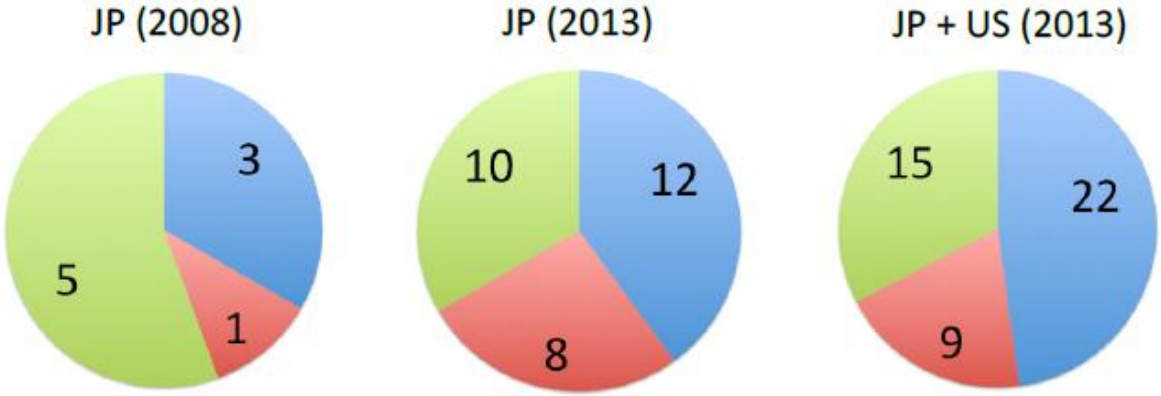
Factors affecting stabilities of biopharmaceuticals in prefilled syringe

Graduate School of Engineering, Osaka University, Japan
Okazaki Integrative Institute of Bioscience, Japan
Guandong University of Technology, China
Susumu UCHIYAMA

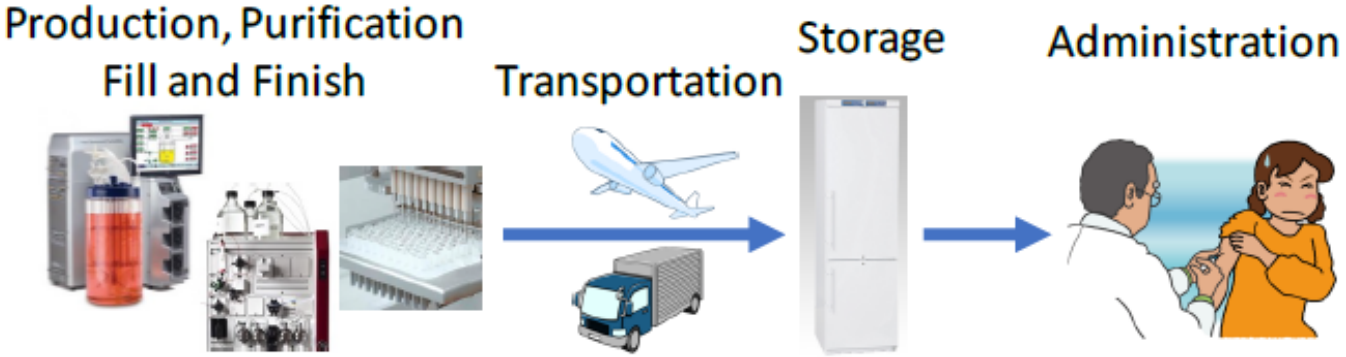
Liquid formulation has increased

- ✓ Liquid formulation has increased
- ✓ Prefilled syringe (PFS) type has increased
- ✓ Changed from lyophilized to PFS (ex. Enbrel)

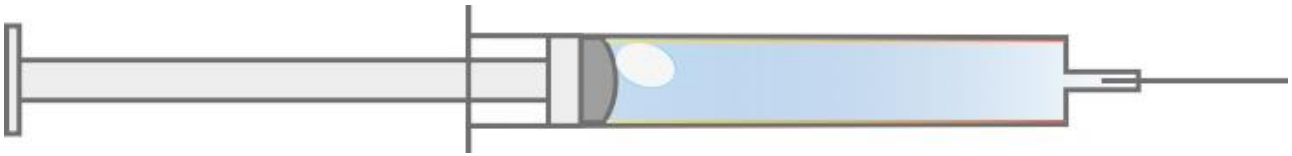


Uchiyama, S. (2014) ***Biochim. Biophys. Acta.*** 1844, 2041-2052.

Possible stresses to Prefillable syringe (PFS)



Stress	Production, Purification Fill and Finish	Transportation	Storage	Administration
Pumping	✓			
Contact w Interface	✓	✓	✓	✓
Sweeping wall				✓
Shaking		✓	✓	
Handling, Impact		✓	✓	
Pressure change	✓	✓		✓
Temperature change	✓	✓	✓	✓
Light irradiation		✓	✓	✓
Oxygen	✓	✓	✓	
pH and <I> change				✓



Design

Materials

Glass	+ Silicone oil coating "covalent" method" "baked silicone oil" "liquid silicone oil" (Gerhardt, 2015)
Polymers	COC: Cyclo Olefin Copolymer COP: Cyclo Olefin Polymer +/- Silicone oil coating
Stopper	Rubber with coating
Needle	Staked stainless needle

Characterization of pharmaceutical antibodies

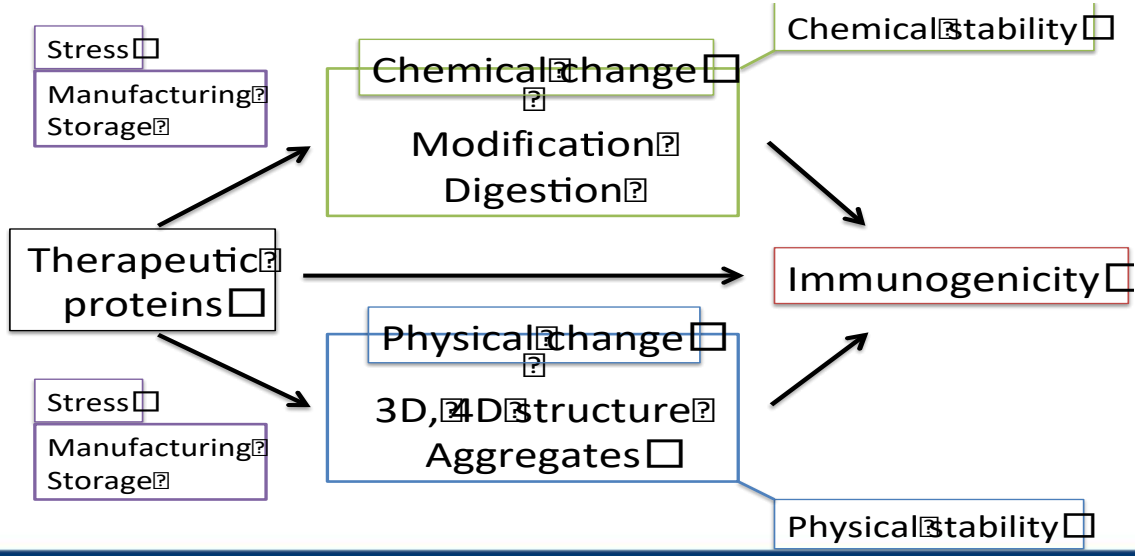
Guidance for Industry

Immunogenicity Assessment for Therapeutic Protein Products

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

Chemical modifications of therapeutic protein products, such as oxidation, deamidation, aldehyde modification, and deimination, may elicit immune responses by, for example,

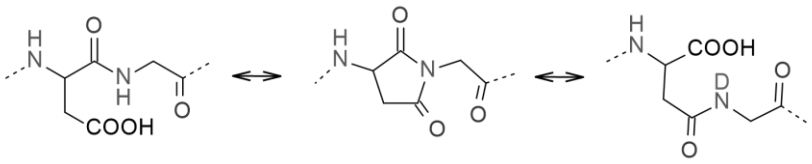
modification, and morphology (Narhi et al. 2012). Aggregates ranging from dimer to visible particles that are hundreds of micrometers in size (Narhi et al. 2012) have been recognized for their potential to elicit immune responses to therapeutic protein products for over a half-century (Gamble 1966). Mechanisms by which protein aggregates may elicit or enhance immune



