New aspects on the degradation mechanisms of polysorbate: complex reaction pathways of a complex surfactant

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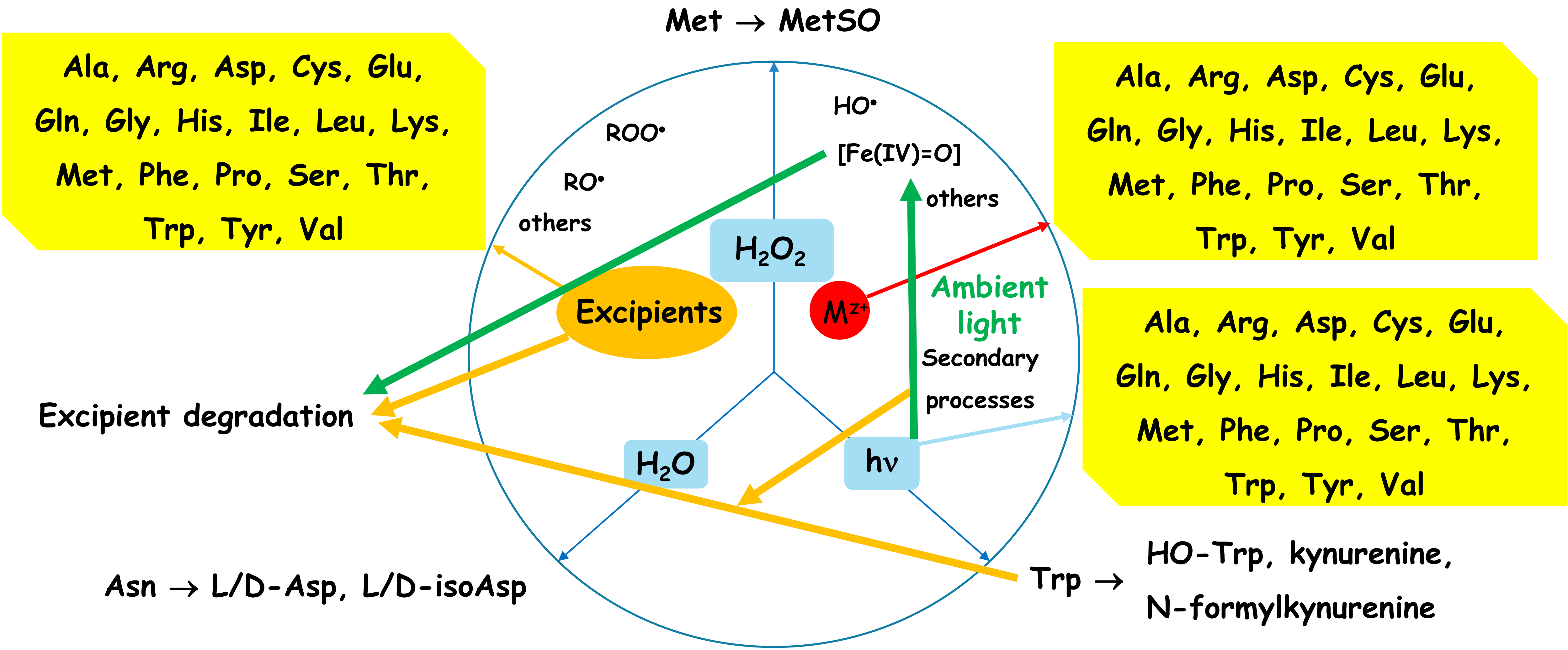
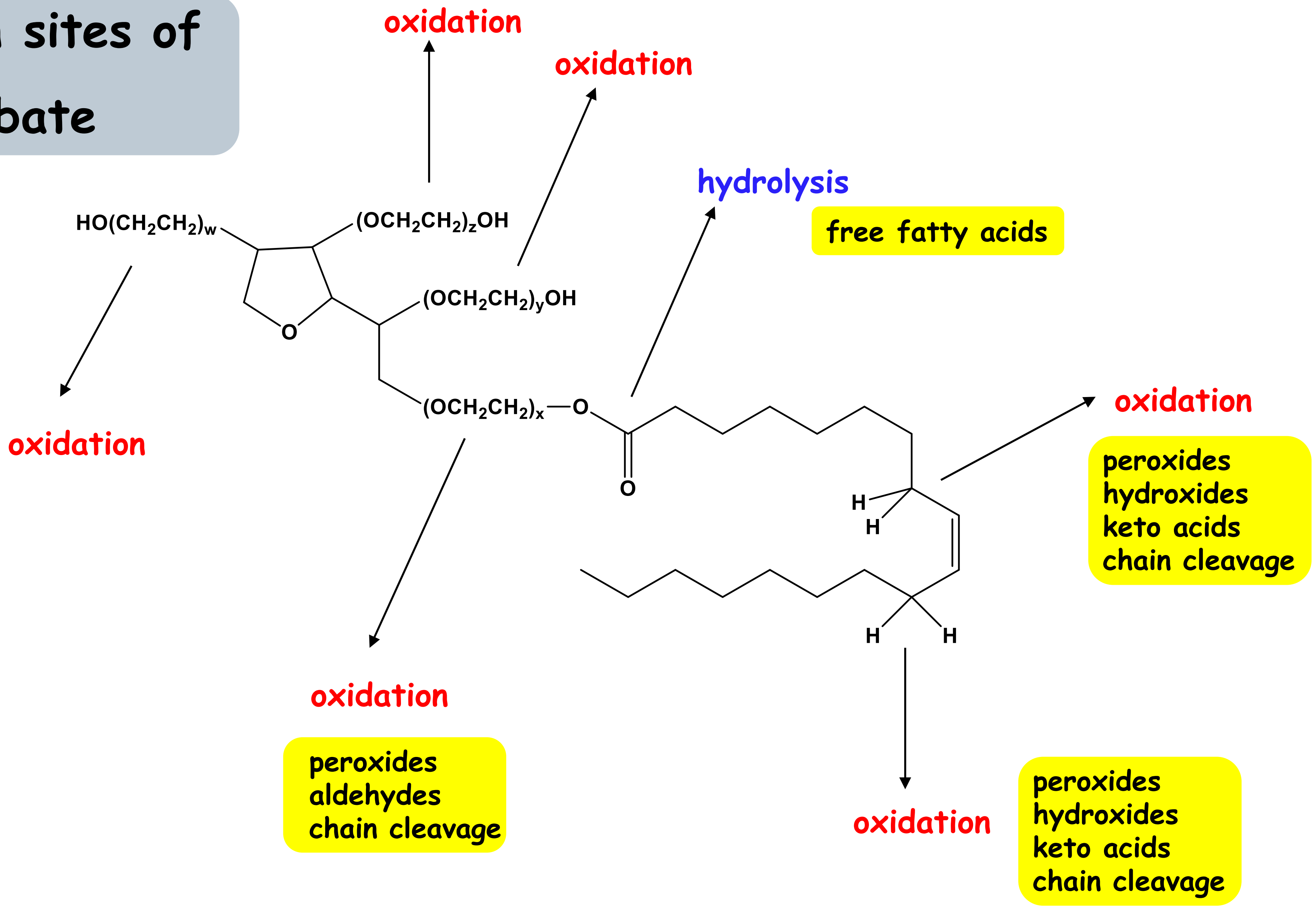
CASSS CMC Strategy Forum

Washington, DC, January 27, 2020

The full scope of chemical degradation of a protein formulation…



Degradation sites of polysorbate



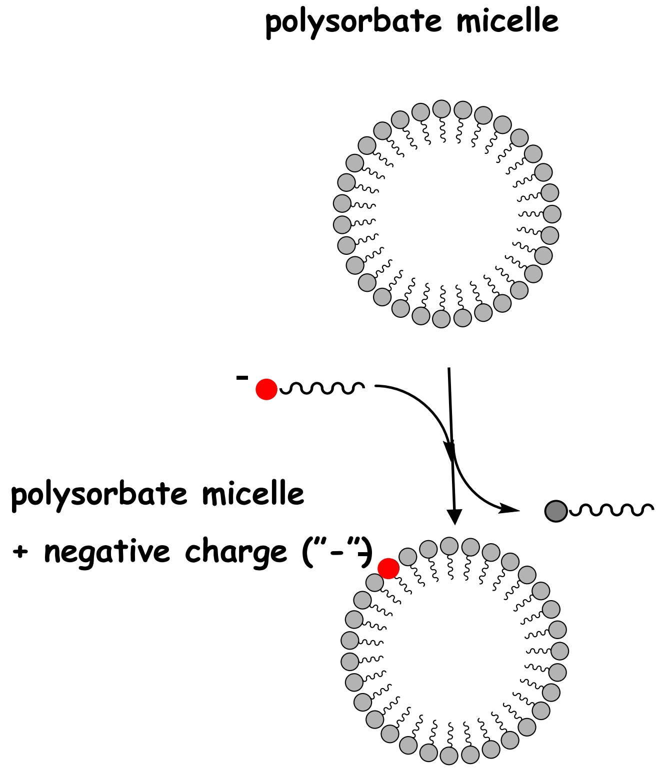
molecular pharmaceutics
DOI: 10.1021/acs.molpharmaceut.5b00310
Mol. Pharmaceutics 2015, 12, 3792−3804
Article
pubs.acs.org/molecularpharmaceutics

Understanding Particle Formation: Solubility of Free Fatty Acids as Polysorbate 20 Degradation Byproducts in Therapeutic Monoclonal Antibody Formulations

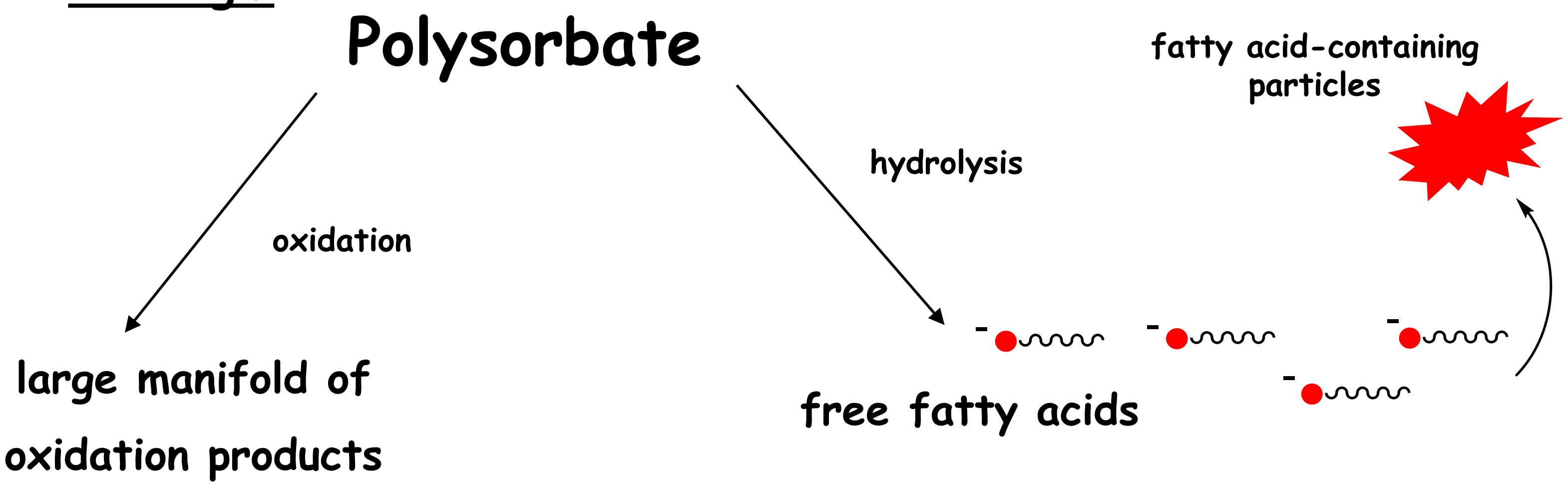
Nidhi Doshi, Barthélemy Demeule, and Sandeep Yadav*
Late Stage Pharmaceutical Development, Genentech Inc., 1 DNA Way, South San Francisco, California 94080, United States

Incorporation of free fatty acid into micelles

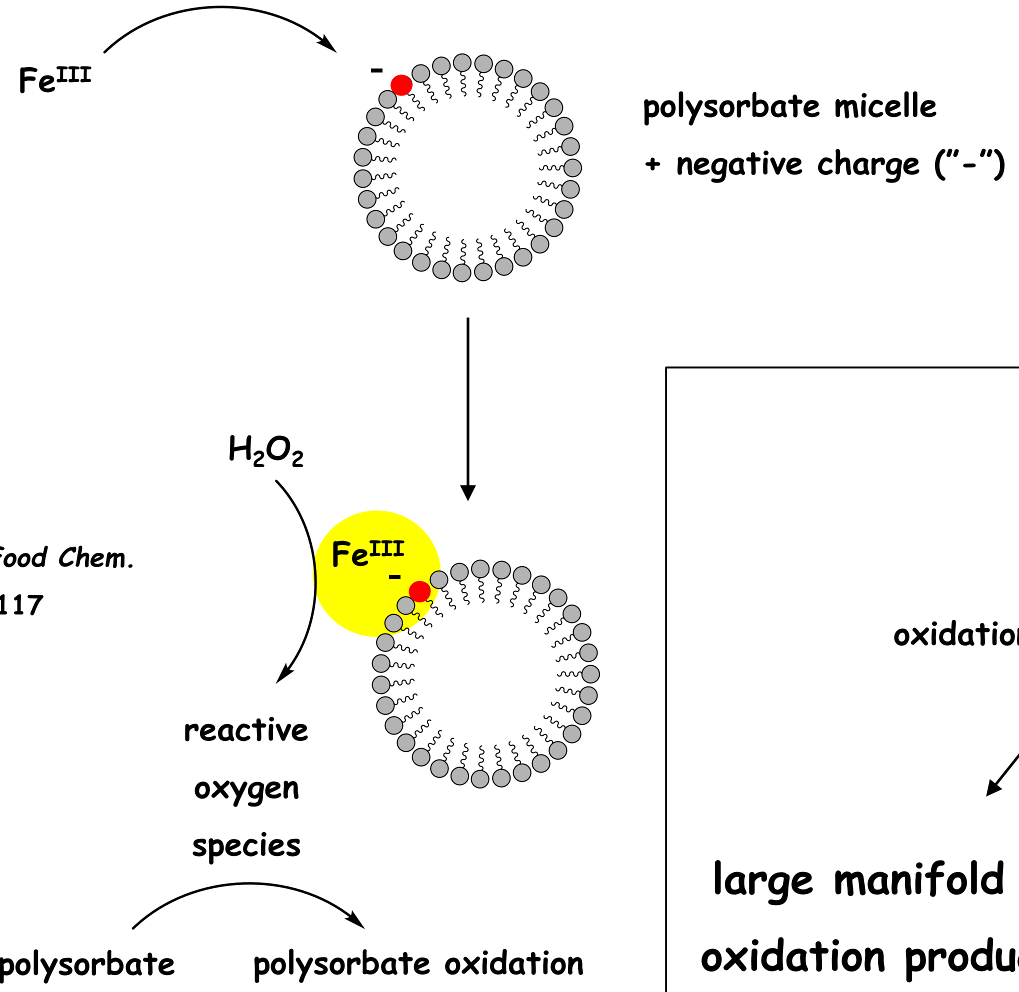
Waraho et al. *J. Agric. Food Chem.* 2009, 57, 7112-7117



