5th International Electronic Conference on Medicinal Chemistry

1-30 November 2019

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by pharmaceuticals

Lactose-based Fatty Acid Monoesters: Synthesis, antimicrobial activity and permeability enhancement studies

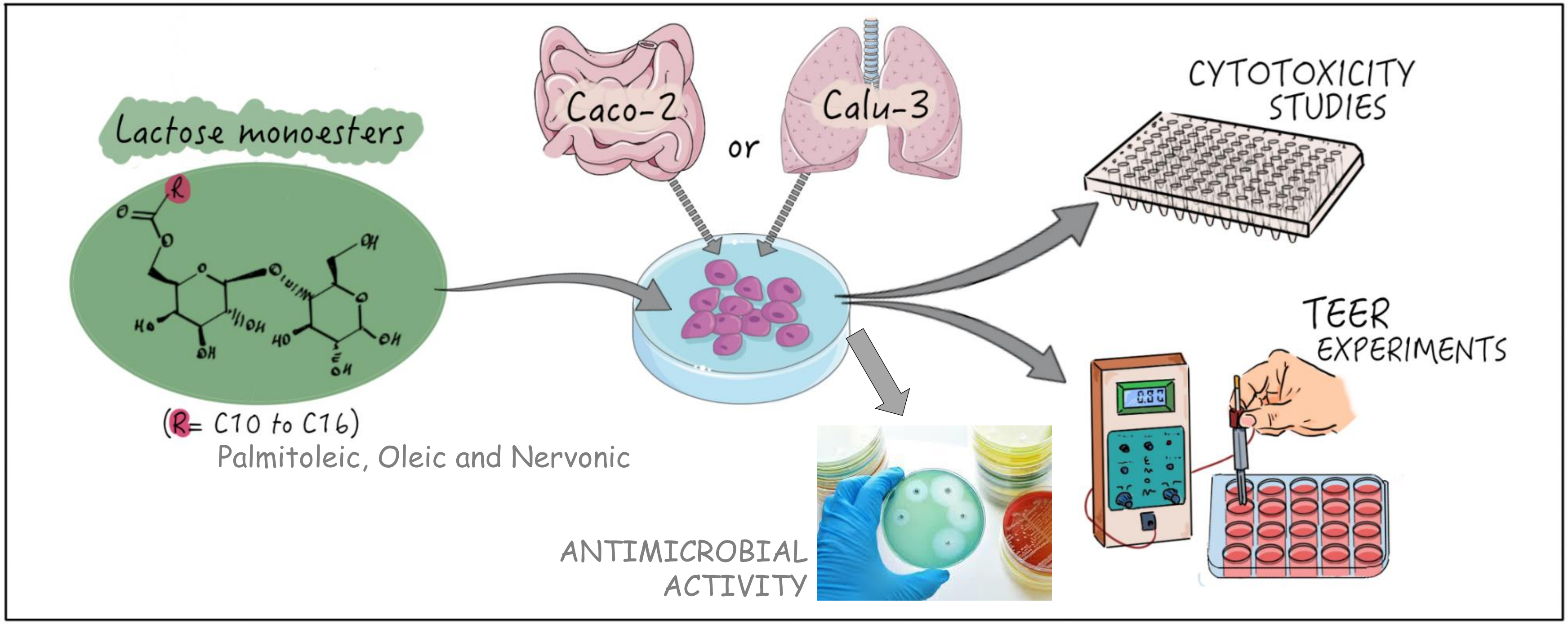
Simone Lucarini[1,*]

[1] Departement of Biomolecular Sciences, University of Urbino Carlo Bo, piazza del Rinascimento 6, 61029-Urbino, Italy;

* Corresponding author: simone.lucarini@uniurb.it

1506 UNIVERSITÀ DEGLI STUDI DI URBINO CARLO BO | DISB DIPARTIMENTO DI SCIENZE BIOMOLECOLARI

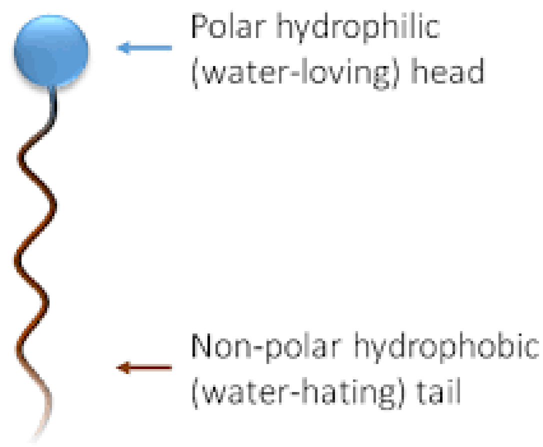
Lactose-based Fatty Acid Monoesters: Synthesis, antimicrobial activity and permeability enhancement studies