Chemical degradation

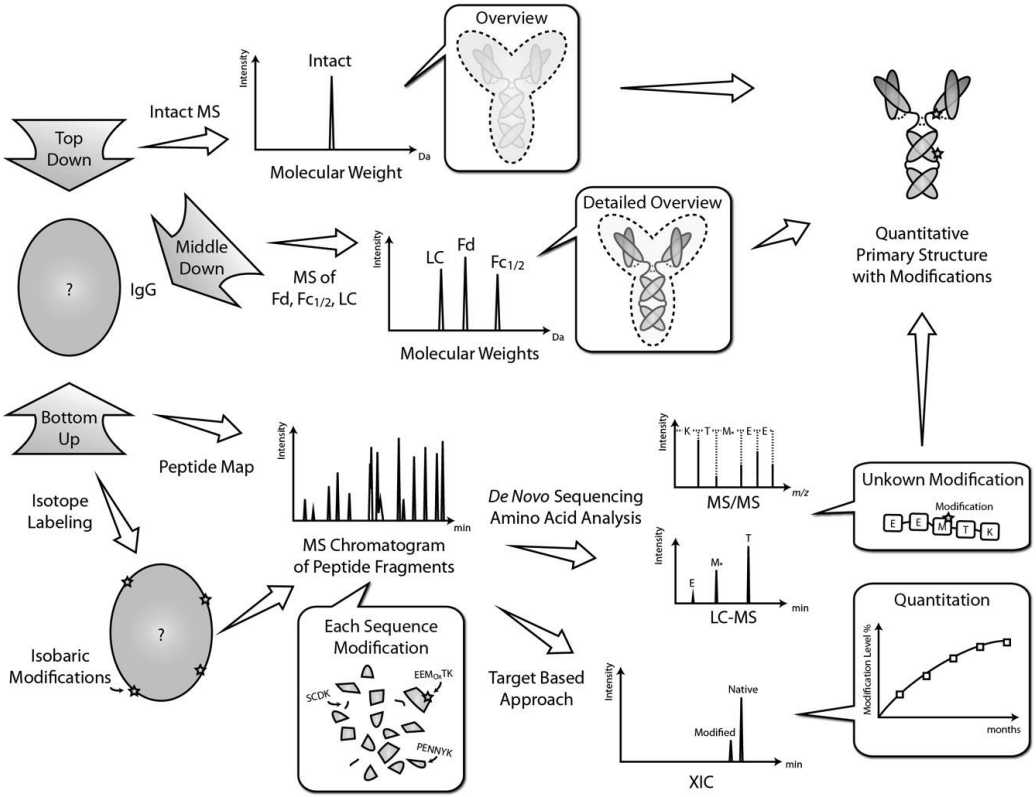
Modification	MS diff.	Notes
N-term pyroglutamate	D17 Da	HC Q ➔ Pyro E
Removal of C-term Lys	D128 Da	Partial or complete
Deamidation of Asn or Gln	+ 1Da	Chumsae C et al., 2007 Liu H et al., 2009
Isomerization		Asp*-Gly
Oxidation	**+ 16 Da**	**Met, Trp, Cys, His**
Glycation	+ 162*n Da	+Hexose (galactose, glucose, fluctose, etc), AGE
Racemization		Asp*-Asp, Ser-Cys*-Asp-Lys

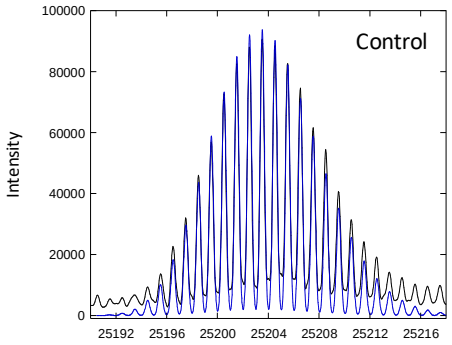
< pH4, pH7 <, Gln, Asn Deamidation is accelarated
Patel, Borchardt, Pharm Res 7, 593, 1990
Bhatt et al., Pharm Res 7, 703, 1990
Asp is racemized and peptides are fragmented under acidic condition
Oliyai, Borchardt, Pharm Res 10, 95 1993
Oliyai, Borchardt, Pharm Res 11, 751, 1994
Neutral-basic conditions increase of SS scrambling
Wang Intl. J. Pharm. 185, 129, 1999



Reaction scheme of aspartic acid isomerization

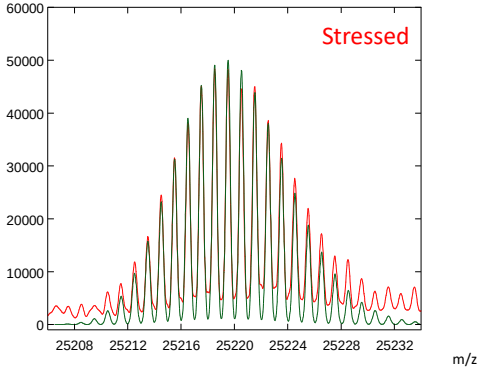
Chemical modification analysis by mass spectrometry





■ Deconvoluted (MaxEnt)
Mr (monoisotopic)＝25188.4781 Da

■C1122H1740N286O361S6(配列から)
Mr (monoisotopic)＝25188.4913 Da
DM_W ＝0.013Da(-0.52ppm)

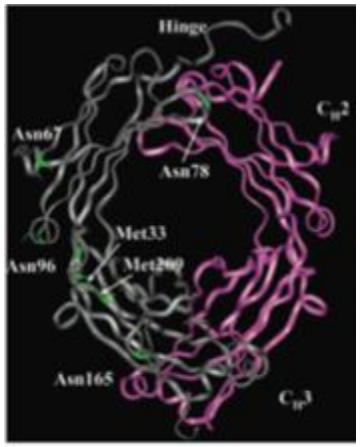


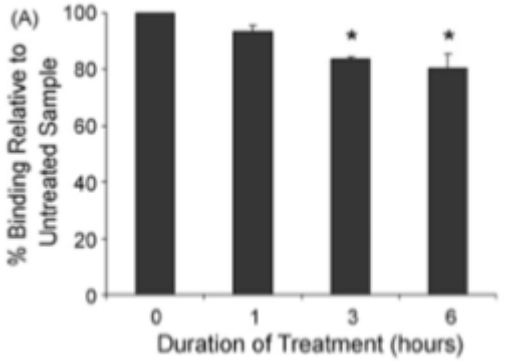
Deconvoluted (MaxEnt)
Mr (monoisotopic)＝25204.4784 Da

C1122H1740N286O362 S6
Mr (monoisotopic)＝25204.4862Da
DM_W ＝0.008Da(-0.31ppm)

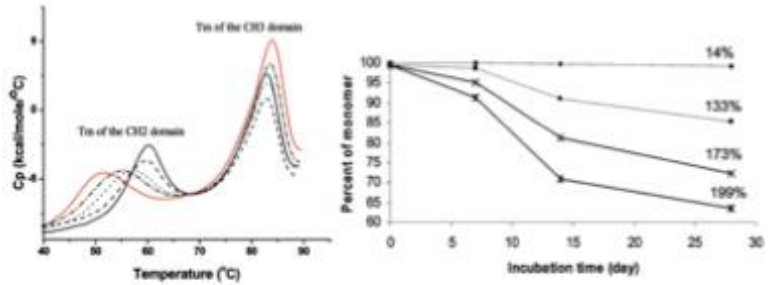
Oxidation changes stability and PK

- ✓ A Met at CH2 is susceptible to oxidation.
- ✓ Oxidation of the Met reduces conformational stability.
- ✓ Also changes effector functions (Kuo TT et al., 2011; Wang W et al., 2011) .
- ✓ binding affinity to FcRn (Liu D et al., 2008).
- ✓ oxidations in the CDR regions may affect the binding activities (Yang J et al., 2007).



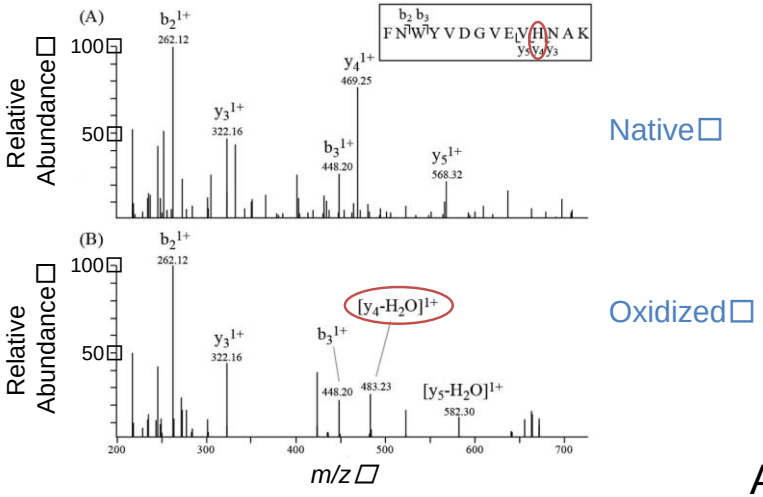


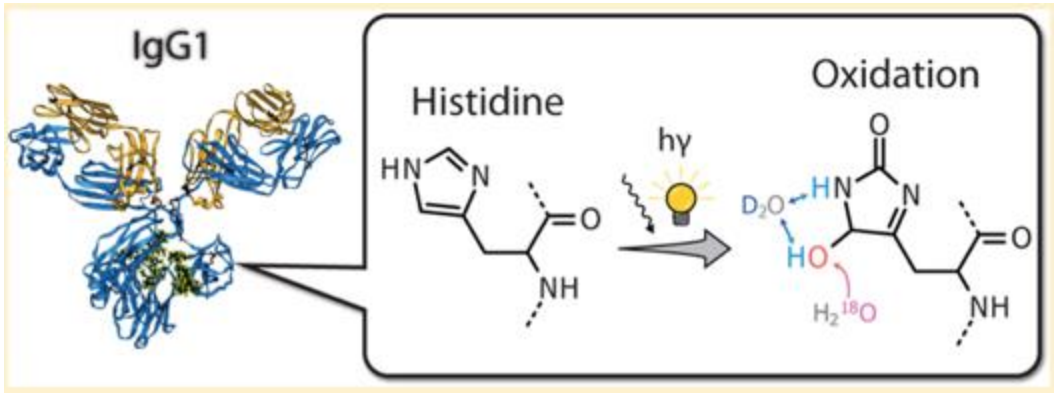
Bertolotti-Ciarlet et al.,
Molecular Immunology 46 (2009) 1878–1882



Liu et al., *Biochemistry* **2008,** *47,* 5088–5100

His is also oxidized



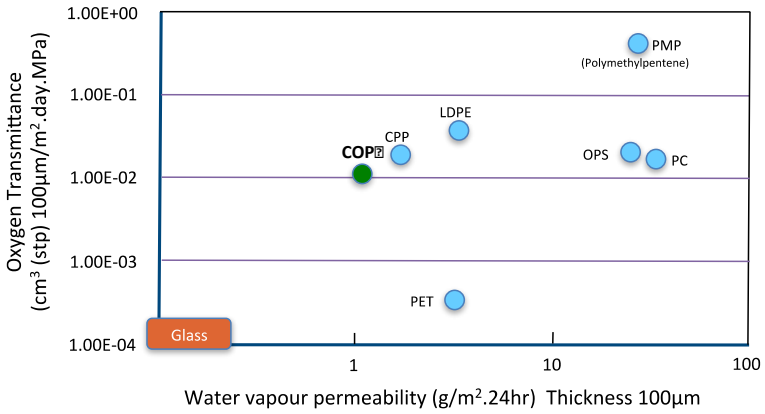


Amano, Uchiyama et al. (2014) *Anal. Chem.* **86**, 7536-43

- His at CH_2 is also oxidized when IgG1 was exposed to light.
- Oxidation could be suppressed by the decrease of the dissolved oxygen since O_2 is involved in His oxidation.