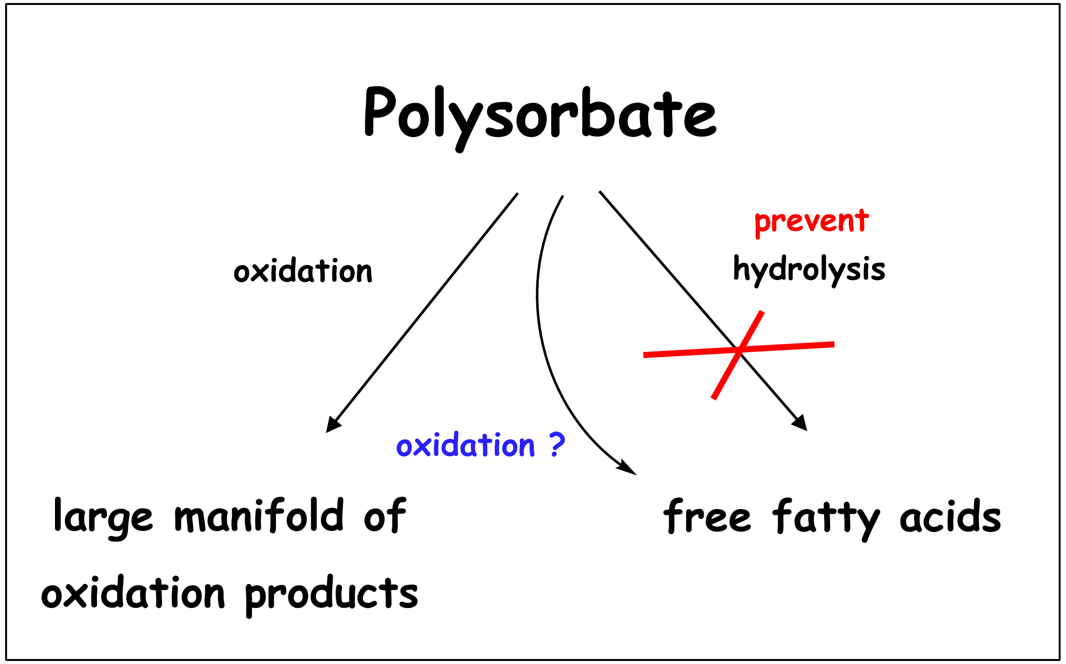
__Paradigm__:



Negatively charged free fatty acids in micelles attract redox active transition metals



Waraho et al. *J. Agric. Food Chem.* 2009, *57*, 7112-7117



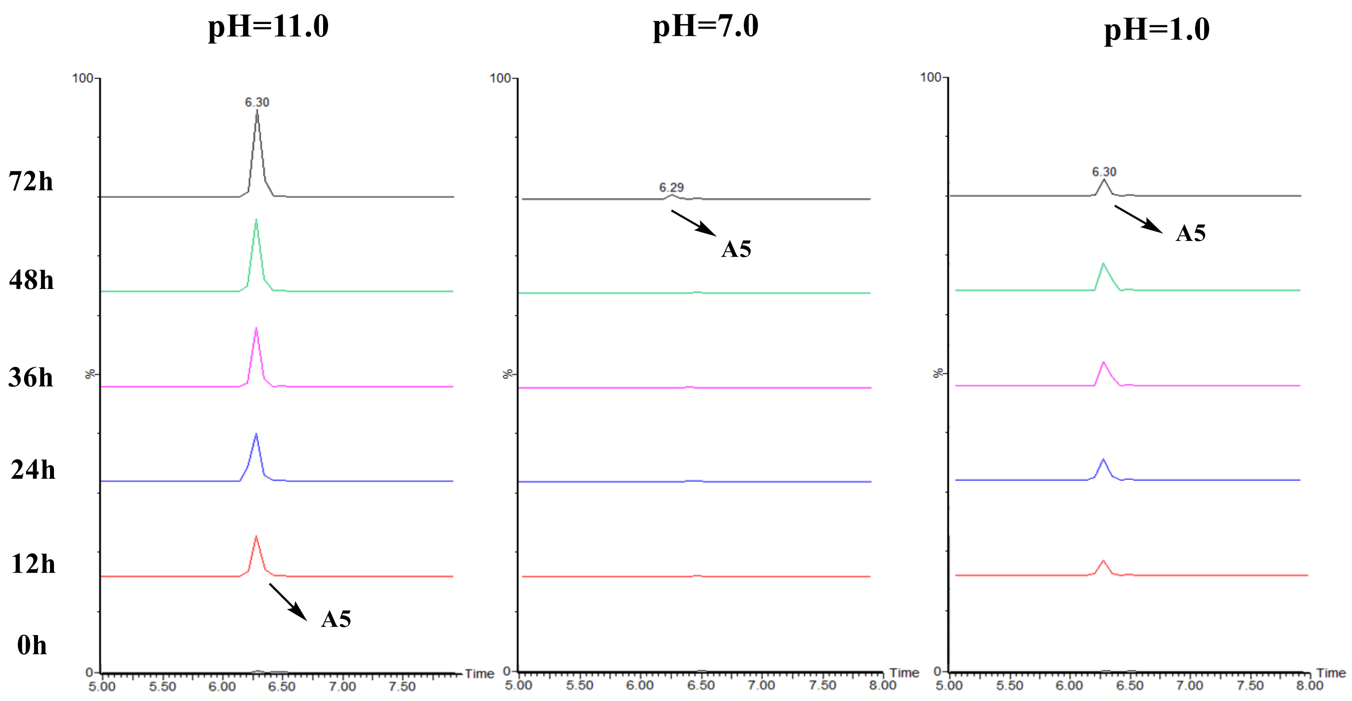
→ Free fatty acid formation via polysorbate oxidation

Degradation Mechanisms of Polysorbate 20 Differentiated by ^{18}O-labeling and Mass Spectrometry

L. Zhang, S. Yadav, B. Demeule, Y. J. Wang, O. Mozziconacci and Ch. Schöneich

Pharm. Res. 2017, *34*, 84-100

72 h hydrolysis



Chromatograms of free lauric acid released from all-laurate PS20 at different pH values at 40°C over 72 hours, shown as Peak A5: $[M-H]^-$ with m/z 199.19

Degradation Mechanisms of Polysorbate 20 Differentiated by ^{18}O-labeling and Mass Spectrometry

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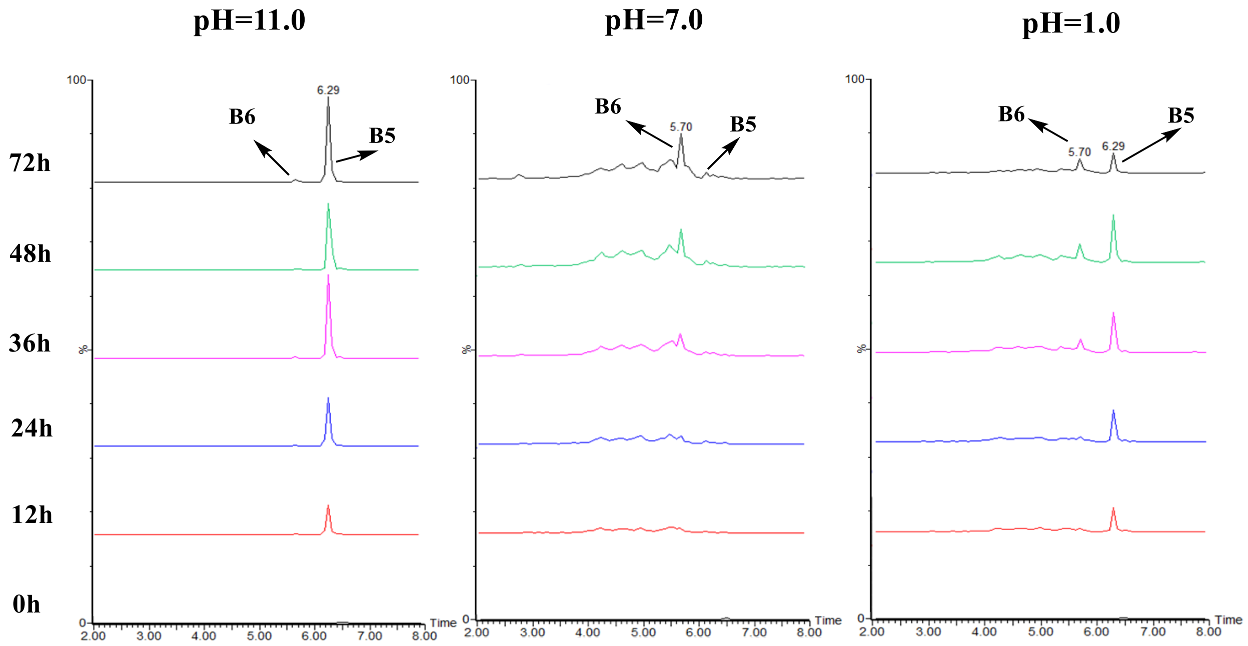
Pharm. Res. 2017, *34*, 84-100

72 h AAPH



Chromatograms of free lauric acid released from all-laurate PS20 exposed to AAPH at 40°C for 72hours

Degradation Mechanisms of Polysorbate 20 Differentiated by ^{18}O-labeling and Mass Spectrometry

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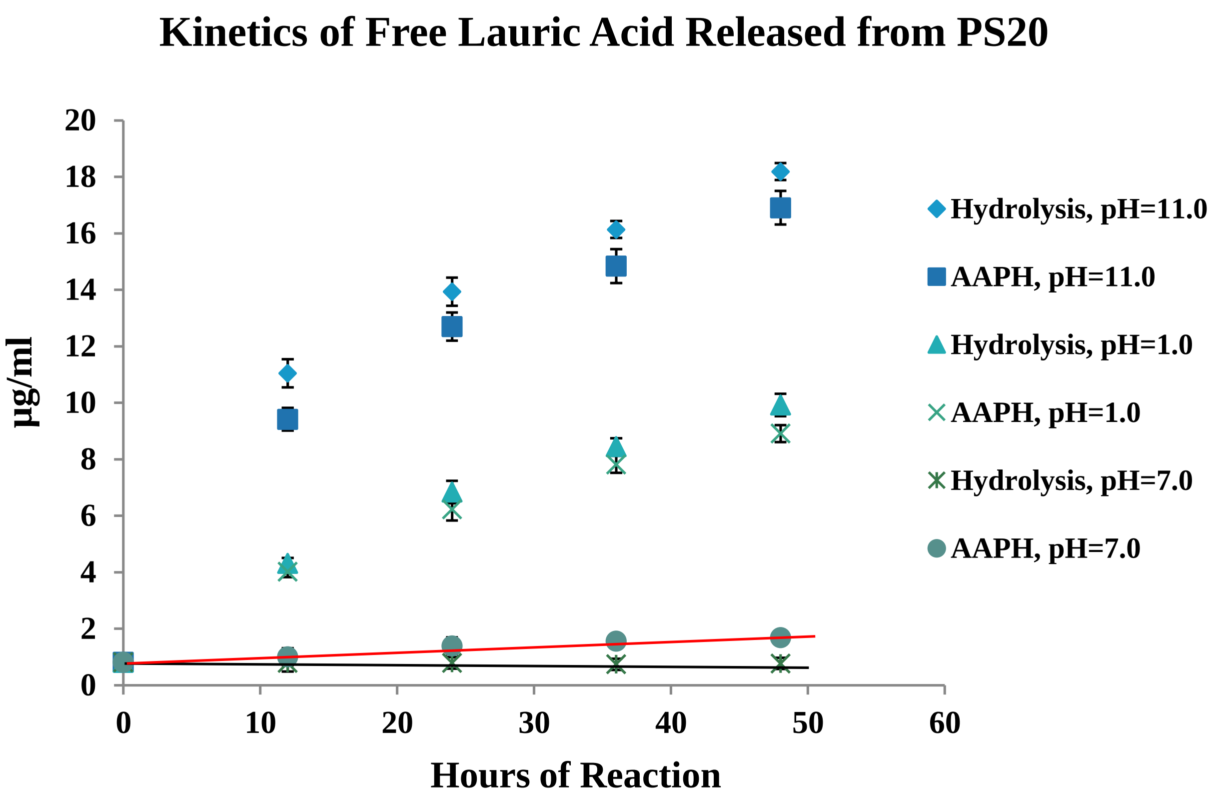
Pharm. Res. 2017, *34*, 84-100

40°C



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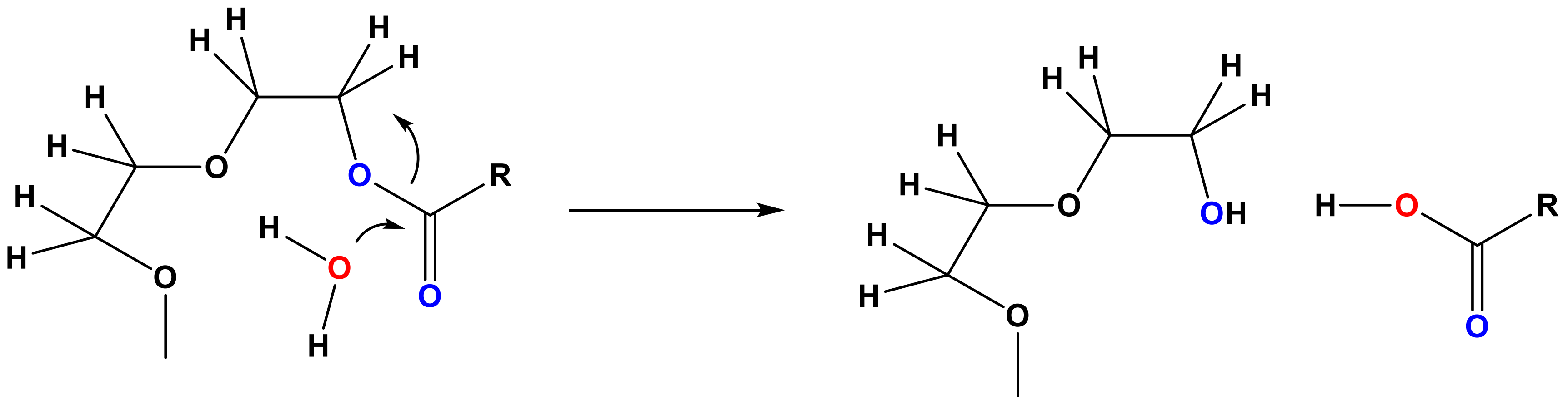
Pharm. Res. 2017, *34*, 84-100

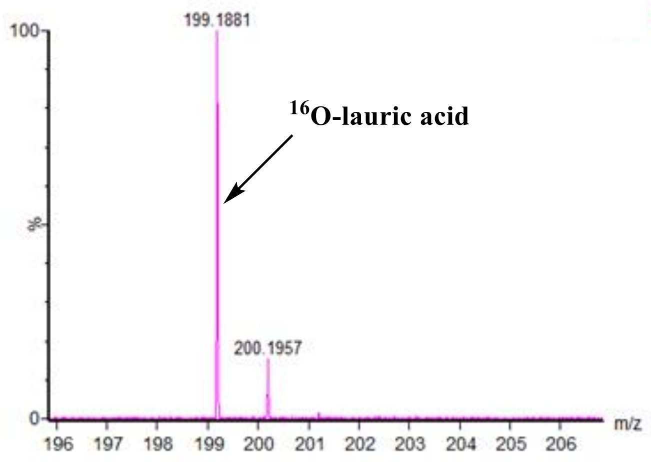
What is the mechanism of oxidative free fatty acid formation ?