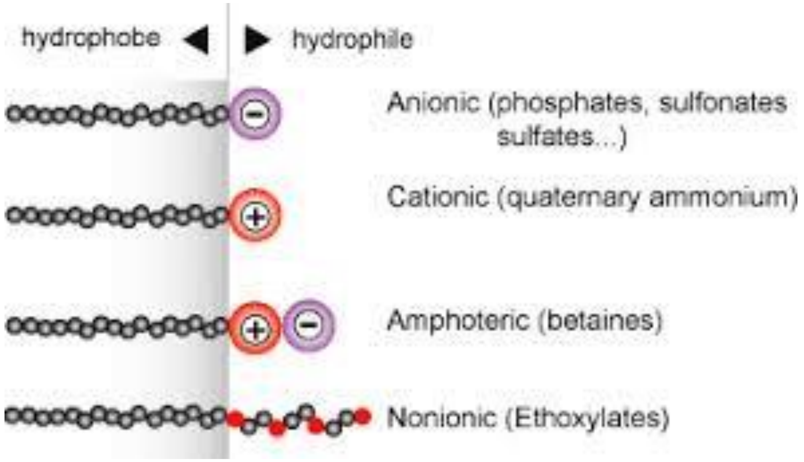


Abstract: We present the synthesis and the in vitro assaying of a series of lactose-based non-ionic surfactants, highlighting the relationship between their structure and biological effect. Using tensiometric measurements the critical micelle concentrations (CMCs) of the surfactants were determined and demonstrate that increasing hydrophobic chain length reduces surfactant CMC. In vitro testing on Caco-2 intestinal and Calu-3 airway epithelia revealed that cytotoxicity is present, for most of the surfactants, at concentrations greater than their CMCs. Importantly, through the culture of epithelial monolayers on Transwell® supports, the surfactants demonstrate the ability to reversibly modulate transepithelial electrical resistance (TEER), and thus open tight junctions, at non-toxic concentrations. The surfactants were then tested for their ability to improve the in vitro permeability of Ovalbumin and Dextran, confirming their potential application as safe permeability enhancers in vivo. Moreover, the synthesized compounds exhibit antimicrobial activity versus eight pathogenic species belonging to Gram-positive, Gram-negative microorganisms and fungi.

Keywords: absorption enhancers; sugar-based surfactants; biocompatibility studies; transmucosal drug delivery, antimicrobial

Surfactants





Sugar-based Surfactants

- **SUGAR FATTY ACID ESTERS** are a class of non-ionic, biodegradable and biocompatible surfactants

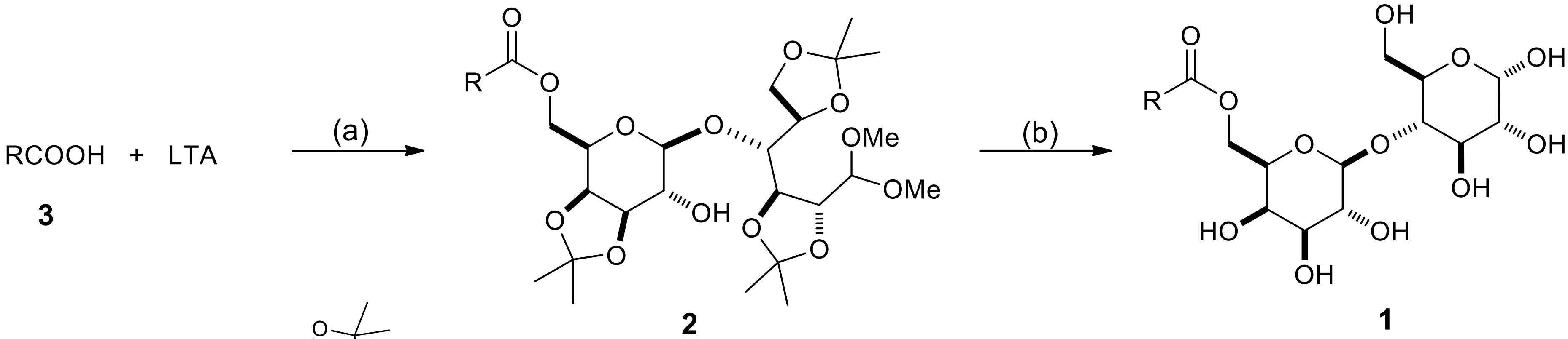
- They have a broad applications in the **food**, **pharmaceutical** and **personal care** industries *(sucrose fatty acid esters have been commercially manufactured at high volume since the early 1960s.)*