Prevention of oxidations

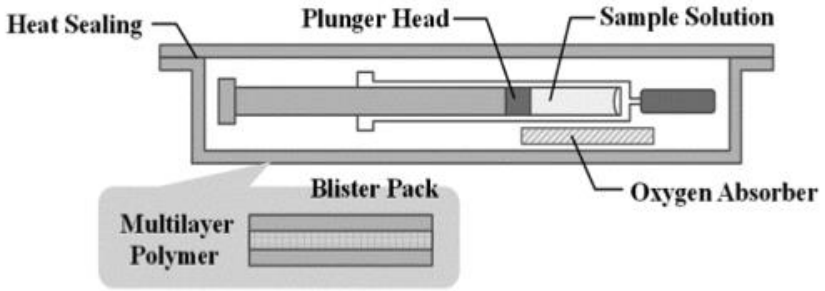
Oxygen transmittance & permeation



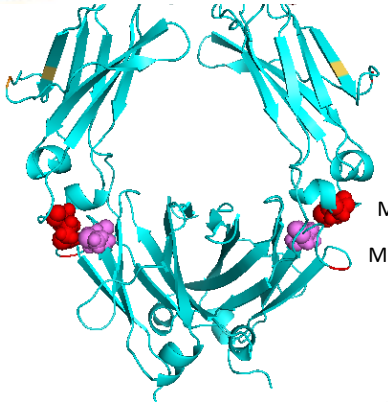
Nakamura K et al. PDA J Pharm Sci Technol 69(1), 88-95 (2015)

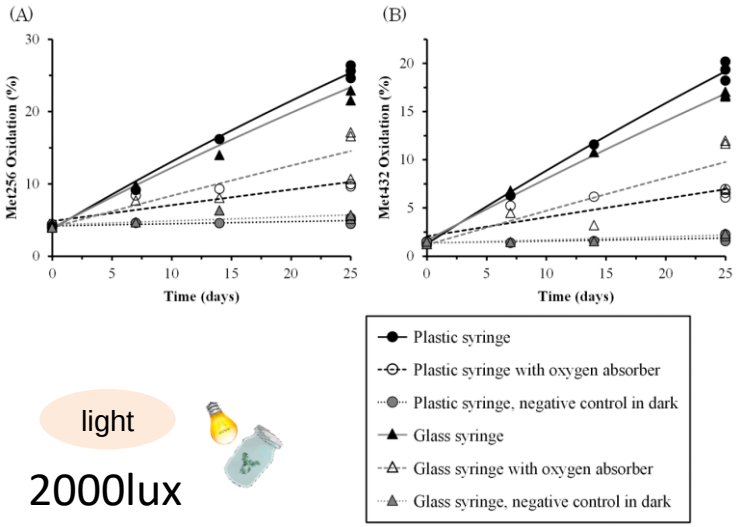
Prevention of Met oxidation
by improved container and closure system

1. Glass vs. COP
2. w oxygen absorber vs. wo oxygen absorber



Met oxidation of IgG by light exposure



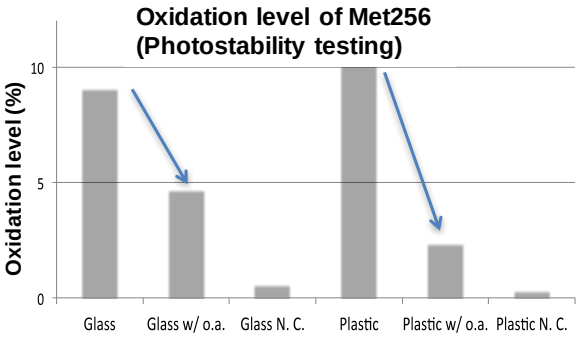


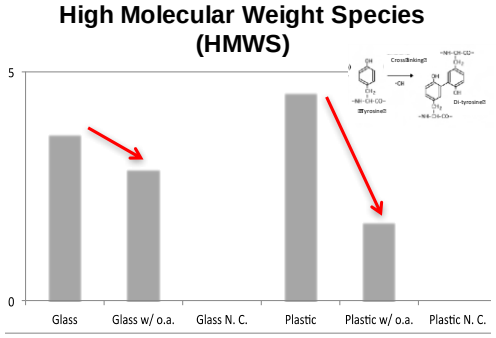
light

2000lux

First reaction constant of each species under the photostability testing.

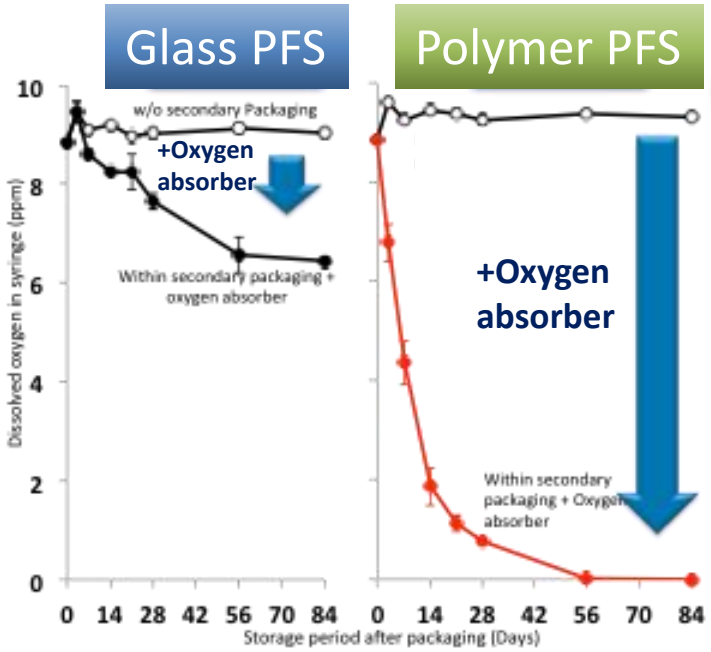
Light Stress	First reaction constant [$\times 10^{-3}$ day^{-1}]				
	Oxidation of Met256	Oxidation of Met432	APG	HMWS	LMWS
Plastic	10.17 (± 0.31)	8.00 (± 0.24)	31.76 (± 1.74)	4.53 (± 0.26)	1.20 (± 0.71)
Plastic w/ o.a.	2.35 (± 0.40)	2.04 (± 0.37)	13.80 (± 1.61)	1.71 (± 0.31)	0.71 (± 0.11)
Plastic N.C.	0.31 (± 0.10)	0.20 (± 0.07)	3.67 (± 0.09)	N/A	0.48 (± 0.08)
Glass	9.05 (± 0.64)	6.73 (± 0.13)	27.45 (± 0.47)	3.62 (± 0.15)	1.01 (± 0.10)
Glass w/ o.a.	4.66 (± 0.83)	3.63 (± 0.73)	19.40 (± 2.45)	2.86 (± 0.41)	0.92 (± 0.08)
Glass N.C.	0.58 (± 0.20)	0.34 (± 0.06)	3.52 (± 0.10)	N/A	0.50 (± 0.04)