Hydrolysis of the ^{16}O-fatty acid ester in ^{18}O-water

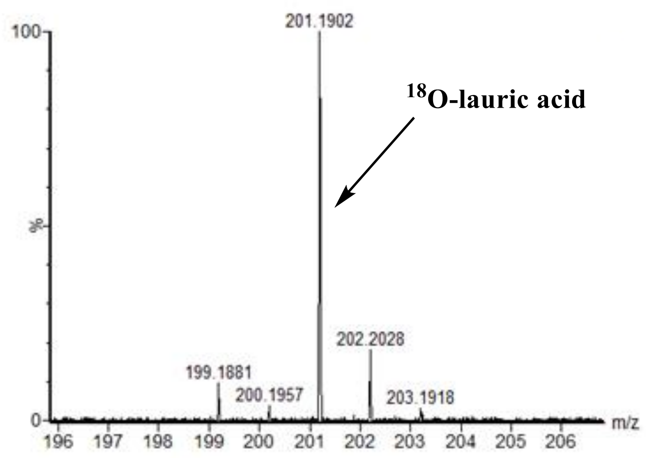


__Expectation__: Full incorporation of 1 eq ^{18}O

(A) **Hydrolysis of PS20 in $H_2{}^{16}O$, pH=11.0, 48h**

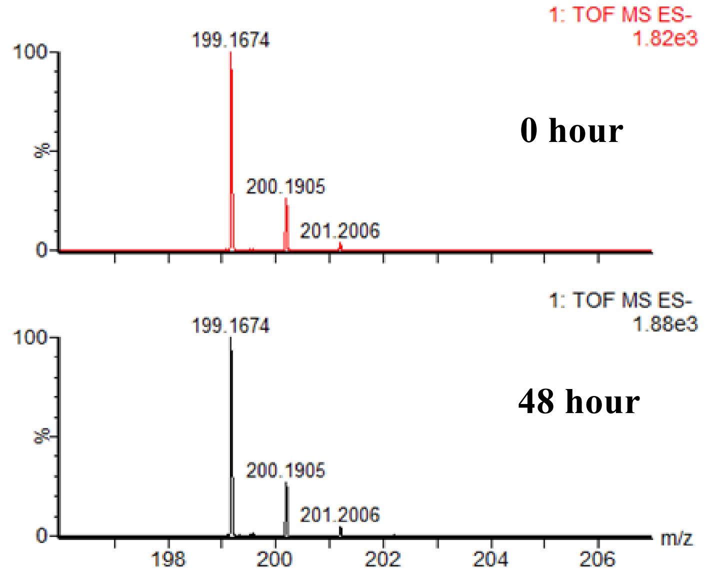


Hydrolysis of PS20 in $H_2{}^{18}O$, pH=11.0, 48h

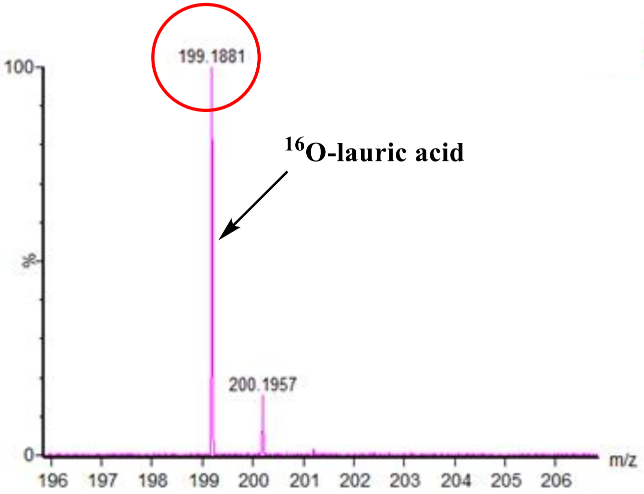


Control:

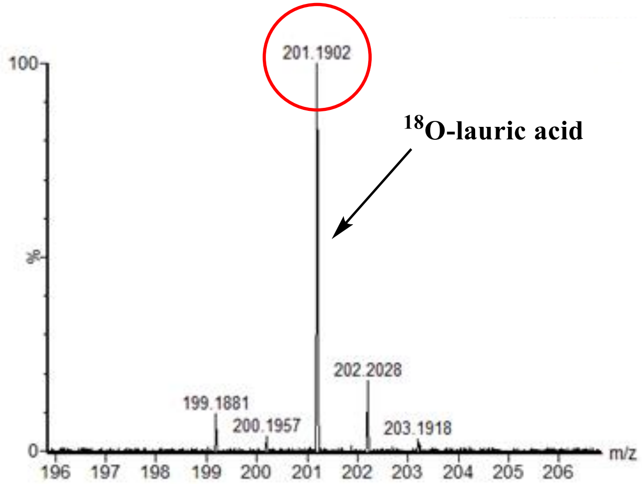
Mass spectrum of free ^{16}O-lauric acid standard incubated in $H_2{}^{18}O$ at pH 11.0 for 48 hours at 40°C



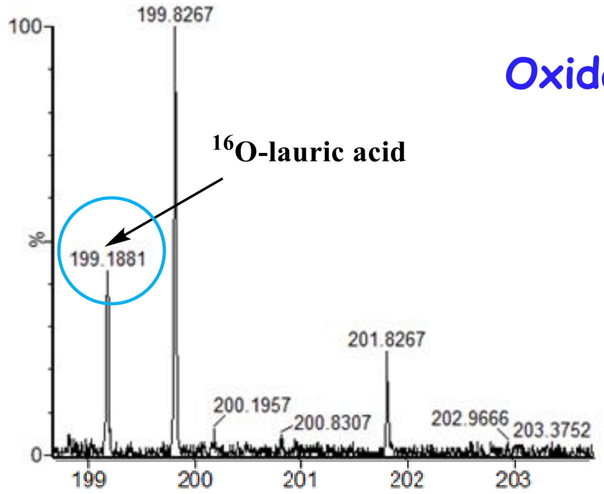
(A) **Hydrolysis of PS20 in $H_2{}^{16}O$, pH=11.0, 48h**



Hydrolysis of PS20 in $H_2{}^{18}O$, pH=11.0, 48h

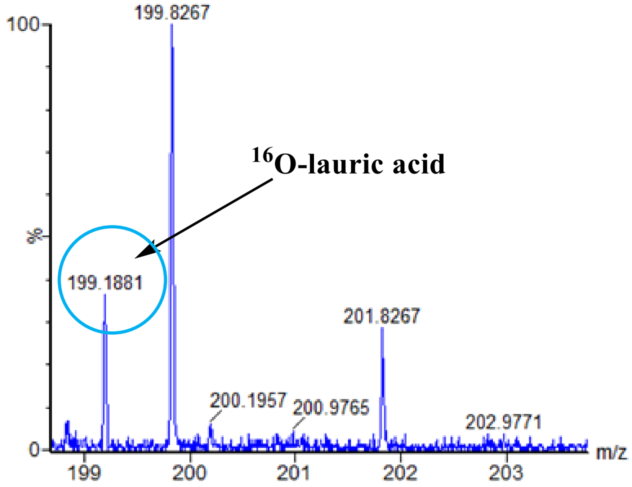


(A) **AAPH exposed PS20 in $H_2{}^{16}O$, pH=7.0, 48h**



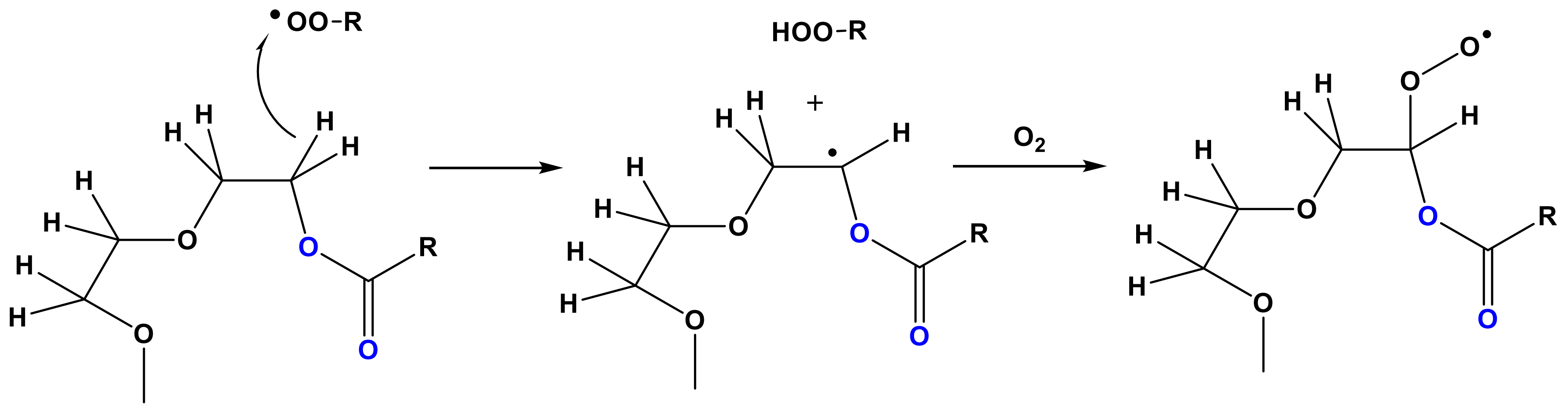
Oxidation

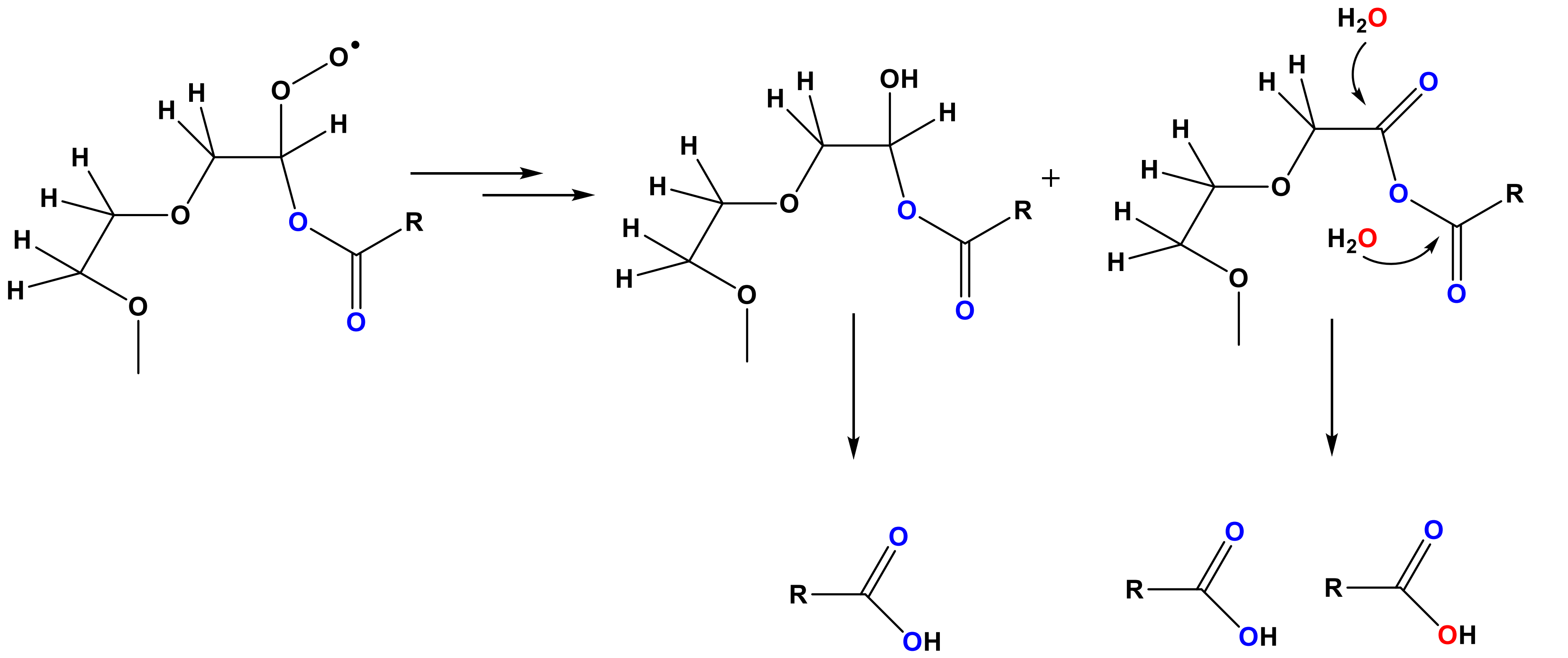
AAPH exposed PS20 in $H_2{}^{18}O$, pH=7.0, 48h



