0.042	104 ± 10
IgGC-PS20	0.040	100 ± 5

Sample	FMA (NPN)		LC-CAD	
	Calculated [PS20]% (W/V)	%Recovery	Calculated [PS20]% (W/V)	%Recovery
PS20	0.044	110 ± 1	0.041	102 ± 4
IgGA-PS20	0.145	362 ± 1	0.041	102 ± 6
IgGB-PS20	0.201	502 ± 1	0.040	99 ± 3
IgGC-PS20	0.115	288 ± 1	0.039	98 ± 3

Martos et al. (2020), J Pharm Sci, 109: 646-655

Provide 5 answers about the DiI assay

01 What is the purpose?

02 How does it work?

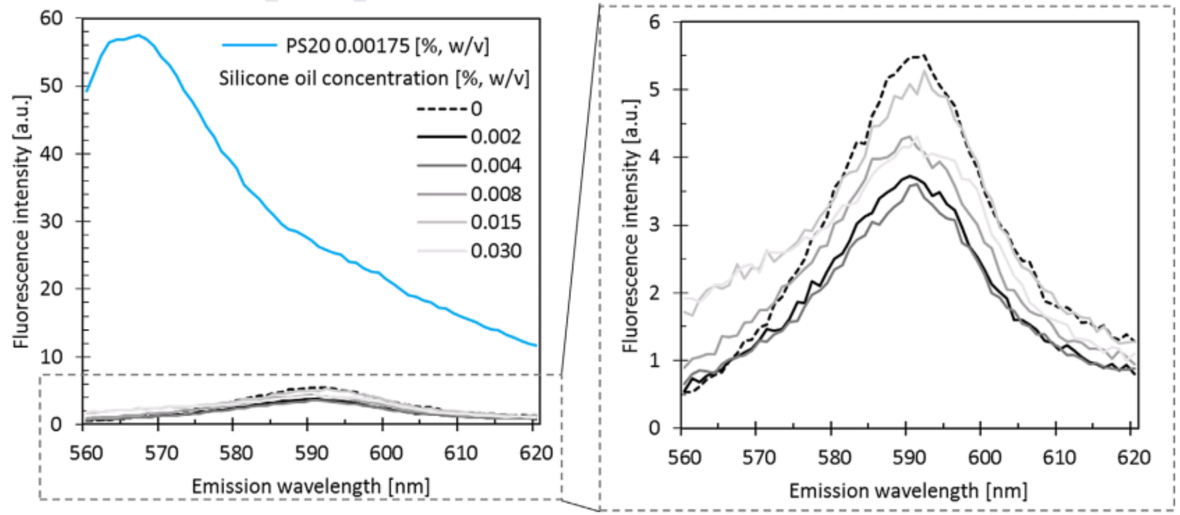
03 Why does it work?

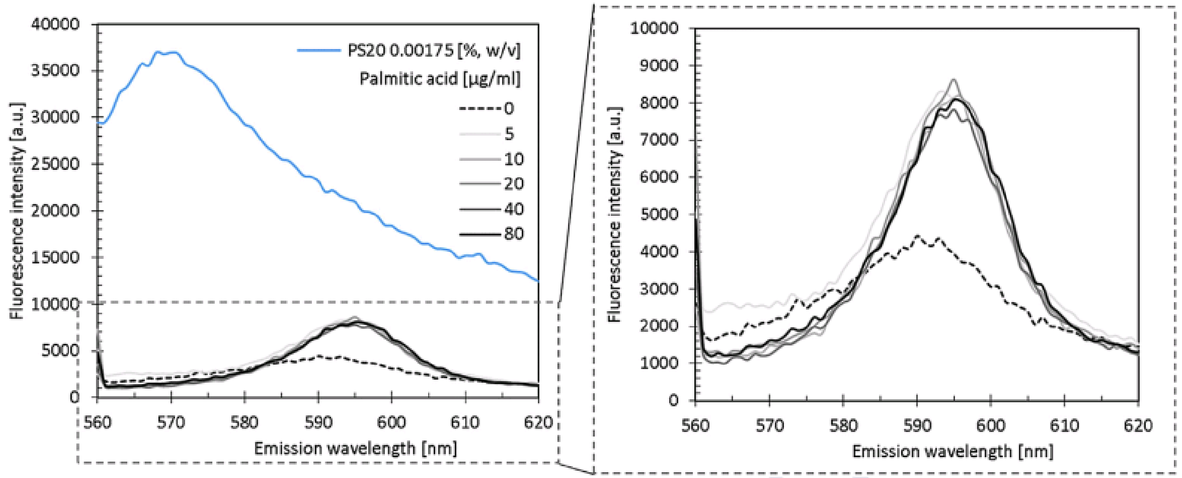
04 How does it perform?