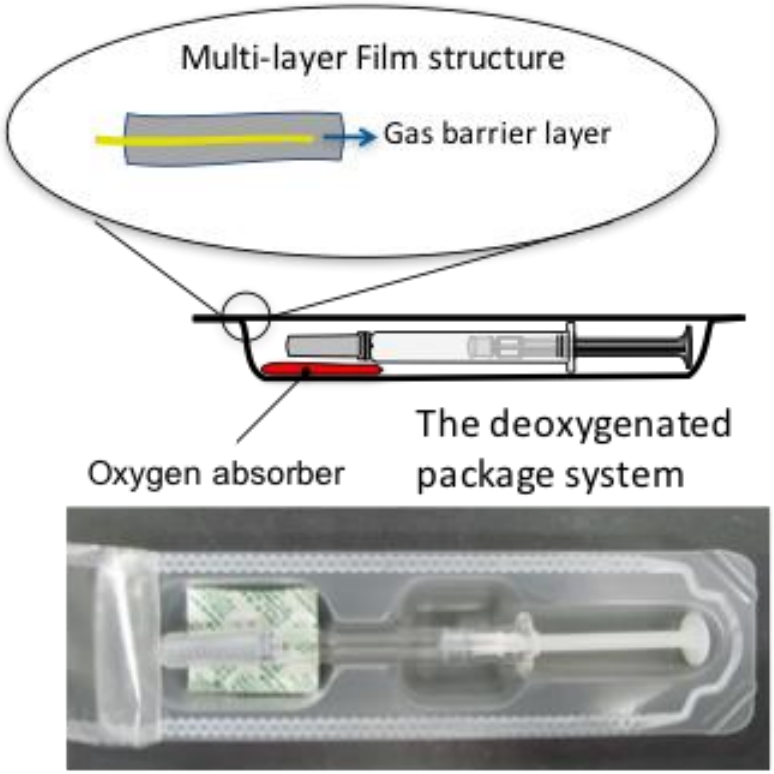




Amano, … Uchiyama, ***J. Pharm. Sci.***, 2016

Optimized PFS packaging system



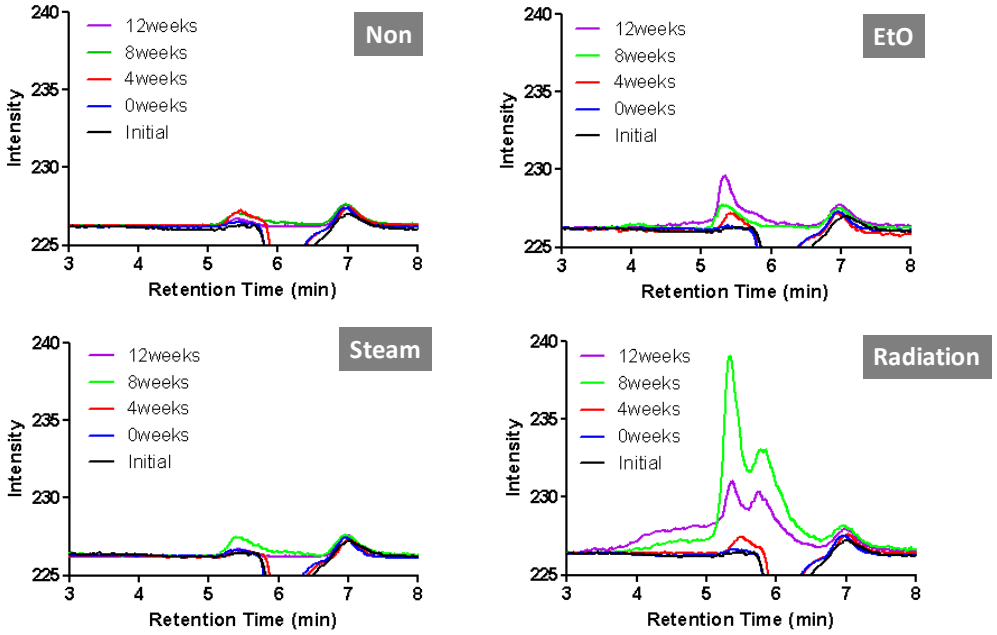


Nakamura K et al. : PDA. ***J. Pharm. Sci. Technol***. 2015

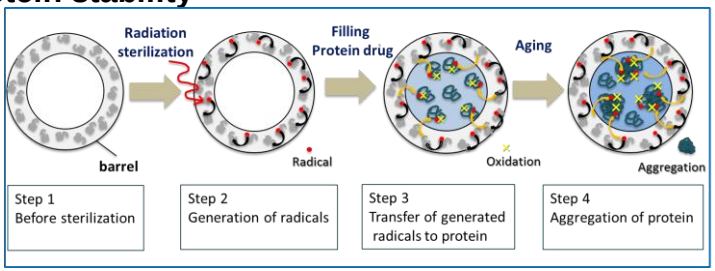
Amano, … Uchiyama, ***J. Pharm. Sci.***, 2016

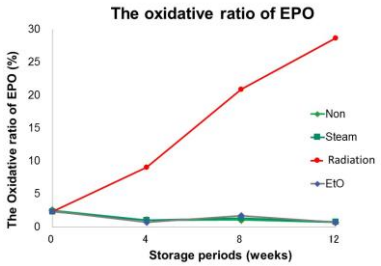
Proper sterilization method is neccessary

HMWS were detected in Radiation and EtO syringes



Steam is preferable sterilization method to keep protein stability





Steam is preferable sterilization method to reduce protein oxidation and particle formation in biopharmaceutical drugs.

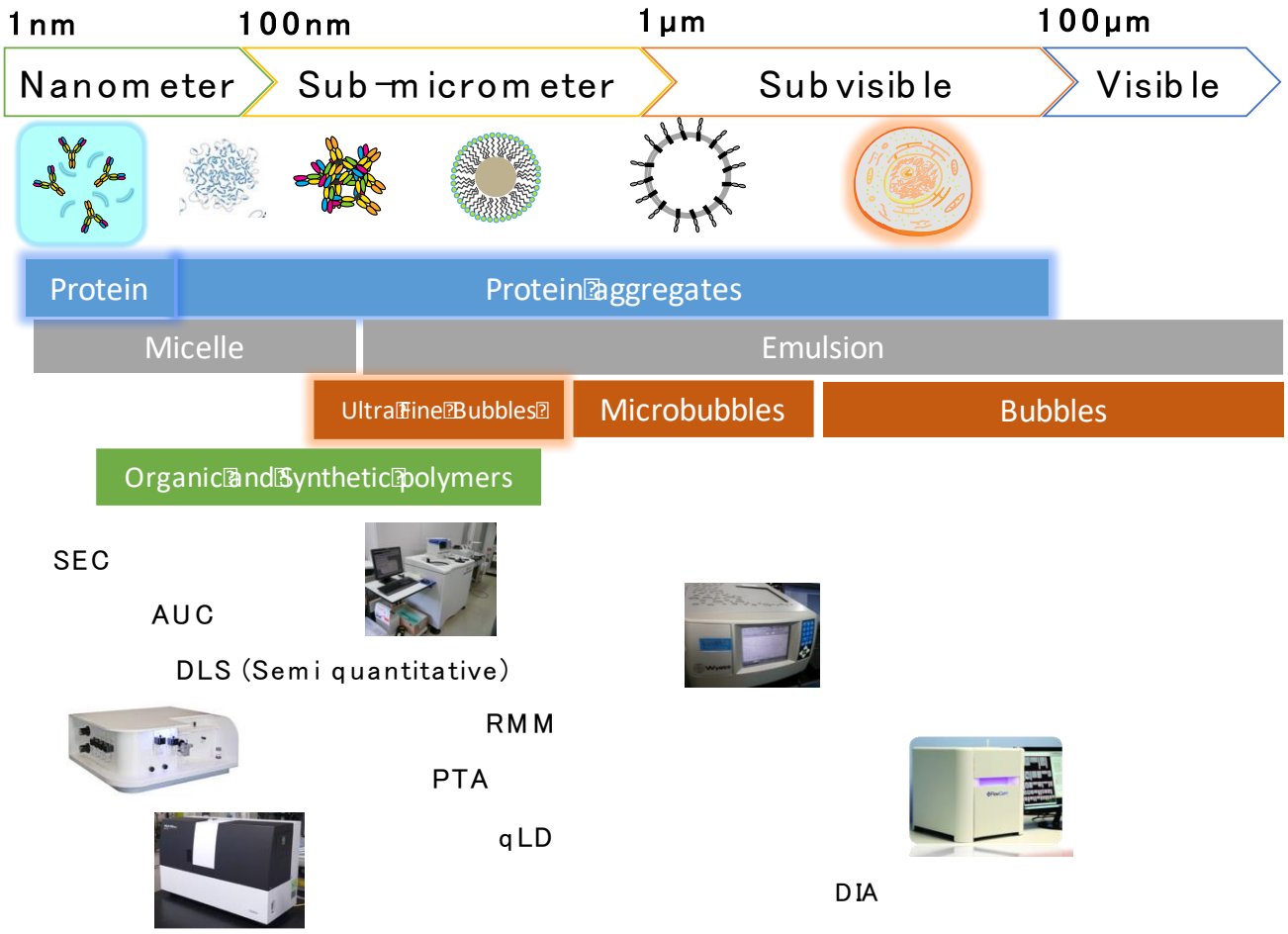
Kiminami H…Carpenter JF et al. : ***J Pharm Sci*** .2017

Summary

- ✓ In Glass syringes, oxygen absorber is effective to prevent oxidation of proteins in solutions.

- ✓ In COP syringes, proper container and closure system can
  (1) reduce oxidation of therapeutic proteins
  (2) reduce covalently bonded aggregates
  through effective removal of dissolved oxygen
  from protein solutions.

Aggregates quantification methods are required depending on the particle sizes

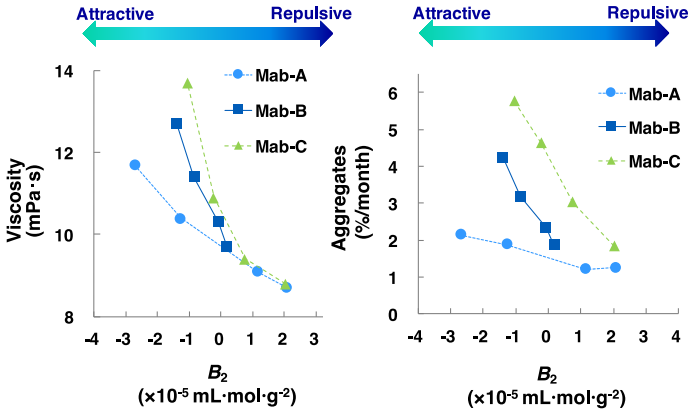


Factors related to protein aggregations

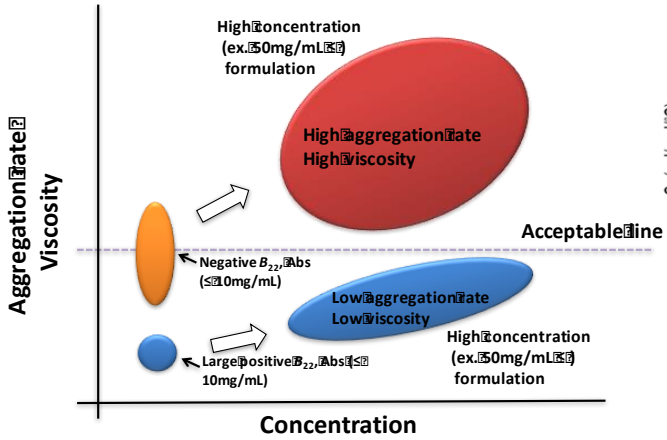
1. Stability attributed to protein solution.