Oxidative formation of free fatty acids

Possibility 1: the Russell mechanism

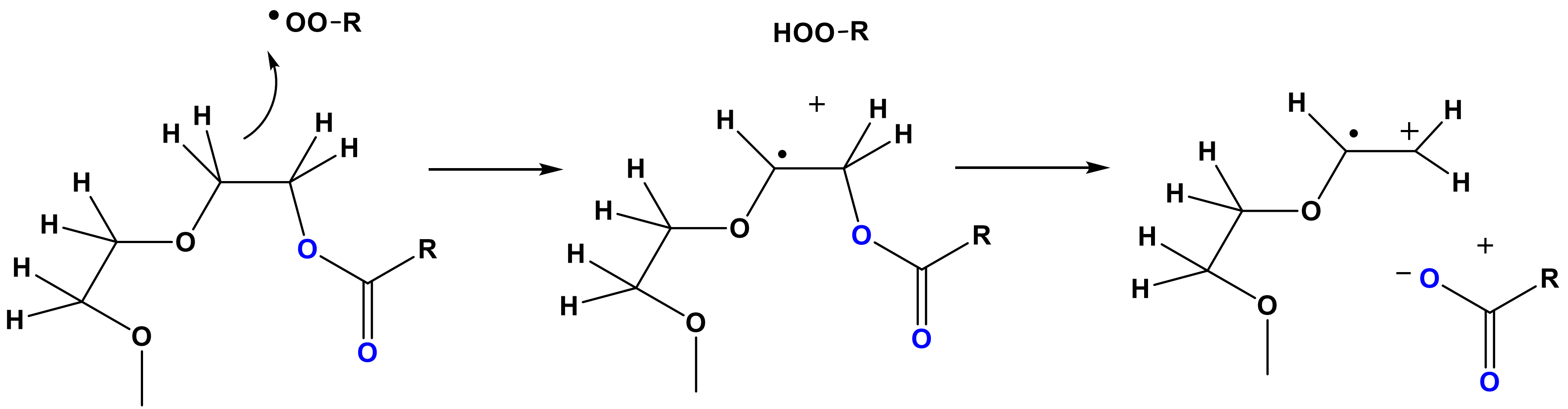




Expectation: [RCOOH] : [RCOOH] = 1.5:0.5

Oxidative formation of free fatty acids

Possibility 2: unimolecular cleavage of initial radical



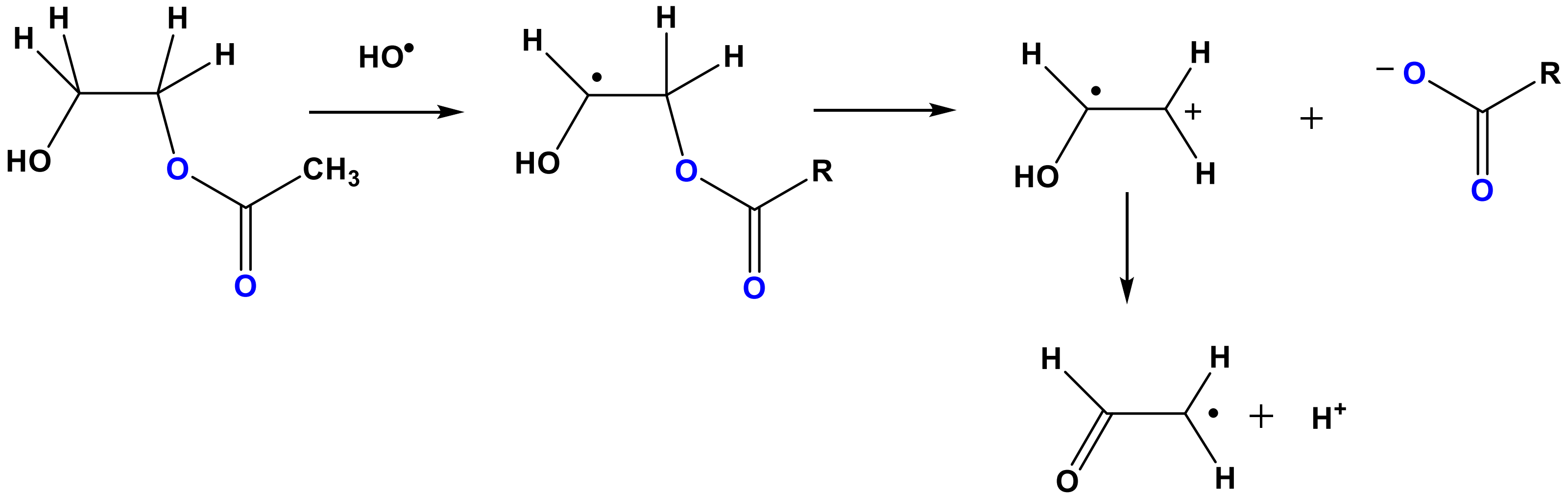
Expectation: No incorporation of ^{18}O

The mechanism of decay of the radical HO-C•H-CH_2-$OCOCH_3$ in aqueous solutions.

A conductometric pulse radiolysis study

G. Koltzenburg, T. Matsushige, D. Schulte-Frohlinde

Z. Naturforsch. 1976, 31b, 960-964

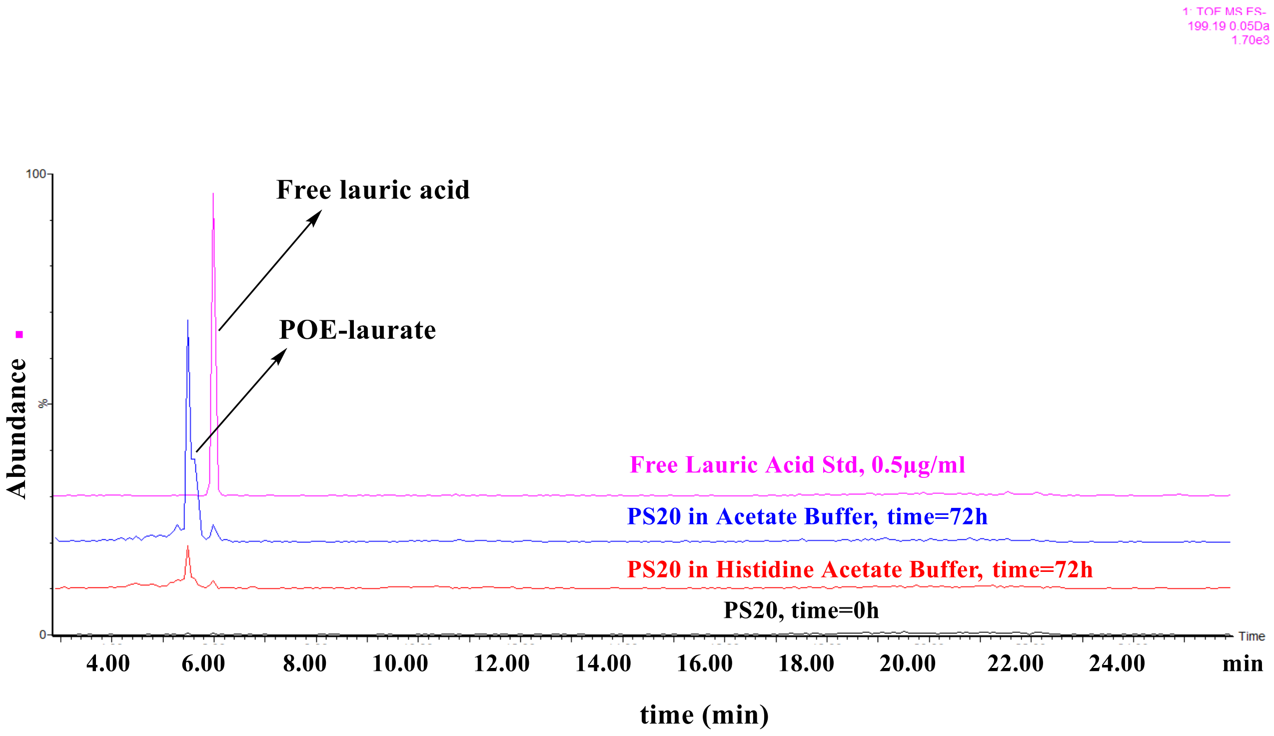


Dual effect of histidine on polysorbate 20 stability: mechanistic studies

L. Zhang, S. Yadav, Y. J. Wang, O. Mozziconacci and Ch. Schöneich

Pharm. Res. 2018

L-His suppresses the formation of AAPH-induced degradation products of PS20, mainly free lauric acids and short-chain POE-laurate

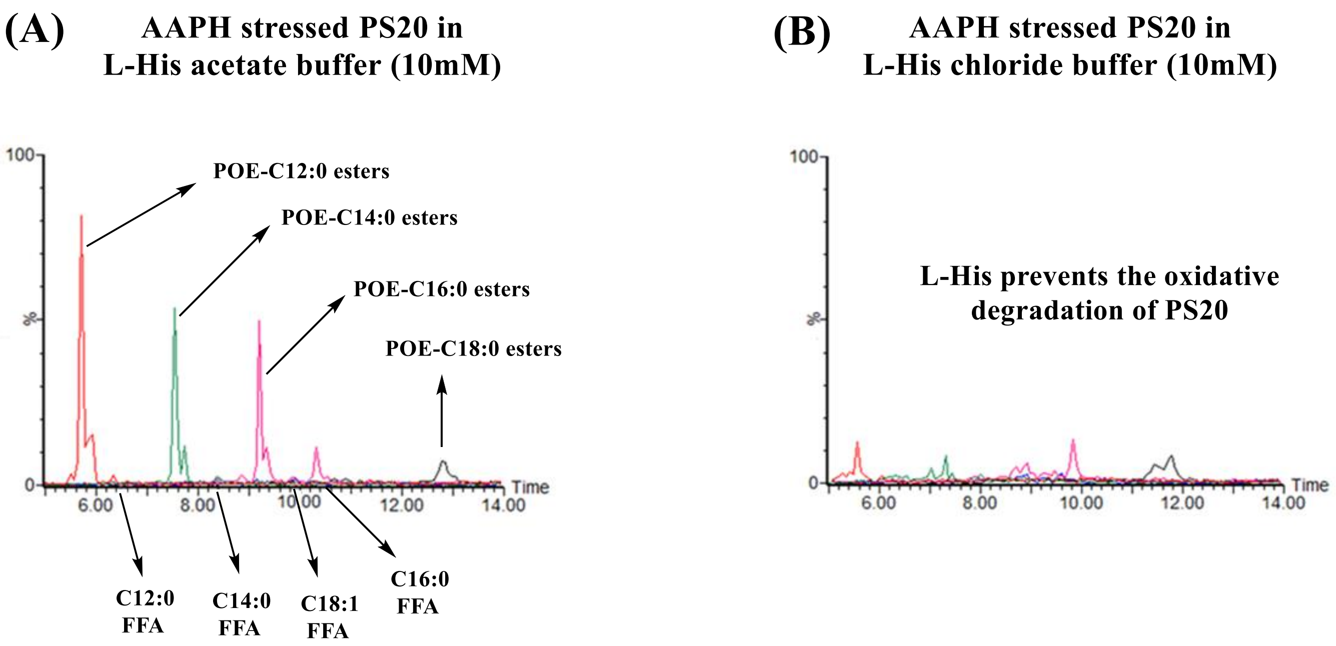


Dual effect of histidine on polysorbate 20 stability: mechanistic studies

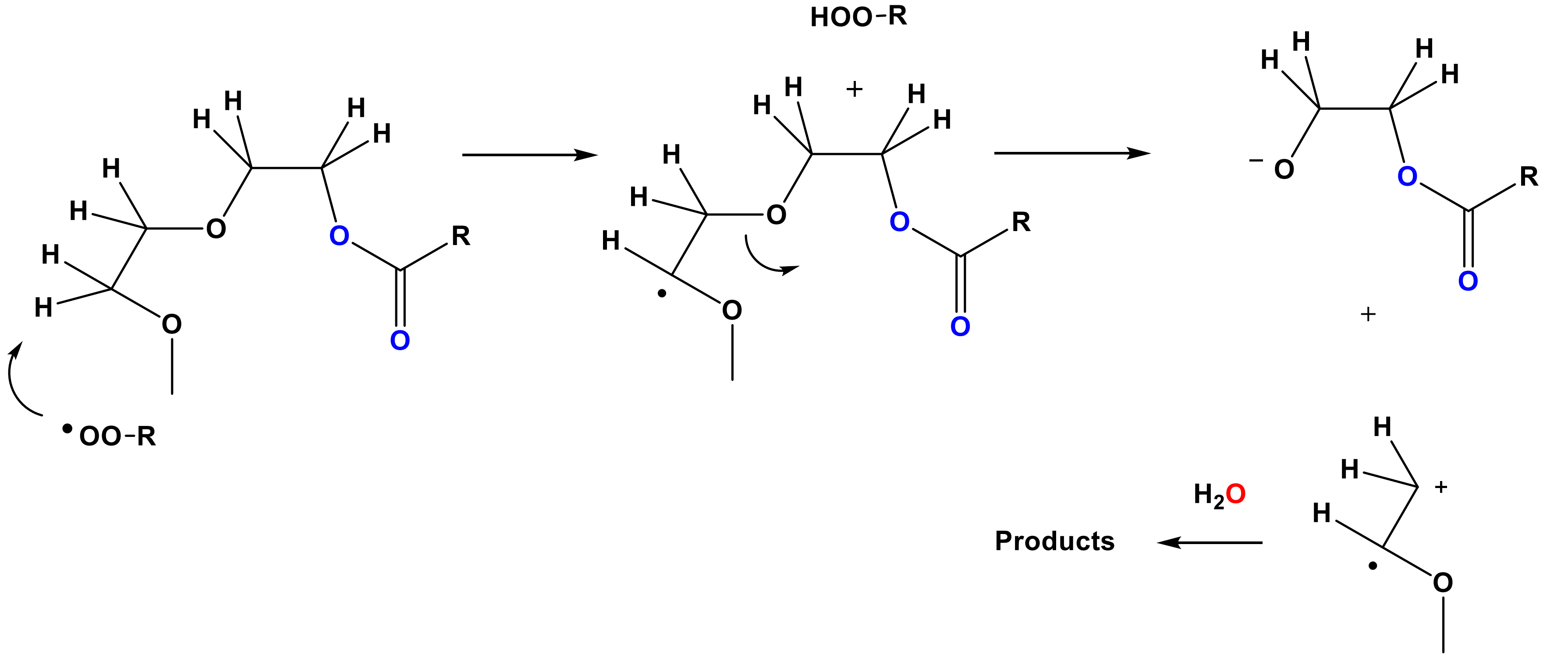
L. Zhang, S. Yadav, Y. J. Wang, O. Mozziconacci and Ch. Schöneich