05 Why do we like it?

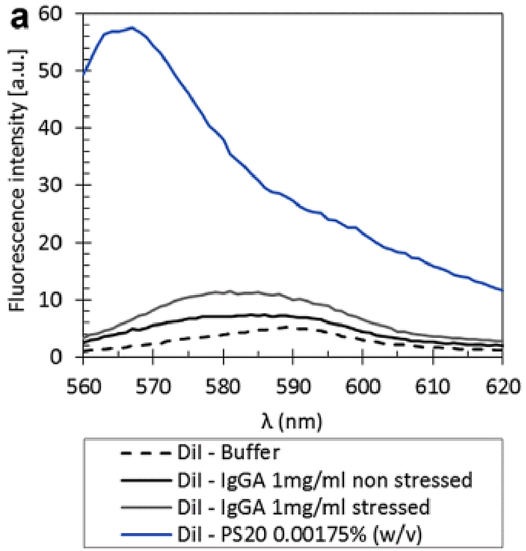
DiI: compatible with protein formulations

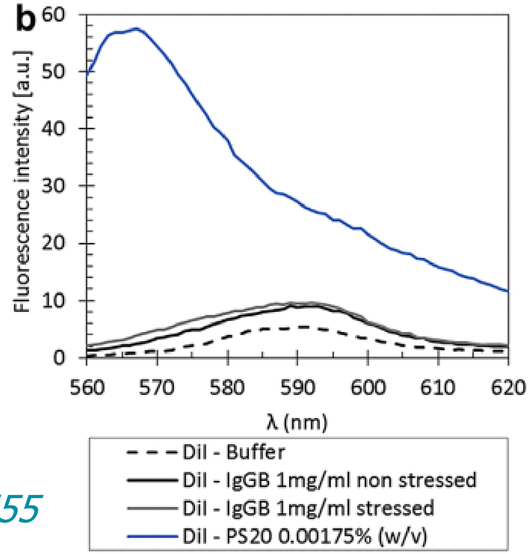
Negligible interference of silicone oil and fatty acids





Compatible with IgG





Coriolis Pharma

Martos et al. (2020), J Pharm Sci, 109: 646-655

Conclusion: Why we like the DiI assay

- ✓No removal of protein required
- ✓Use of conventional spectrofluorometer
- ✓High-throughput, low volume ready (well plates & liquid handling)
- ✓Works with usual formulations (presence of silicone oil)
- ✓Acceptable limitations (high protein + low PS concentrations)

Acknowledgments

Journal of Pharmaceutical Sciences 109 (2020) 646-655

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutical Biotechnology

Novel High-Throughput Assay for Polysorbate Quantification in Biopharmaceutical Products by Using the Fluorescent Dye DiI

Check for updates

Ariadna Martos [1], Michelle Berger [1], Wendelin Kranz [1], Anna Spanopoulou [1], Tim Menzen [1], Wolfgang Friess [2], Klaus Wuchner [3], Andrea Hawe [1,*]

[1] *Coriolis Pharma, Fraunhoferstr. 18b, 82152 Martinsried, Germany*
[2] *Department of Pharmacy, Pharmaceutical Technology and Biopharmaceutics, Ludwig-Maximilians-Universität München, Butenandtstr. 5-13, 81337 Munich, Germany*
[3] *Janssen Research&Development, Pharmaceutical Development & Manufacturing Sciences, Large Molecule Analytical Development, Hochstrasse 201, 8205 Schaffhausen, Switzerland*

Coriolis Pharma

Note: John Carpenter & Ted Randolph dedicated issue is open access.

Contact us

Coriolis Pharma Research GmbH
Fraunhoferstraße 18 b
82152 Martinsried | Germany

Phone: +49 (0) 89 41 77 60 – 0

www.coriolis-pharma.com
contact@coriolis-pharma.com

Coriolis Pharma