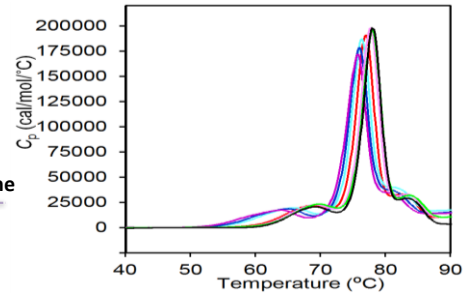
Colloidal stability & Conformational stability

150 mg/mL



< 10 mg/mL





Uchiyama, S. (2014) ***Biochim. Biophys. Acta.*** 1844, 2041-2052.
Saito, Uchiyama et al., (2013) ***Pharm Res.*** 30, 1263-1280
Saito, Uchiyama et al., (2012) ***Pharm Res.*** 29, 397-410
Nishi, Uchiyama et al., (2010) ***Pharm. Res.*** 27, 1348–1360

2. Silicone oil/water interfaces.

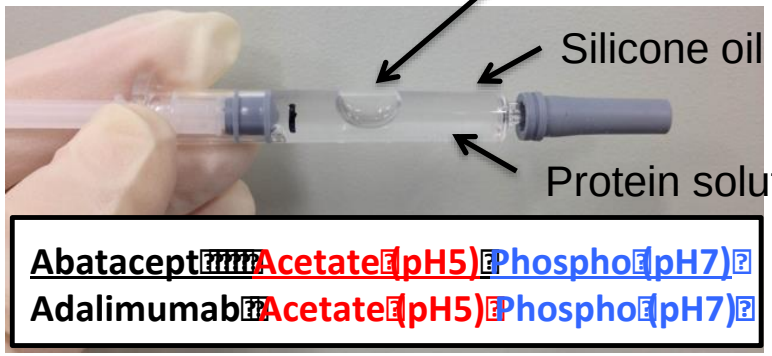
3. Protein adsorption.

Silicone oil induces protein aggregates

- Silicone oil affect insulin stability (disposable syringe, Bernstein, Diabetes Care, 1987).
- Silicone oil induced aggregation of proteins (Jones et al., J Pharm Sci. 2005).
- Silicone oil- and agitation-induced aggregation (Thirumangalathu R, et al., J Pharm Sci. 2009)
- Protein aggregation and particle formation in prefilled glass syringes (Gerhardt A et al., J Pharm Sci. 2014).

Influence of aggitation and silicone oil coating in PFS

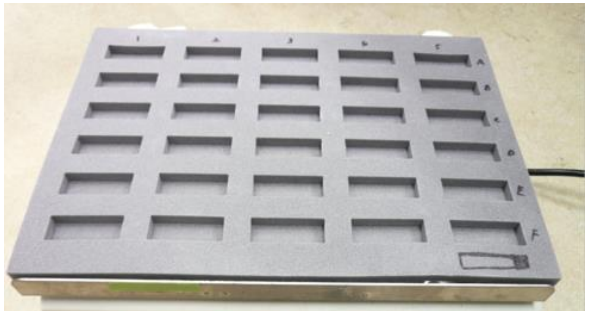
COP (Cyclo-olefin polymer) syringe



Abatacept Acetate (pH5) Phospho (pH7)
Adalimumab Acetate (pH5) Phospho (pH7)

Conditions

1. Glass (borosilicate) + SO (silicone oil)
2. Glass (borosilicate) SO Free
3. COP + SO (silicone oil)
4. COP SO Free

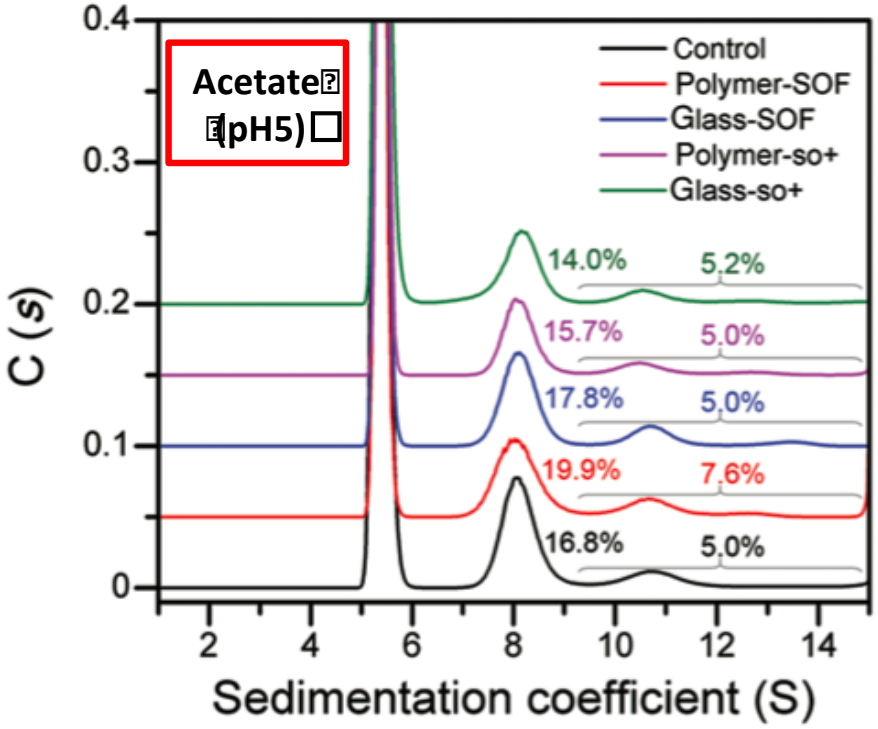


500 rpm/min shaking
4℃
1 week

Effect of silicone oil on small aggregates formation –SEC and SV-AUC–

Table 2. Aggregates Quantification Results by SEC

Sample	Insoluble Aggregates (%)[a]	Soluble Species (%)[b]		
		Monomer	Dimer	Higher-Order Aggregates
Acetate Buffer				
Control	4	93.2 ± 0.0	6.8 ± 0.0	–
Polymer-SOF	4	88.5 ± 0.0	11.5 ± 0.0	–
Glass-SOF	4	90.9 ± 0.0	9.1 ± 0.0	–
Polymer-so+	14	89.3 ± 0.1	10.7 ± 0.1	–
Glass-so+	19	92.6 ± 0.0	7.4 ± 0.1	–

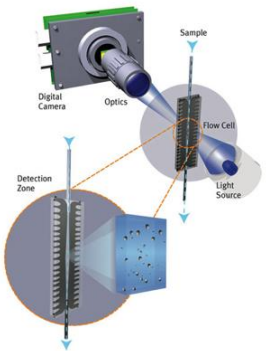


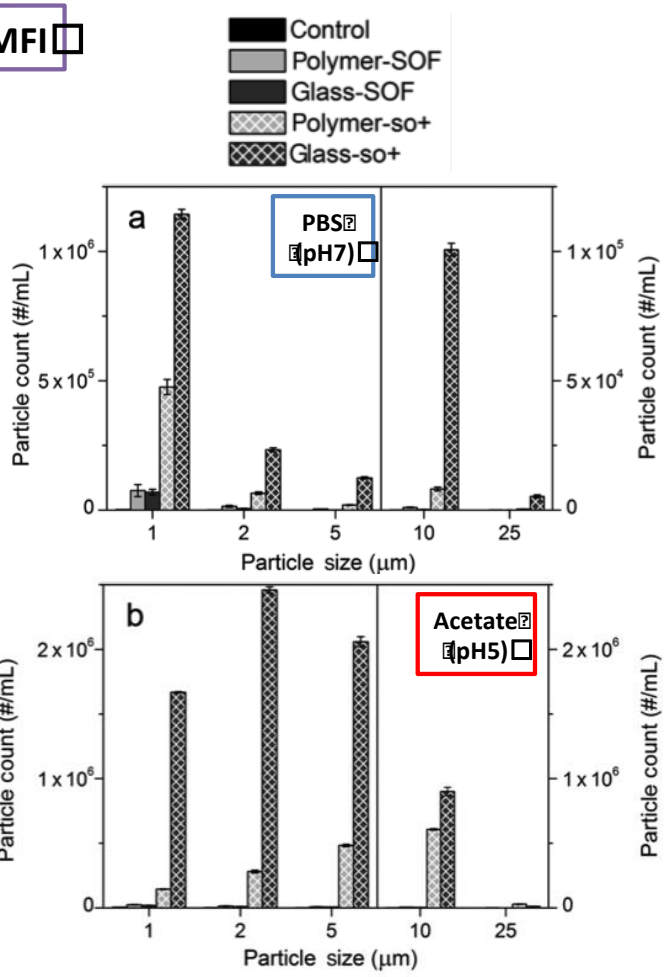
- ✓ SV-AUC detects small aggregate with higher efficiency than SEC
- ✓ Small aggregates was slightly increased