Pharm. Res. 2018

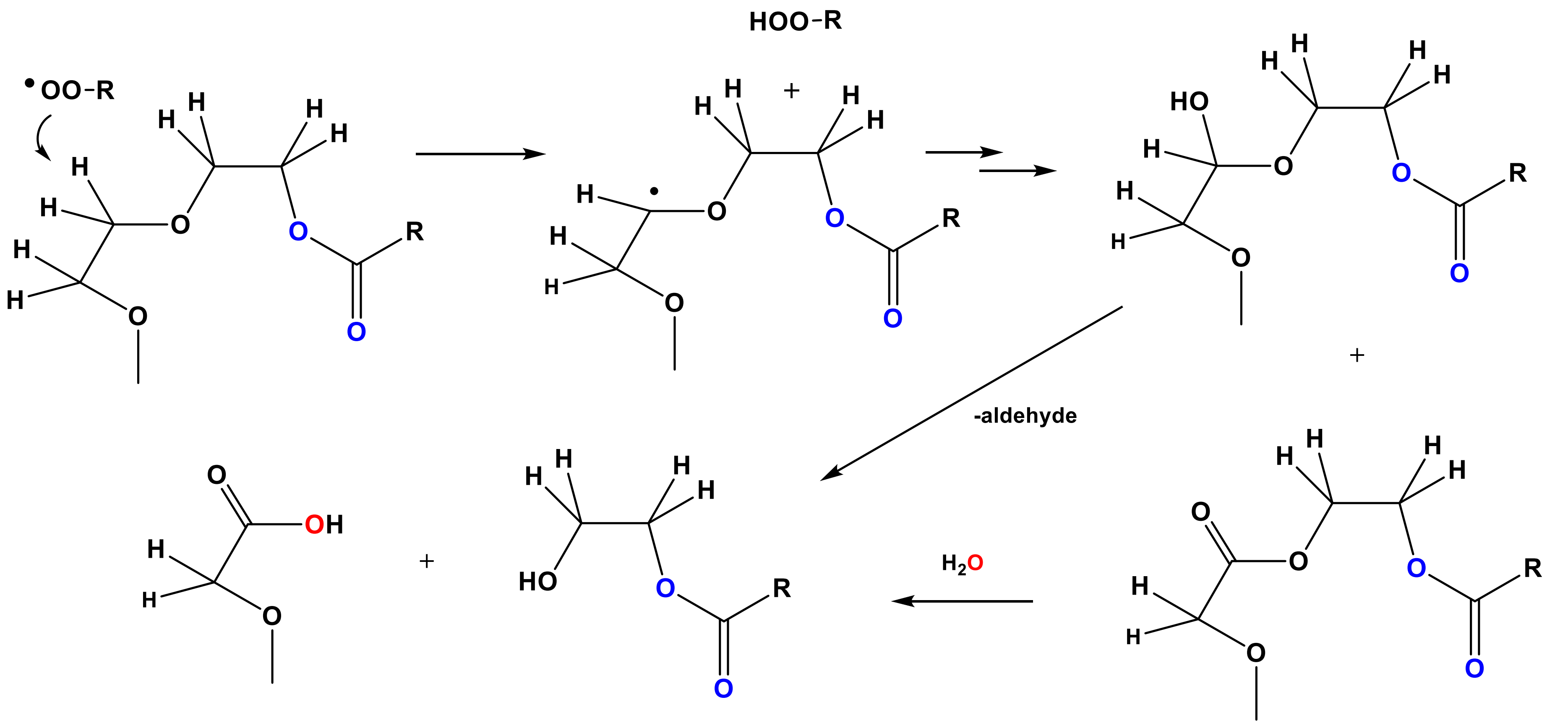
L-His chloride suppresses AAPH-induced PS20 oxidation more efficiently compared to L-His acetate



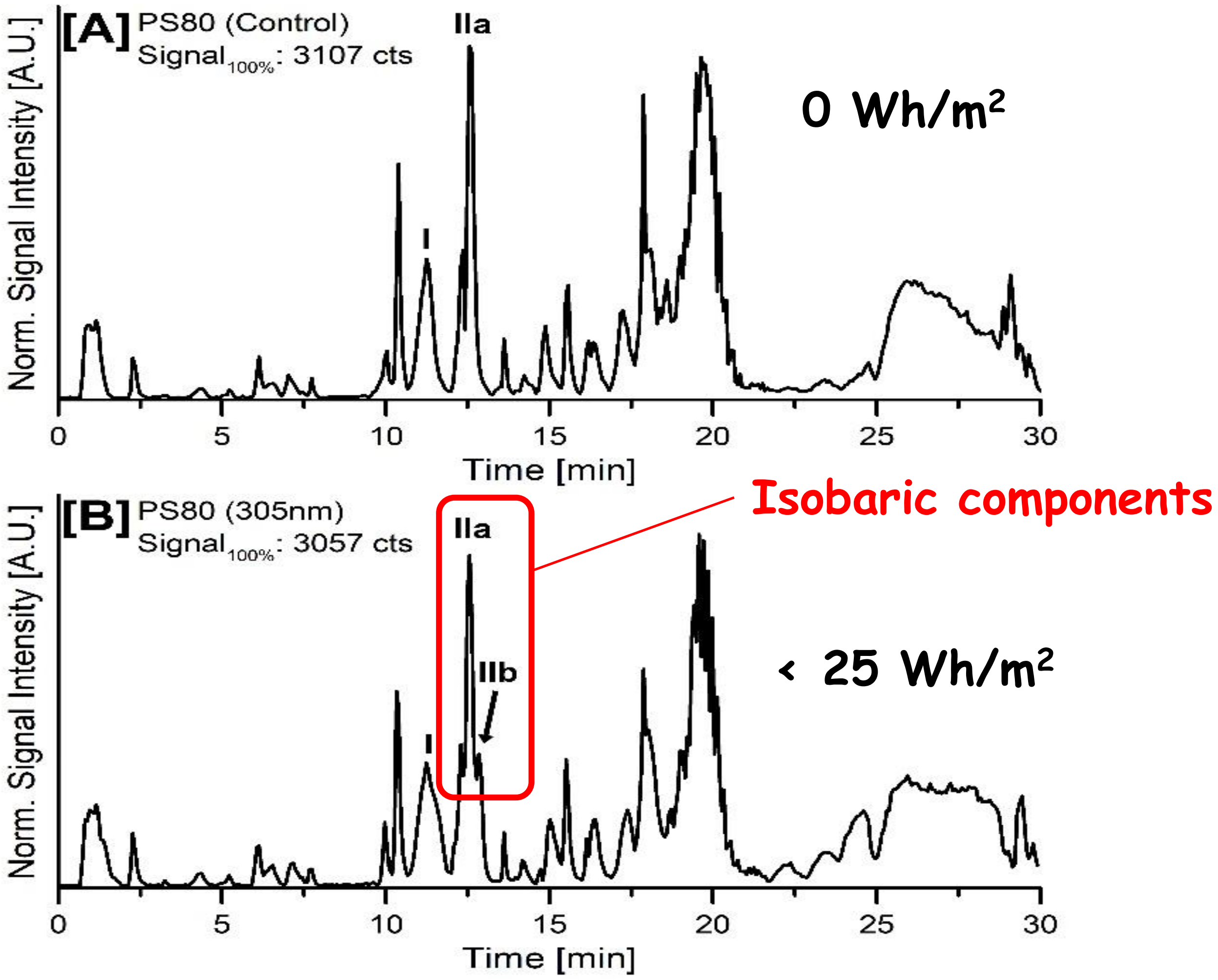
Possible pathway to small POE- esters (1)



Possible pathway to small POE- esters (2)



Secondary effects of light-induced protein degradation on polysorbate 80



Positive mode LC-MS chromatograms of PS80 after extraction from the mAbZ formulation: Dark control sample (A) and samples photo-irradiated (1hr) with λ_{max} = 305 nm (B); Peak I: POE sorbitan oleic acid, Peak IIa: POE oleic acid, Peak IIb: Isobaric product to IIa; y-axis scaling: scaling of each sub-plot is normalized to its own respective maximum signal intensity

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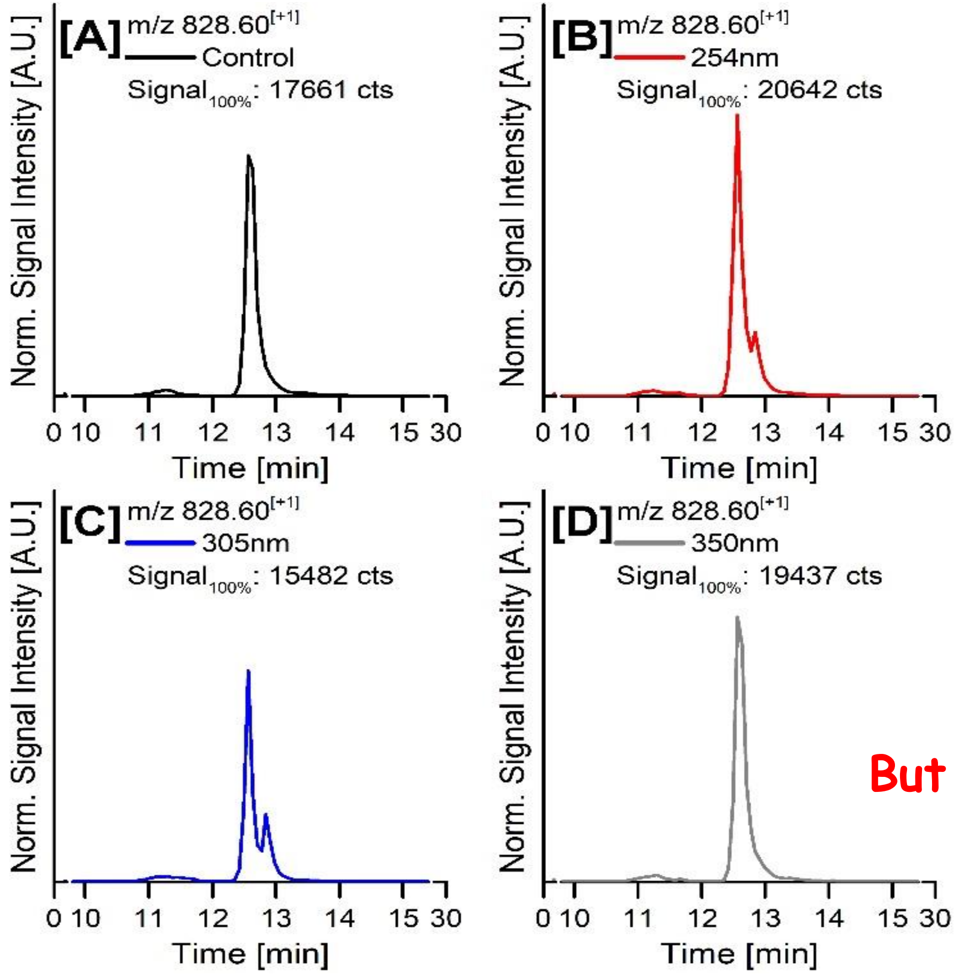
Extraction of POE (12) oleic acid



305 nm

< 25 Wh/m²

254 nm

< 40 Wh/m²

350 nm

< 25 Wh/m²

But observed for < 200 Wh/m²

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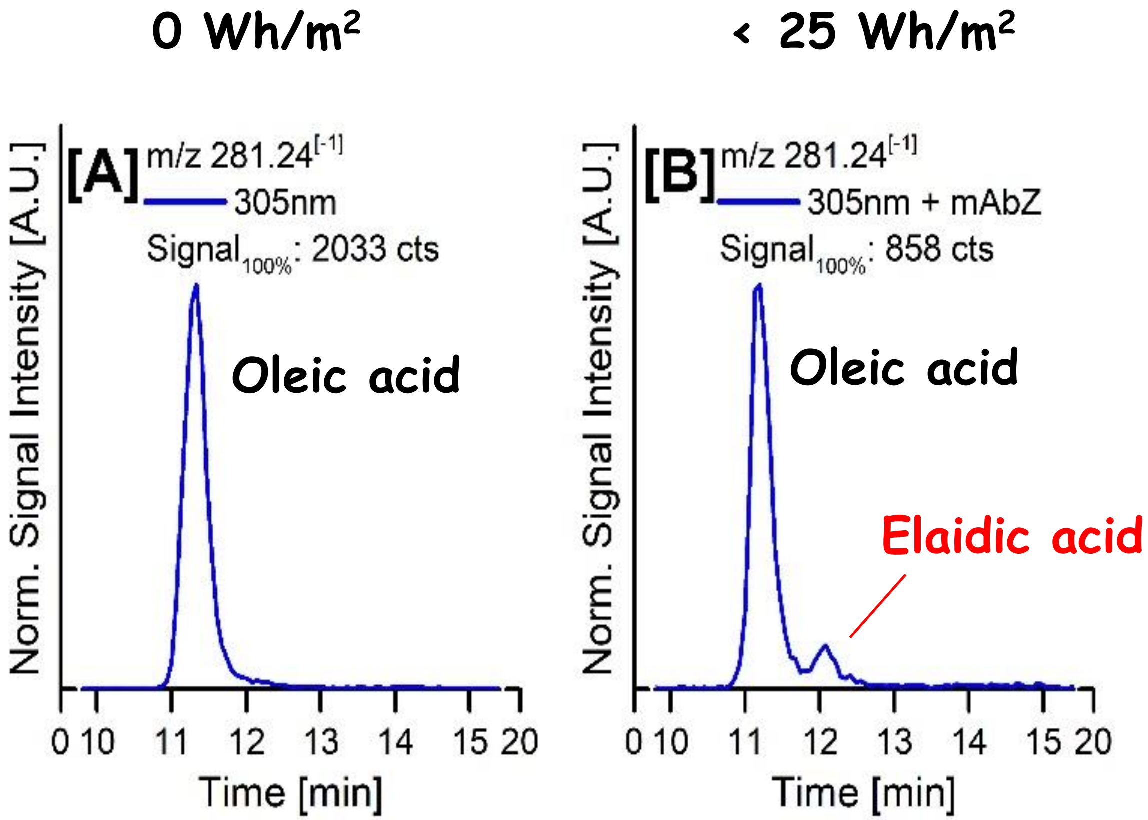
FFA analysis after esterase-catalyzed hydrolysis