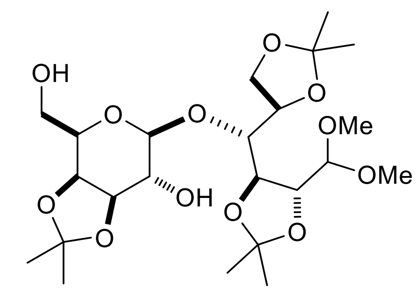
- Emerging sugar-based surfactants: Sucrose esters, alkyl glycosides, alkyl maltosides and rhamnolipids

- New antimicrobial agents

- Limited studies on Lactose–based surfactants

Synthesis of Lactose-based Fatty Acid Monoesters





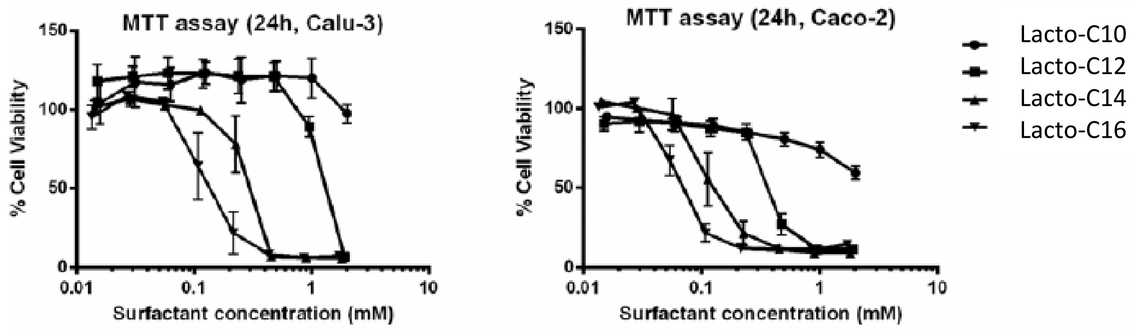
R = alifatic (Capric C10, lauric C12, myristic C14 and palmitic C16)
monoinsaturated (palmitoleic C16, oleic C18 and nervonic C24)

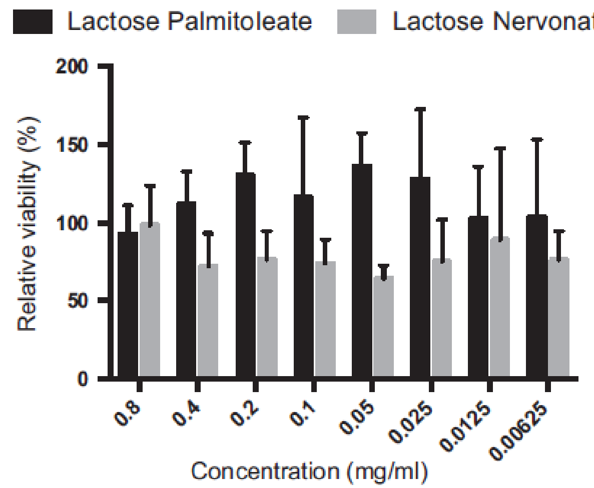
Reaction conditions: (a) Lipozyme, toluene, 75°C, 12 h; (b) HBF_4 Et_2O, CH_3CN, 30°C, 3 h.

Lucarini S. et al., *Eur J Pharm Biopharm* **2016**, *107*, 88-96; Perinelli D. R. et al., *Eur J Pharm Biopharm* **2018**, *124*, 55-62; Lucarini S. et al., *Pharmaceutics* **2018**, *10 (3)*, 81.