Krayukhina E., et al (2015) *J. Pharm. Sci* 104, 527-535

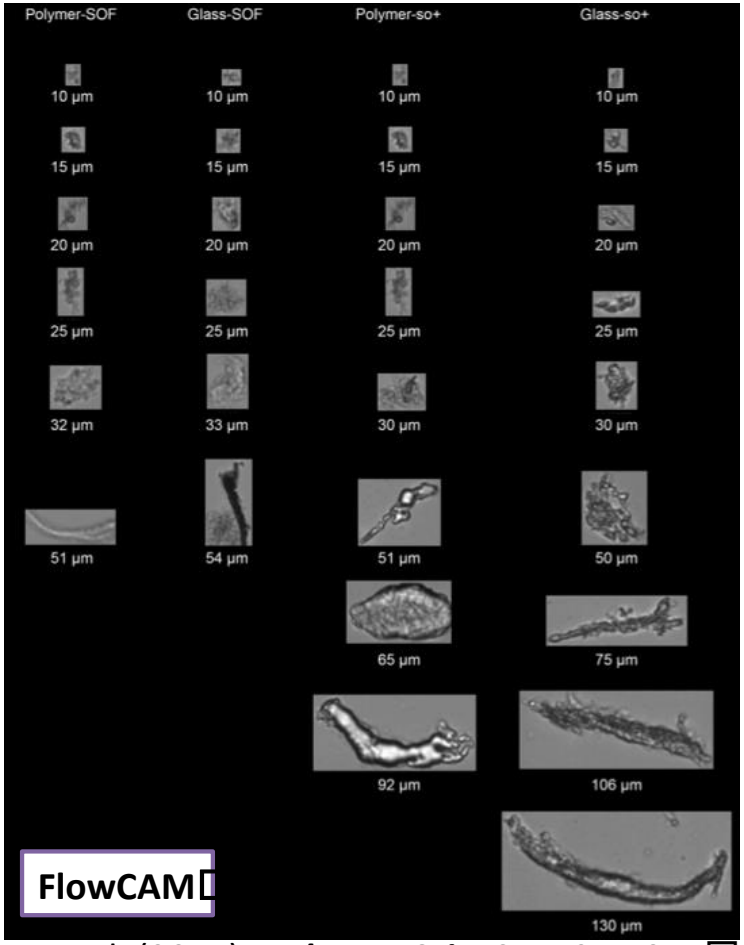
Effect of silicone oil on SVP formation –FI–

MFI





PBS (pH7)
Polymer-SOF Glass-SOF Polymer-SO+ Glass-SO+



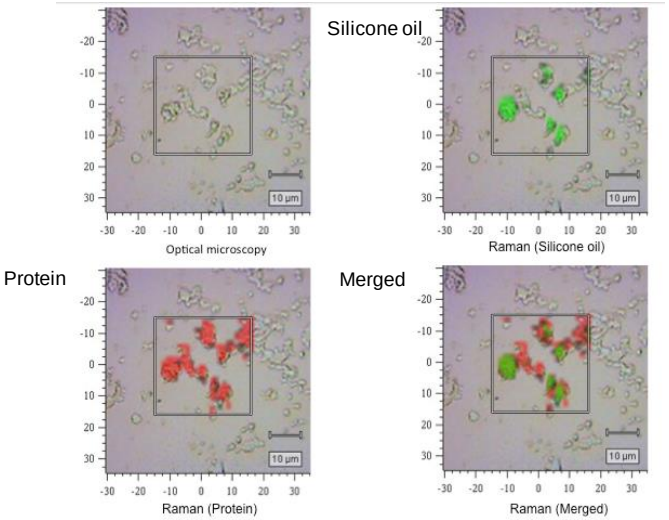
FlowCAM

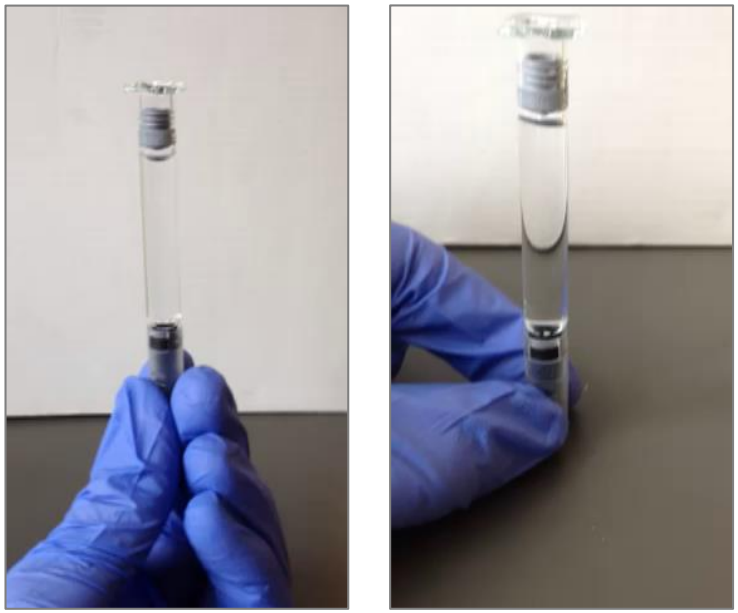
COP<Glass<<COP+SO<Glass+SO

Krayukhina, E., et al. (2015) *J. Pharm. Sci.* 104, 527-535.

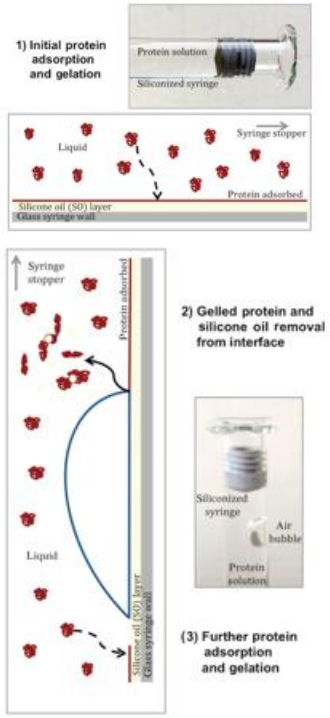
Possible mechanism of aggregates formation by silicone oil

- ✓ Silicone oils induce SVP formation composed of protein aggregates. (Not small aggregates)
- ✓ COP syringe can reduce protein aggregates formation.
- ✓ Glass syringe can also reduce protein aggregates formation if silicone oils are not lubricated on it.





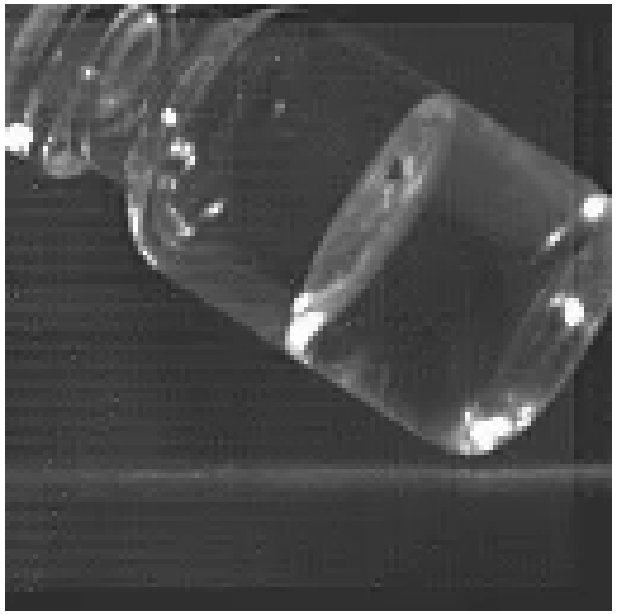
courtesy of **Prof. Randolph**

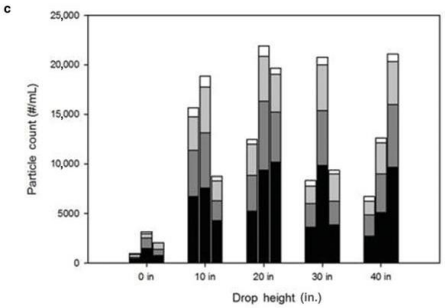


Gerhardt et al., JPS 2014

Vials

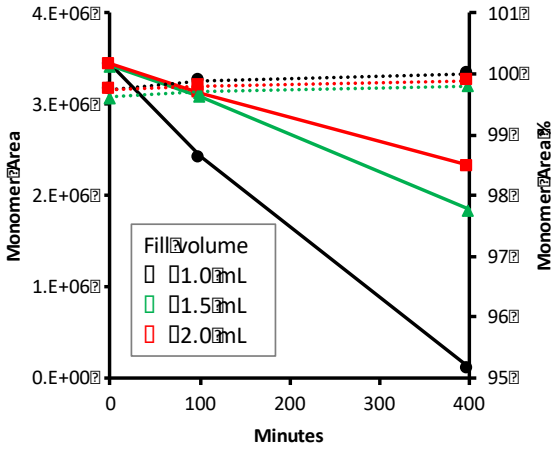
Cavitation by dropping induces aggregation



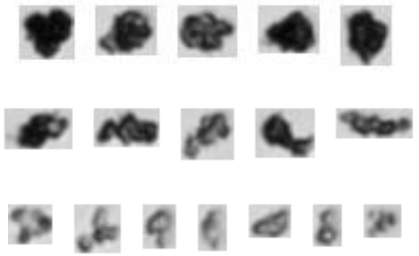


Randolph et al., 2015

Friability testing
dropping and shaking induces aggregation



Solid line: Monomer Area
Dotted line: Monomer Area %

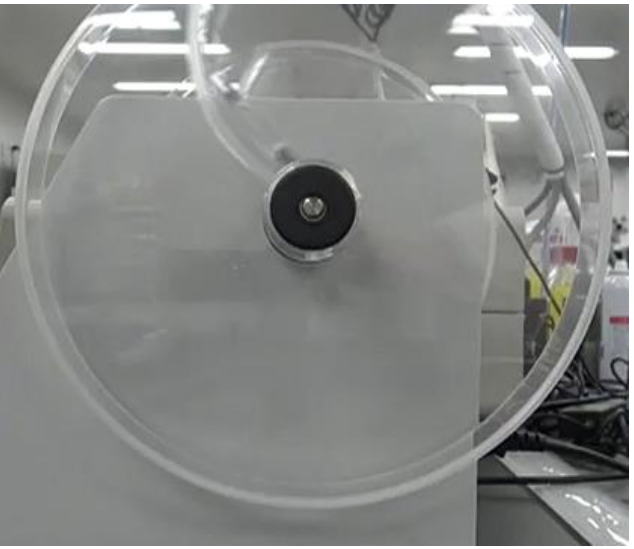


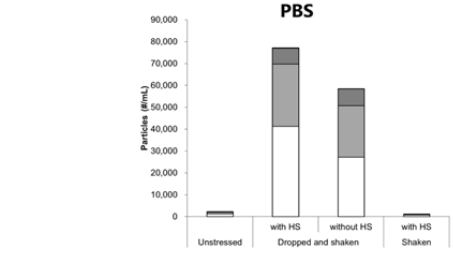
Torisu, T., et al., *J. Pharm. Sci.* **2017**, *106*, 521-529.