Negative mode LC-MS chromatogram of oleic acid (m/z 281.24, $[M-H]^{-1}$) after FFA extraction from PS80 present in the mAbZ formulation: Dark control sample (A) and sample photo-irradiated (1hr) with λ_{max} = 305 nm (B); y-axis scaling: scaling of each sub-plot is normalized to its own respective maximum signal intensity

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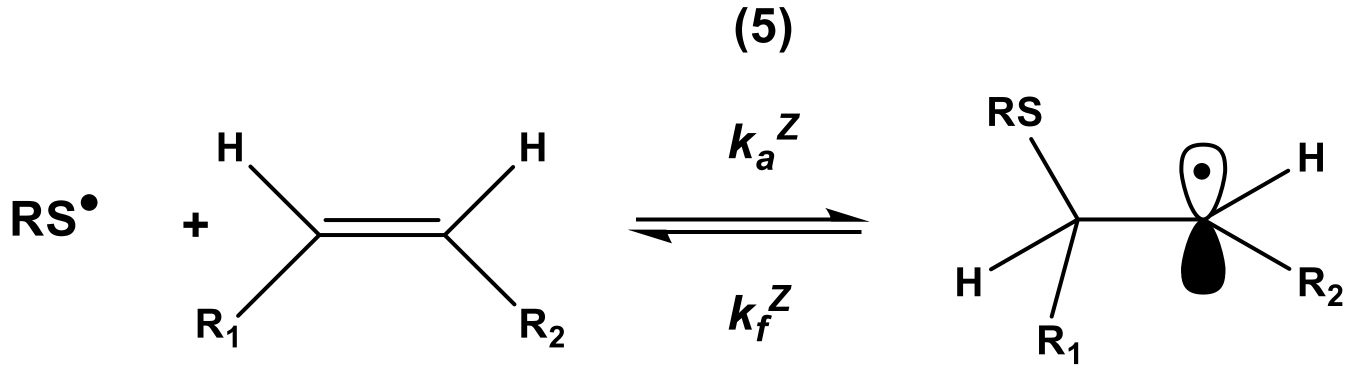
Cis/trans isomerization of unsaturated fatty acids by thiyl radicals

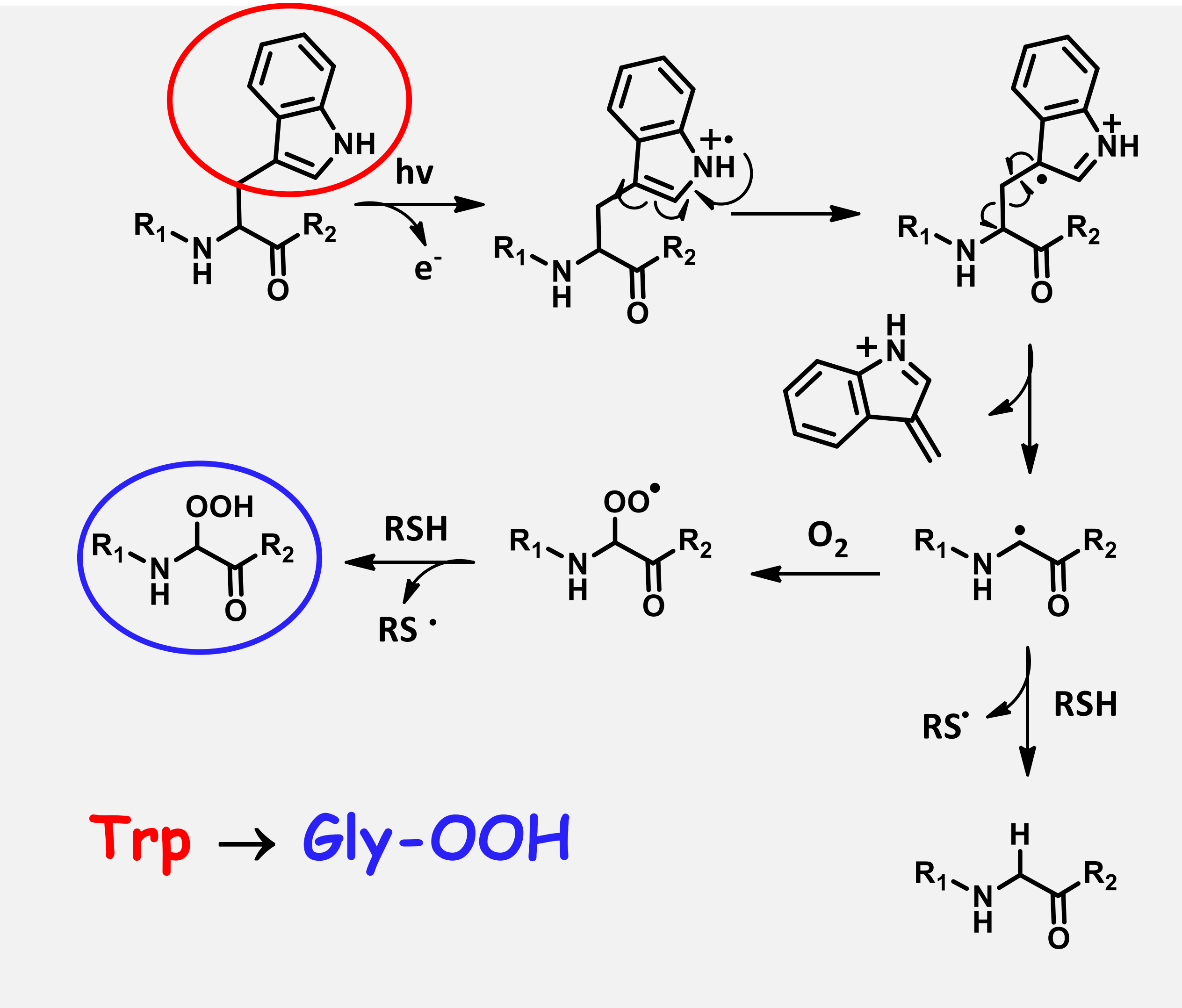


Chatgilialoglu C, Ferreri C, Melchiorre M, Sansone A, Torreggiani A.

Lipid Geometrical Isomerism: From Chemistry to Biology and Diagnostics.

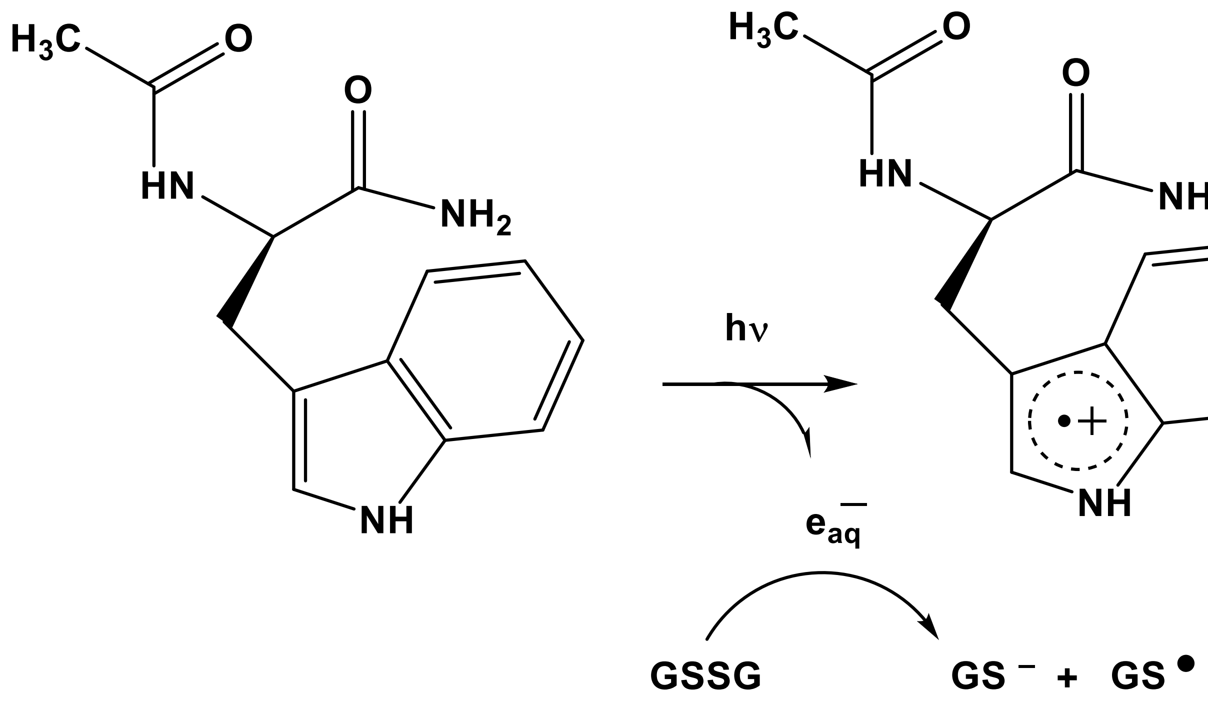
Chem Rev. 2014;114:255-284.

How do we generate thiyl radicals by ligt exposure of a mAb?