Citotoxicity

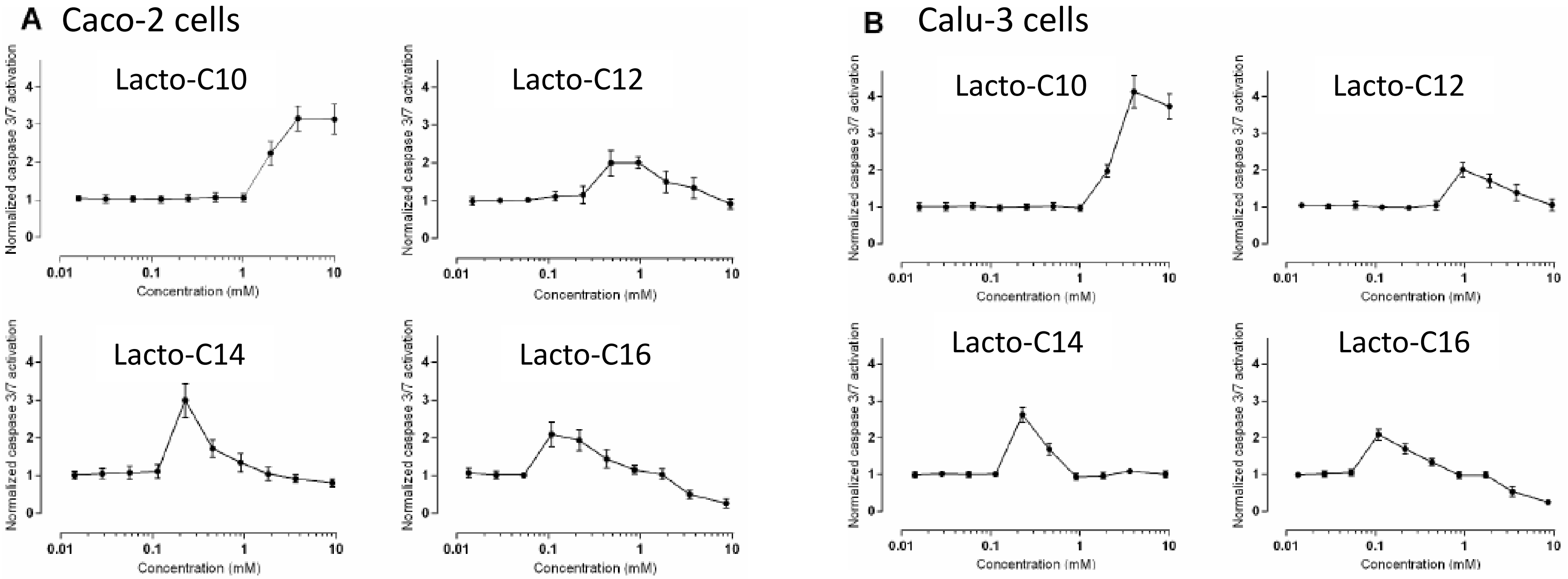
Surfactant	MTT Assay IC_{50} mM (mg/mL)	
	Calu-3	Caco-2
Lacto-C10	> 2 (> 0.99)	> 2 (> 0.99)
Lacto-C12	1.07 (0.56)	0.38 (0.20)
Lacto-C14	0.26 (0.14)	0.11 (0.06)
Lacto-C16	0.12 (0.07)	0.06 (0.03)
Lacto-C16 1W	-	> 1.40 (> 0.8)
Lacto-C18 1W	-	0.23 (0.14)
Lacto-C24 1W	-	> 1.16 (> 0.8)





Lucarini S. et al., *Eur J Pharm Biopharm* **2016**, *107*, 88-96; Perinelli D. R. et al., *Eur J Pharm Biopharm* **2018**, *124*, 55-62; Lucarini S. et al., *Pharmaceutics* **2018**, *10 (3)*, 81.

Activated Caspase 3/7 Detection: Apoptosis?



Responses are displayed normalized to those induced by vehicle control (HBSS buffer).