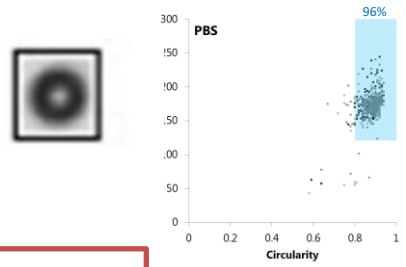
Friability test of mAbs in PFS [SO-]
A large amount of protein aggregates are generated.

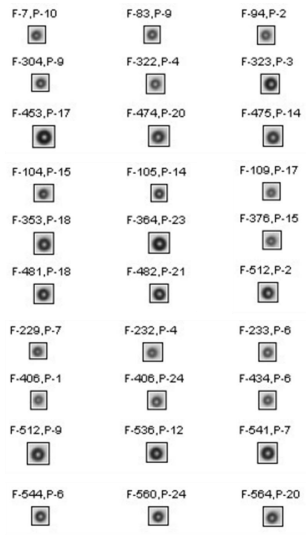
Prefilled syringe

(SO+, no protein) >> SO droplets increase

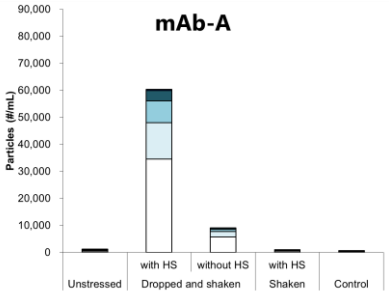


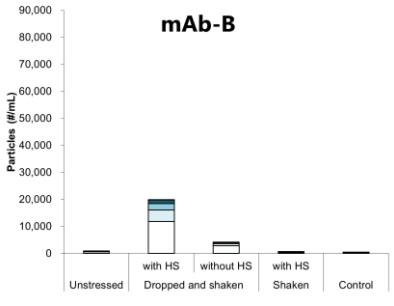




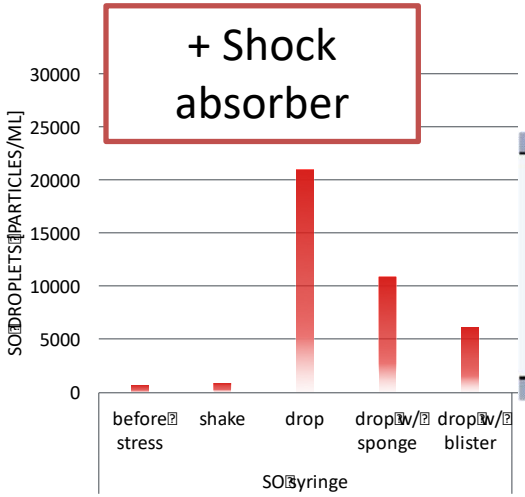


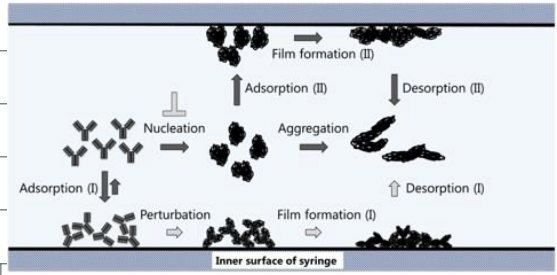
SO- syringe >> protein aggregates increase





+ Shock absorber





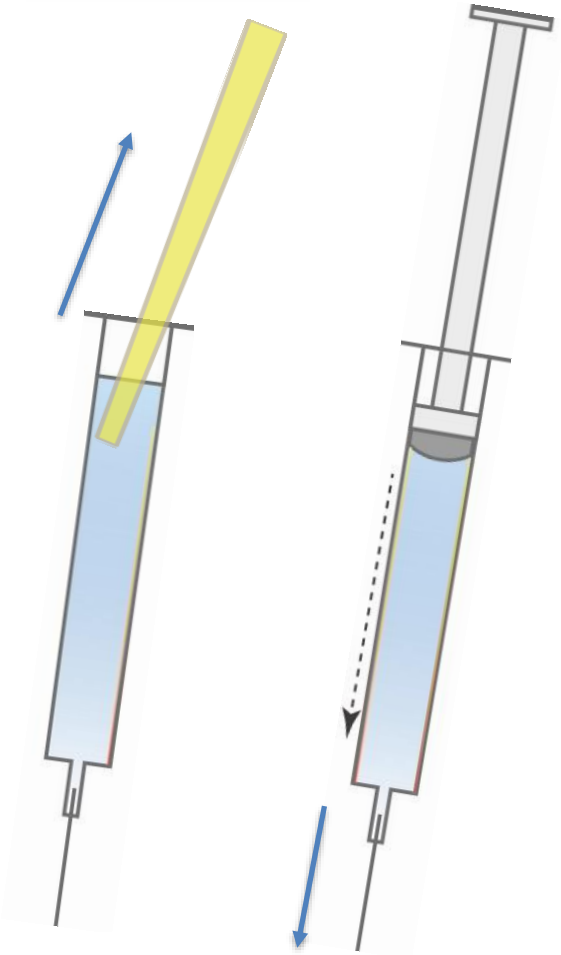
Torisu T, et al., Friability testing as a new stress-stability assay for biopharmaceuticals. *J. Pharm. Sci.* **2017**, *106*, 2966-2978.

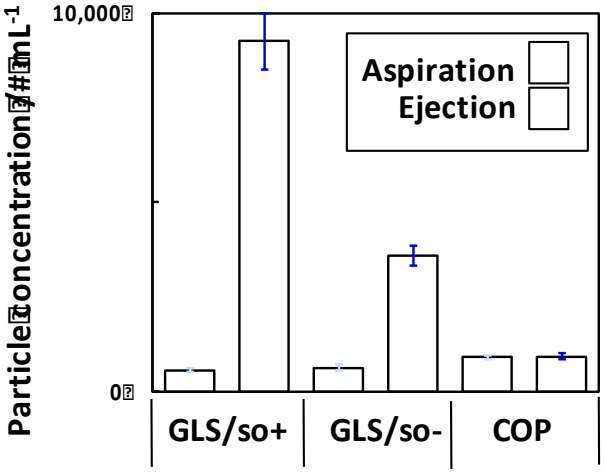
Adsorption leads to protein aggregates generation