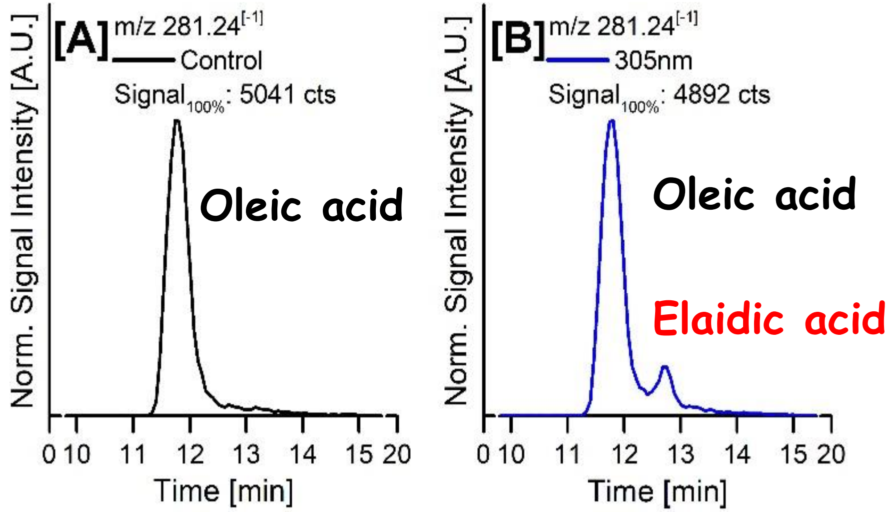


J. Haywood, O. Mozziconacci, K. M. Allegre, B. A. Kerwin, and Ch.Schöneich,
Mol. Pharm. 2013, *10*, 1146-1150

Simple mimic of protein Trp-disulfide system: NATA and GSSG



FFA analysis



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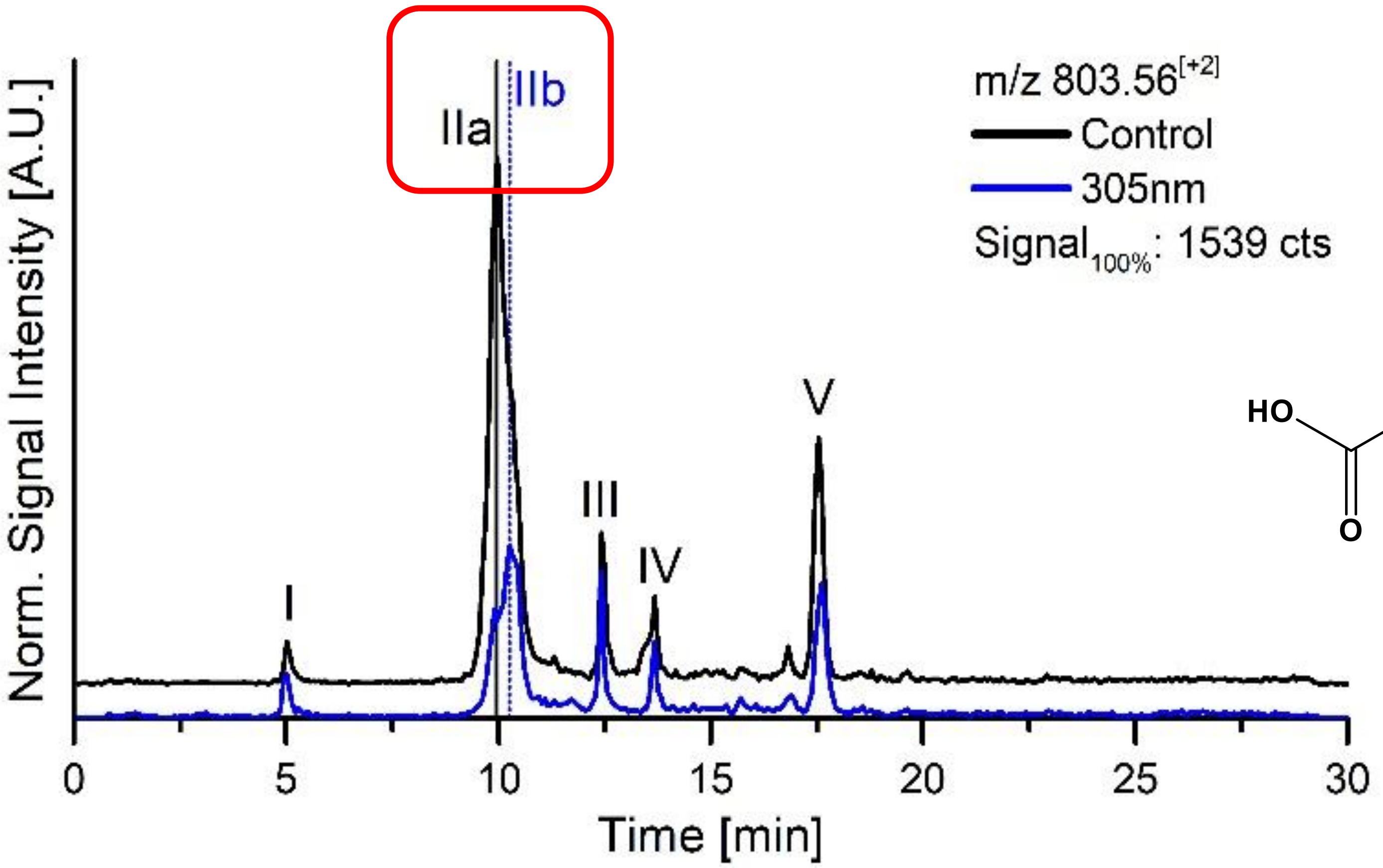
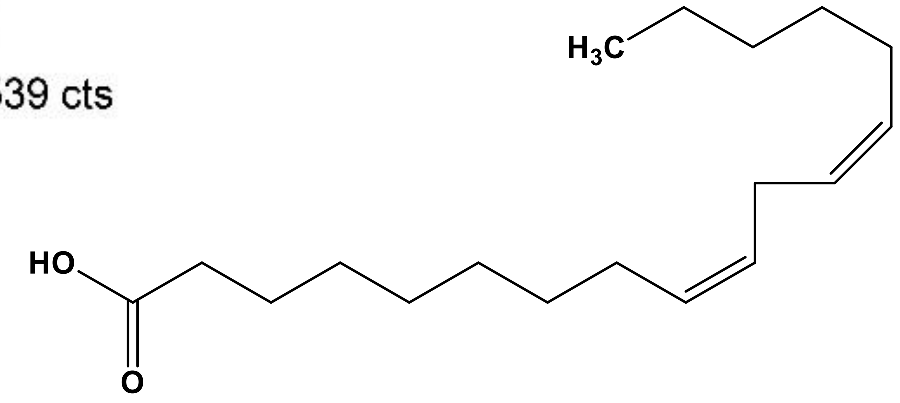
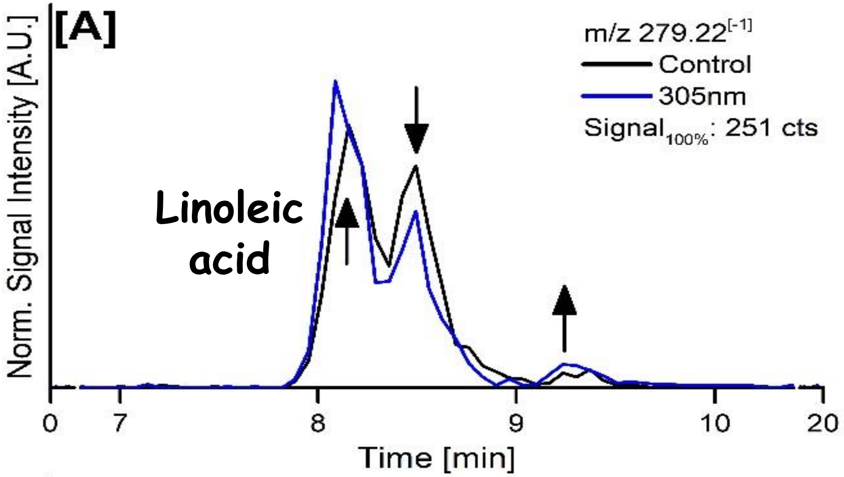
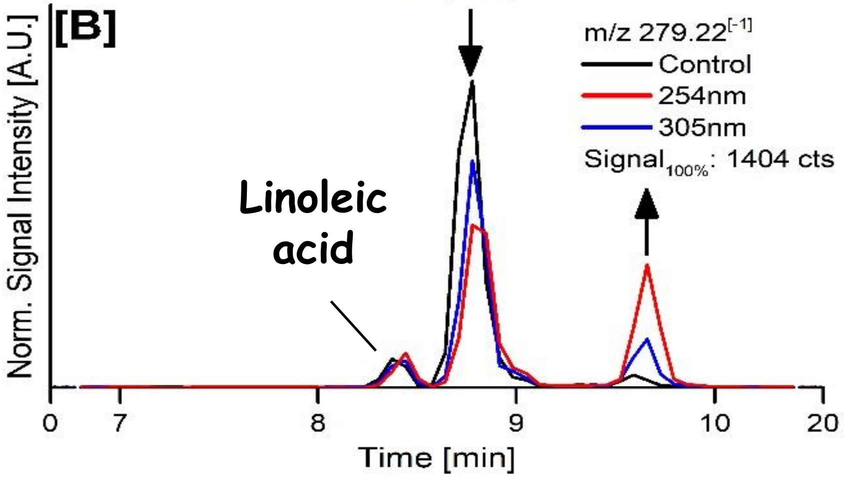
POE (26) sorbitan linoleic acid





FFA extraction: linoleic acid





3 isobaric species interconverting

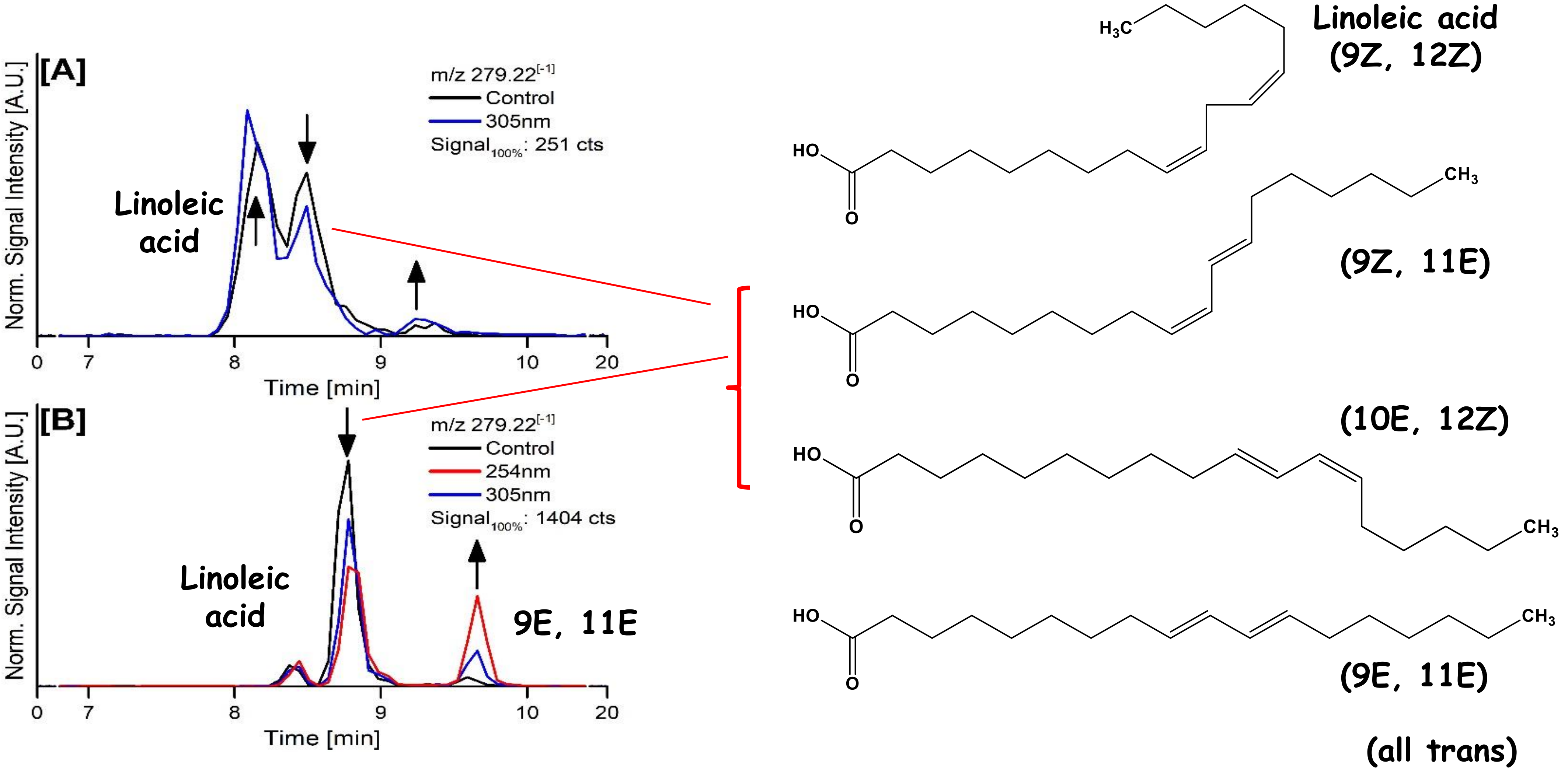
mAbZ with 0.01% PS80

mAbZ with 0.01% PS80 + 0.2% PS80

Different lots from J.T. Baker

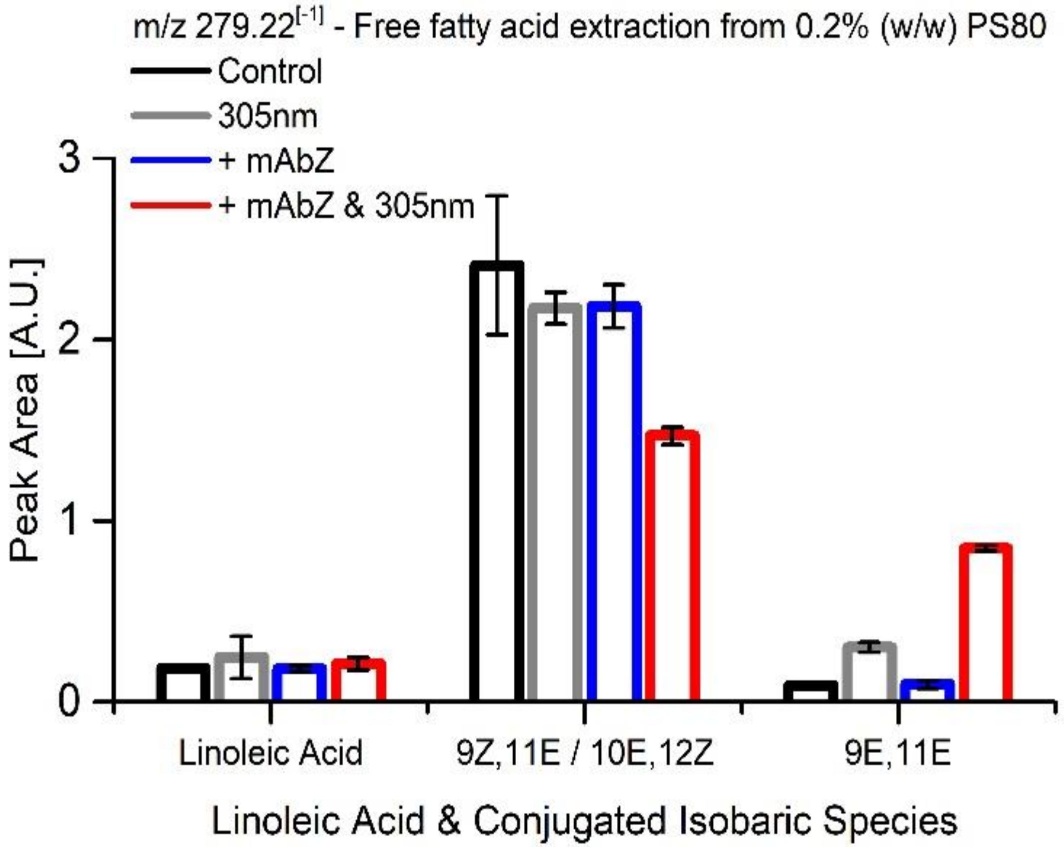
* Absolute retention time shift because of aging column

FFA extraction: linoleic acid



* Absolute retention time shift because of aging column

Linoleic acid



Quantitative comparison of linoleic acid and its isobaric species (m/z 279.22, $[M-H]^{-1}$) after FFA extraction from PS80 alone and from PS80 spiked into the mAbZ formulation: Dark control samples (black and blue lines, respectively) and photo-irradiated samples (1hr) with λ_{max} = 305nm (gray and red lines, respectively); '9Z, 11E' refers to (9Z, 11E)-octadeca-9,11-dienoic acid, '10E, 12Z' refers to (10E, 12Z)-octadeca-10,12-dienoic acid, and '9E, 11E' refers to (9E, 11E)-octadeca-9,11-dienoic acid

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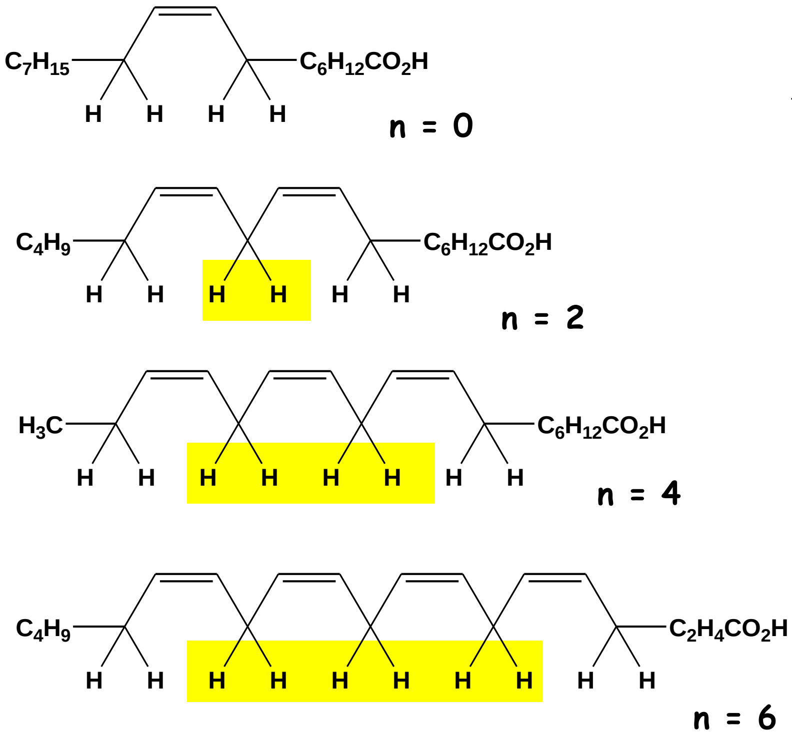
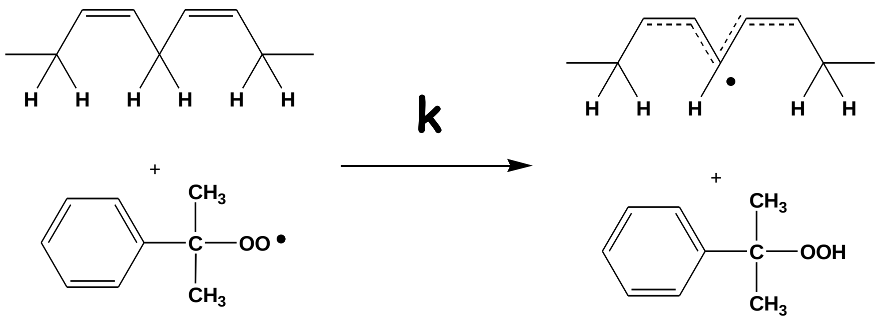
What does this mean ?