Lucarini S. et al., *Pharmaceutics* **2018**, *10 (3)*, 81

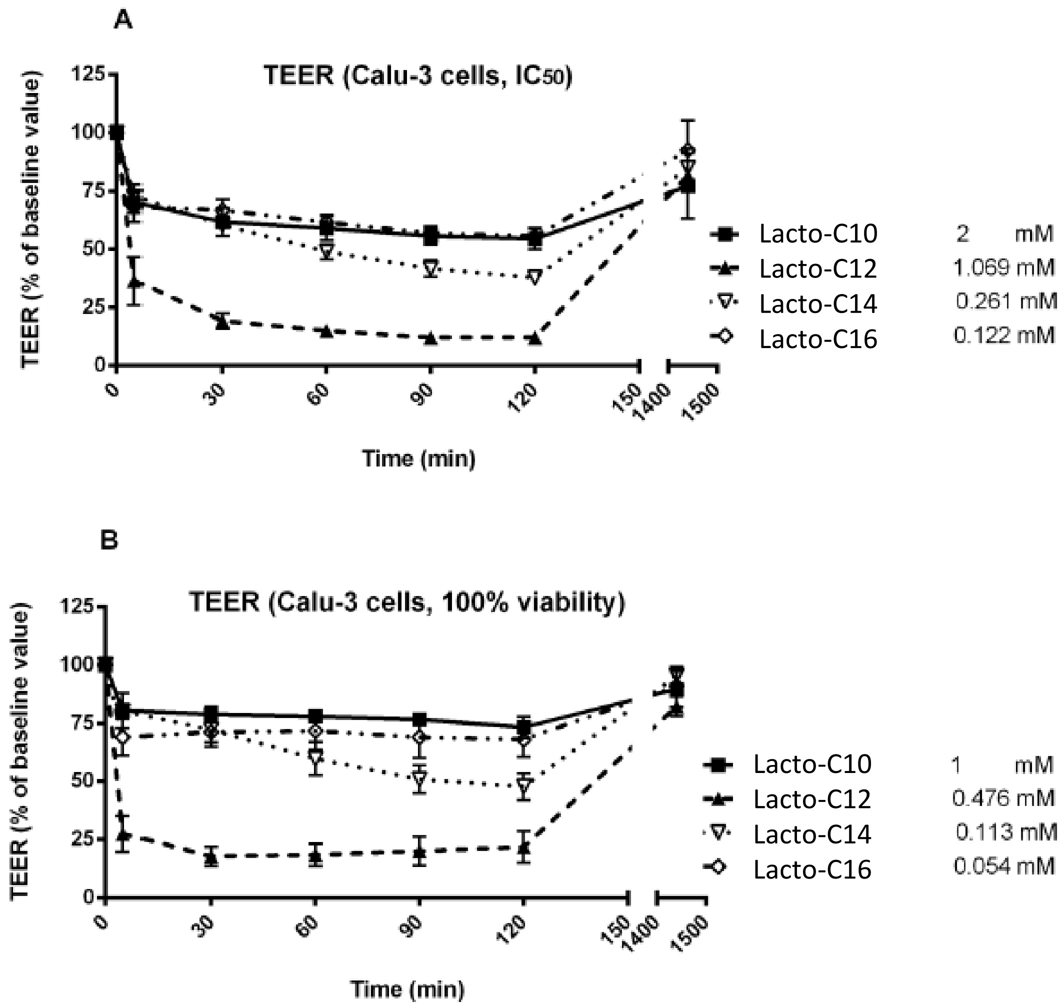
Critical Micelle Concentration (CMC) by Tensiometer

Surfactant	CMC (mM)	Toxicity Caco-2 IC_{50} (mM)
Lacto-C10	2.58	> 2
Lacto-C12	0.55	0.38
Lacto-C14	0.14	0.11
Lacto-C16	0.08	0.06
Lacto-C16 1W	-	> 1.40
Lacto-C18 1W	0.24	0.23
Lacto-C24 1W	-	> 1.16

Lactose derivatives are safe surfactants

Perinelli D. R. et al., *Eur J Pharm Biopharm* **2018**, *124*, 55-62; Lucarini S. et al., *Pharmaceutics* **2018**, *10 (3)*, 81.

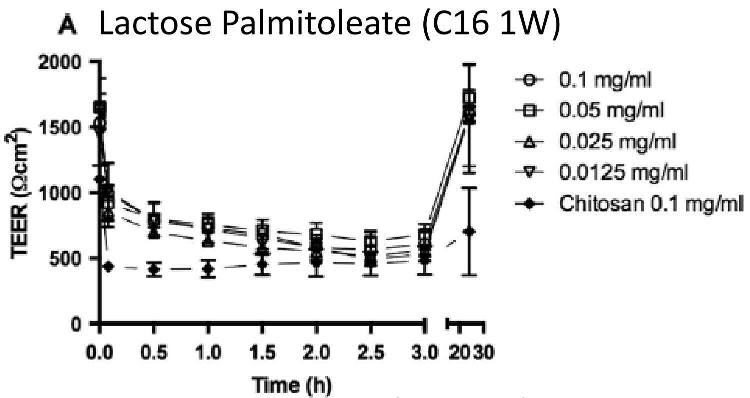
Cell monolayer Trans-epithelial electrical resistance (TEER) - Part 1