1 week quiescent incubation

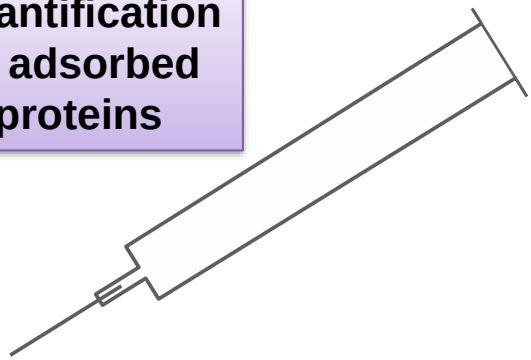
Aspiration

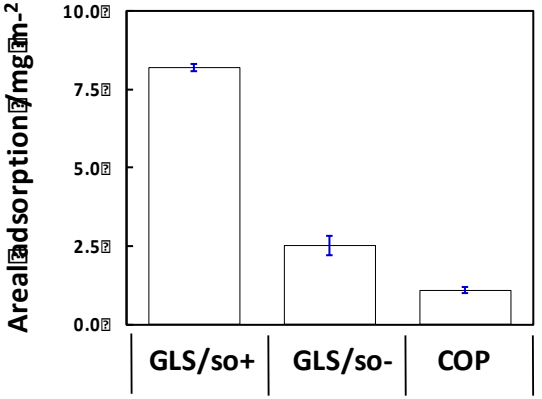
Ejection

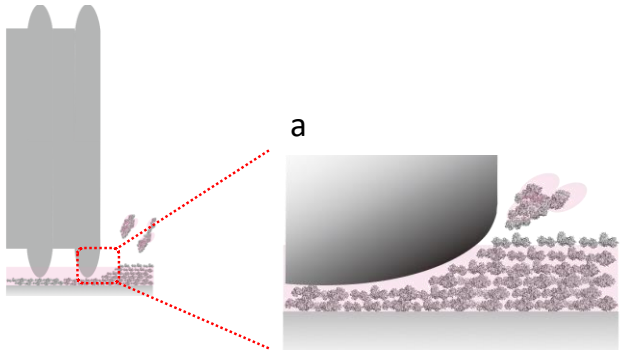




Quantification of adsorbed proteins

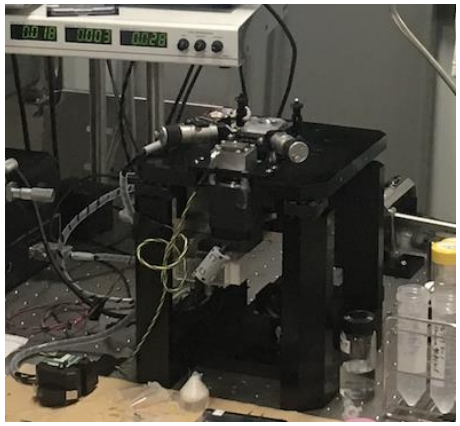




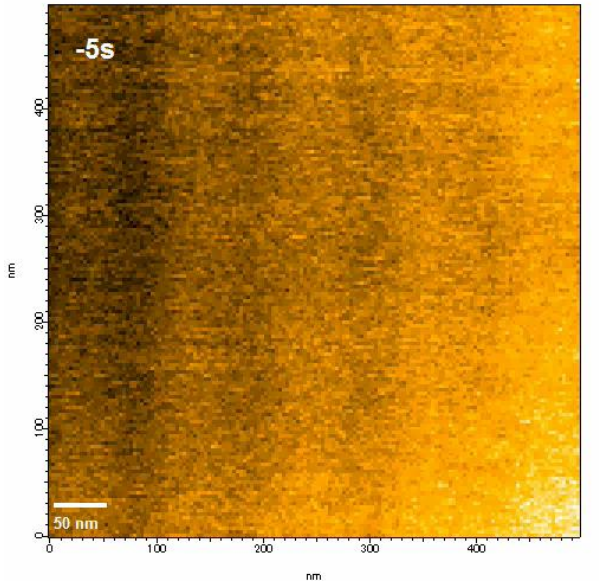


Real time observation of mAb adsoption on surfaces

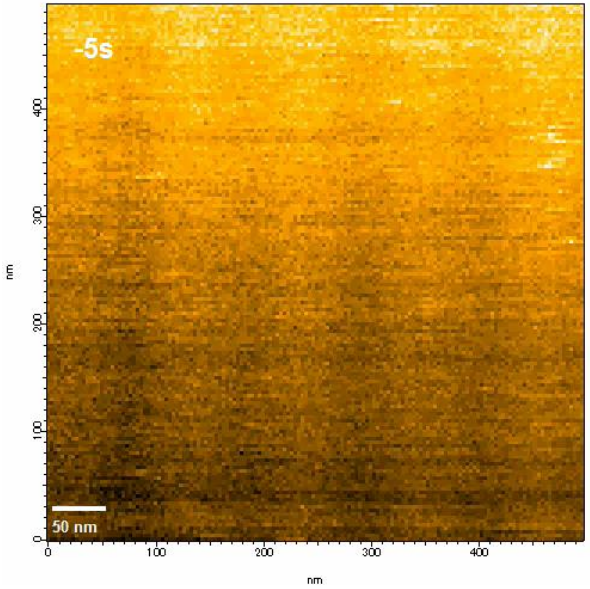
Real time monitoring of adsorption by
***In Solution* High Speed Atomic Force Microscopy**



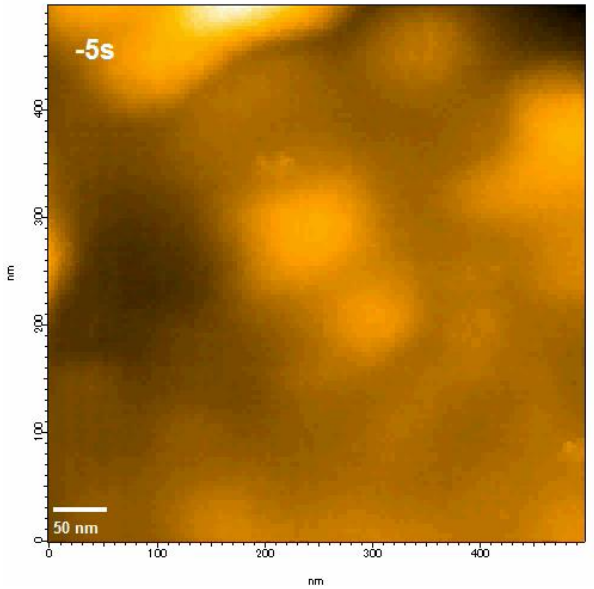
Glass/so-



Glass/so+



COP

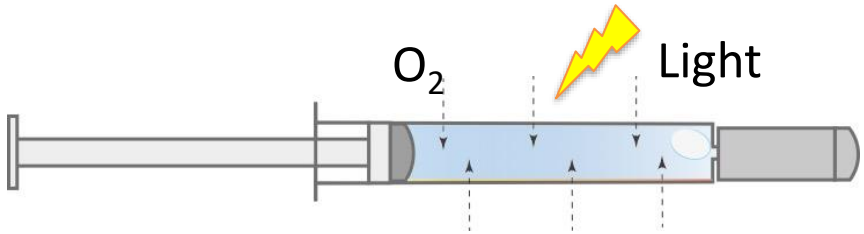


Summary:

Factors affecting stabilities of biopharmaceuticals in prefilled syringe

Besides, formulation optimization

Oxidation can be prevented by proper CCS.
Dark condition for storage is also important

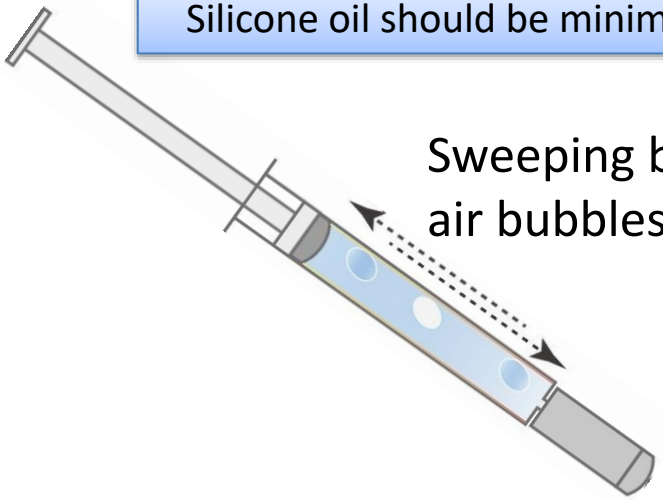


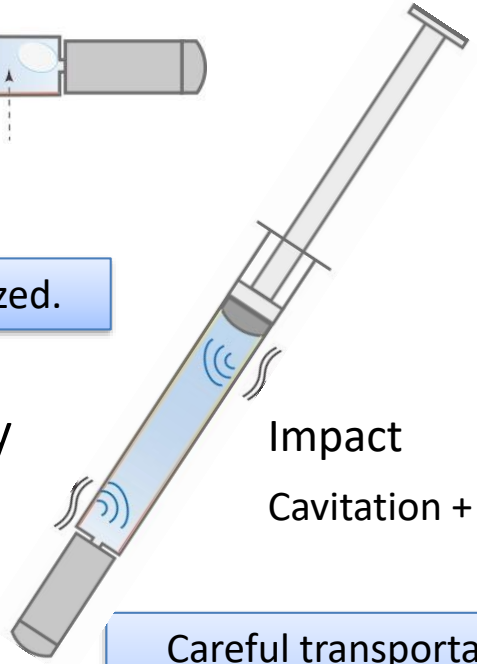
Steam is effective

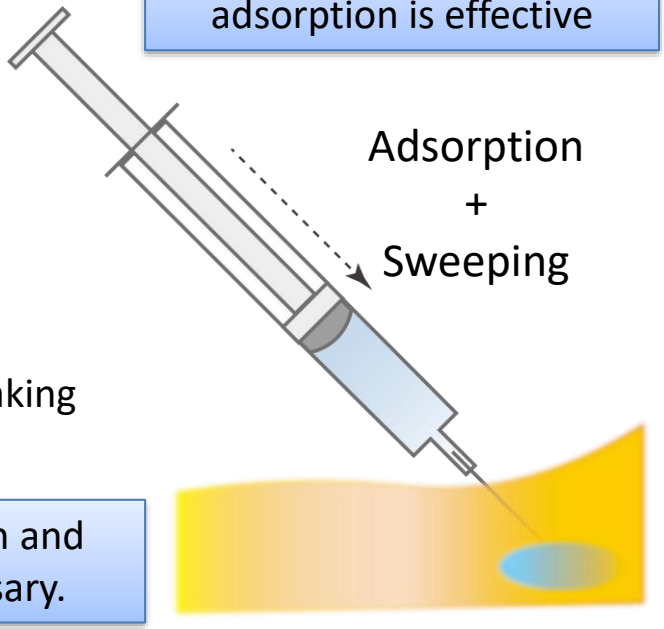
Sterization

Minimizing protein adsorption is effective

Silicone oil should be minimized.







Careful transportation and handling are neccessary.

Terumo

Keisuke Yoshino
Yoshihiko Abe
Koji Nakamura
Sayaka Shinoda

Osaka University
U-Medico Inc.

Masanori Noda
Elena Krayukhina
Masami Yokoyama
Takahiro Maruno

Nihon ZEON

Satoru Adachi
Kunihito Arai
Taichi Sawaguchi

Acknowledgements

Daiichi Sankyo Inc.

Hirotaka Nishi
Shuntaro Saito
Masato Amano
Jun Hasegawa
Naoki Kobayashi

Takeda Pharm. Inc.

Tetsuo Torisu
Yoshinori Hamachi

DNP

Tadashi Nakajima
Masanori Kagota

Nagoya University

Takayuki Uchihashi
Hiroki Watanabe

University of Tokyo

Kohei Tsumoto