Hydrogen transfer from unsaturated fatty acids:

additivity rule for polyunsaturated fatty acids





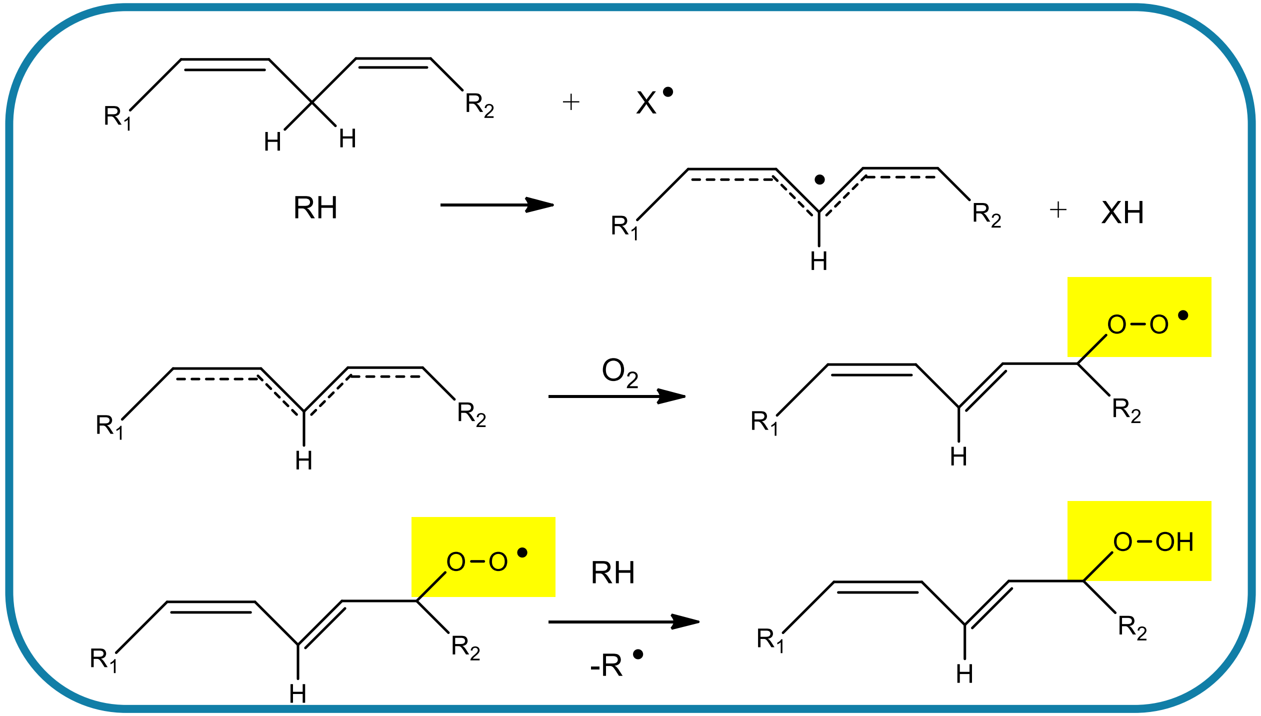
Linear relationship

$$k = k_{H,allyl} + n\, k_{H,pentadienyl}$$

Kitaguchi et al., *Chem. Commun.* 2006, 979-981

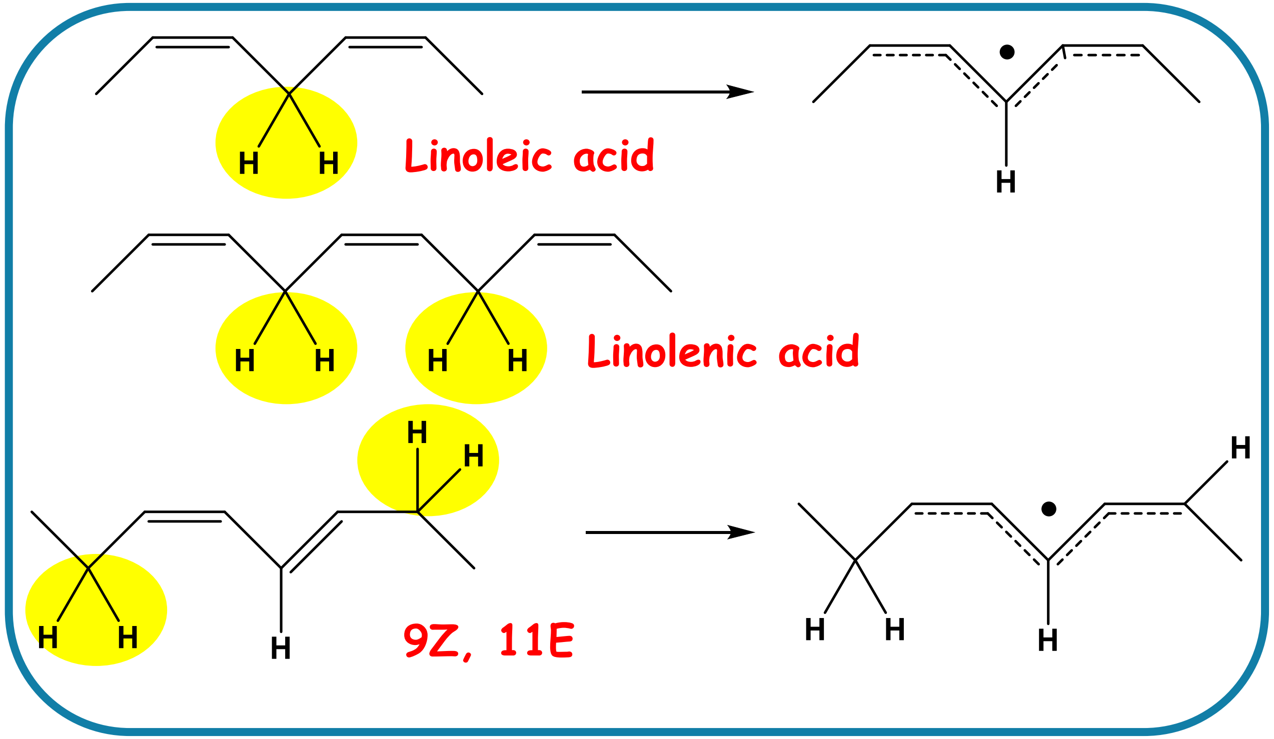
"Additivity rule holds in the hydrogen transfer reactivity of unsaturated fatty acids with a peroxyl radical: mechanistic insight into lipoxygenase"

Simplified mechanism of chain peroxidation of unsaturated fatty acids

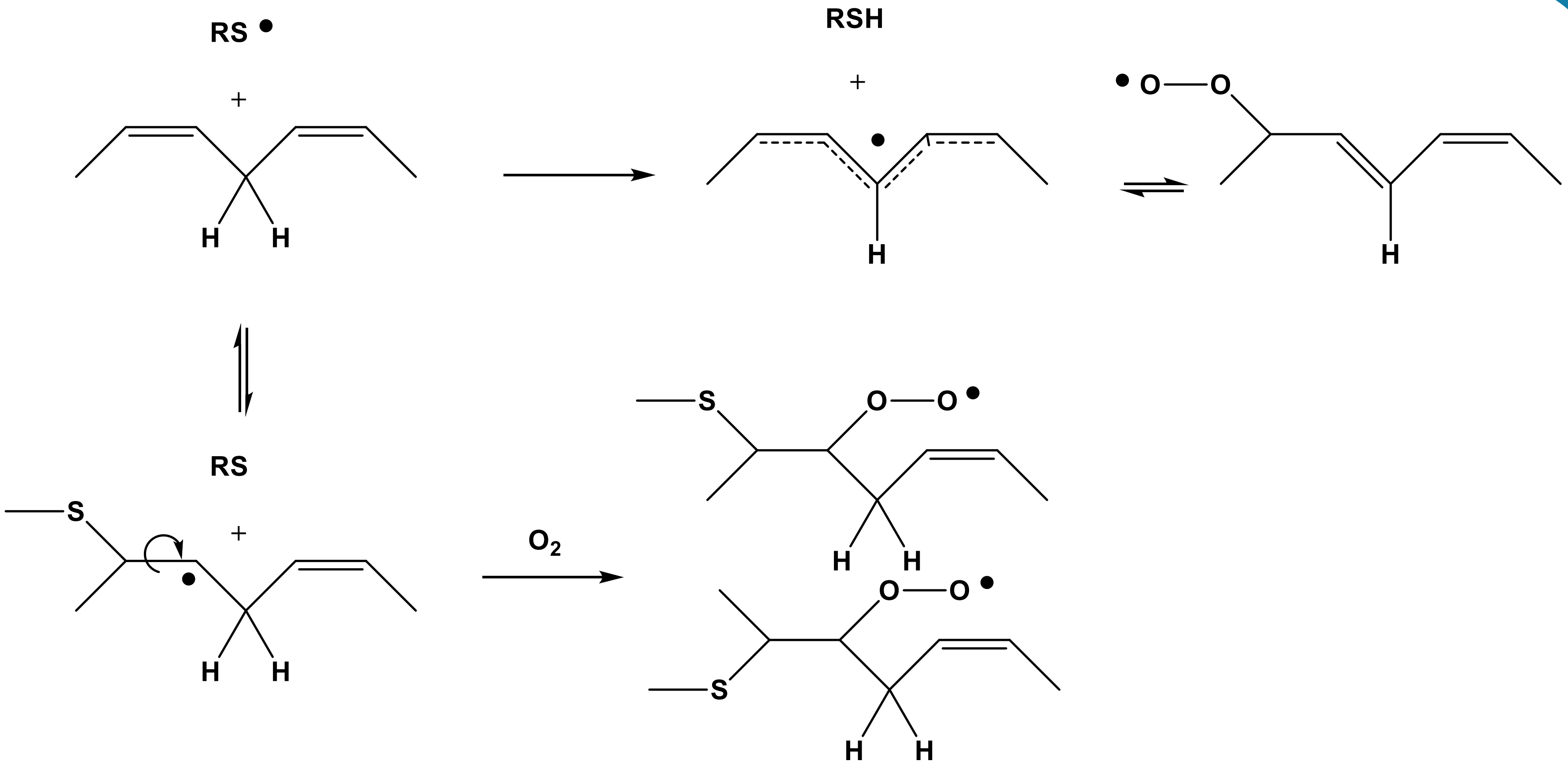


Conjugated fatty acids contain more highly susceptible C-H bonds

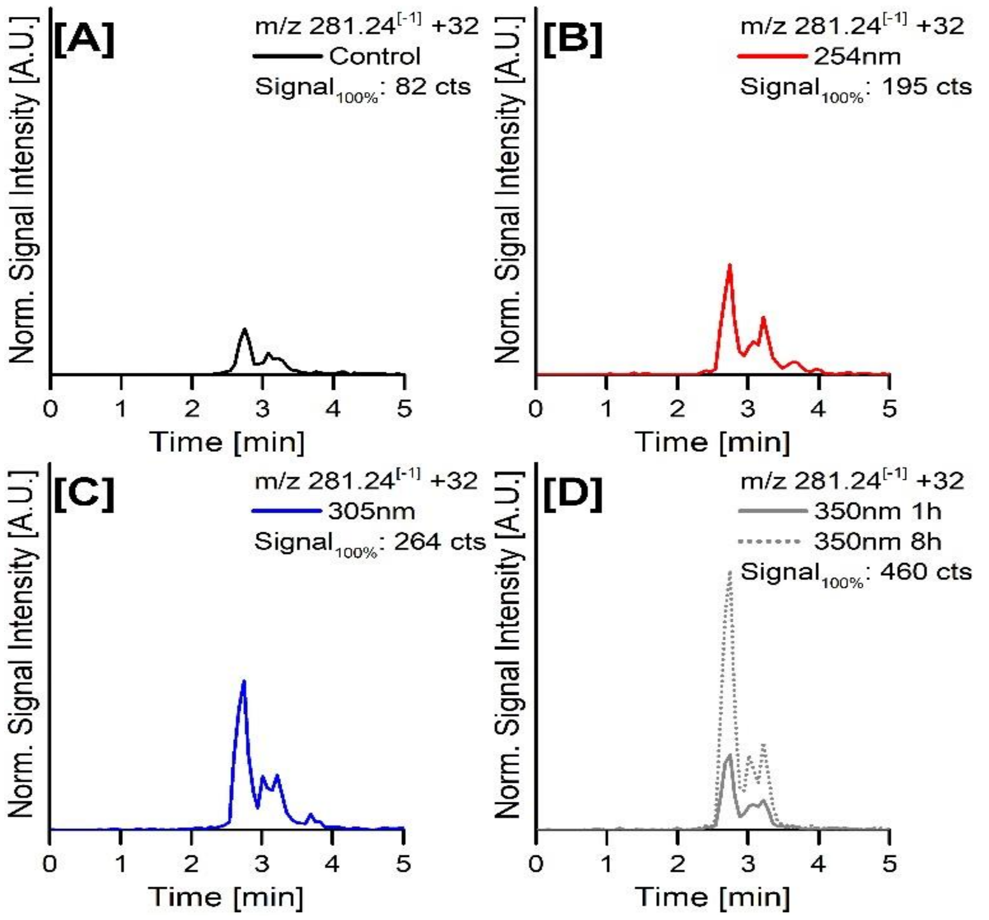
9Z, 11E equivalent to linolenic acid



Oxidation products



Oleic acid hydroperoxides



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Acknowledgements

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Lin Zhang Olivier Mozziconacci Indira Prajapati Björn Peters

Nicholas Larson C. Russell Middaugh Yangjie Wei

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MedImmune/AstraZeneca

Reza Esfandiary Sureshkumar Choudhary

Cavan Kalonia Suzanne Hudak