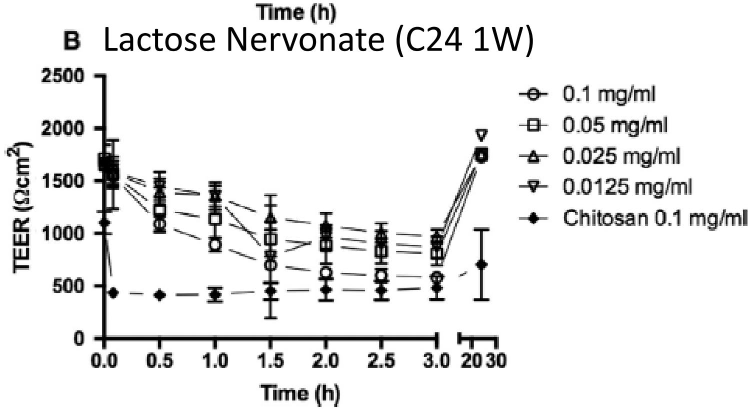


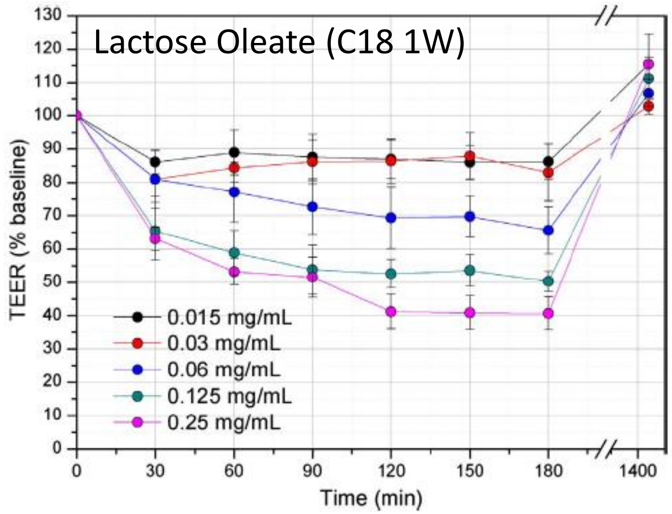
Surfactant	MTT Assay IC_{50} (mM)
	Calu-3
Lacto-C10	> 2
Lacto-C12	1.07
Lacto-C14	0.26
Lacto-C16	0.12

Lucarini S. et al., *Pharmaceutics* **2018**, *10 (3)*, 81.

Cell monolayer Trans-epithelial electrical resistance (TEER) - Part 2



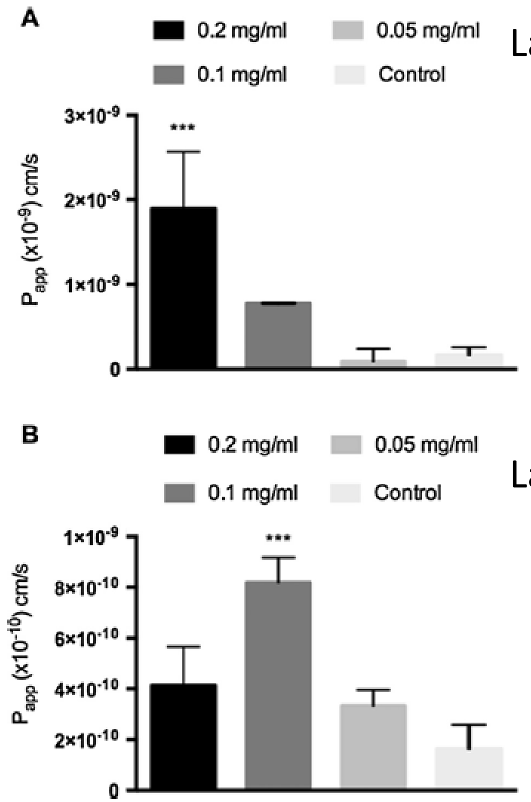




Surfactant	MTT Assay IC_{50} (mg/mL)
	Caco-2
Lacto-C16 1W	> 0.8
Lacto-C18 1W	0.14
Lacto-C24 1W	> 0.8

Lucarini S. et al., *Eur J Pharm Biopharm* **2016**, *107*, 88-96; Perinelli D. R. et al., *Eur J Pharm Biopharm* **2018**, *124*, 55-62.

FITC-Ovalbumin Permeability across Caco-2 cell monolayer



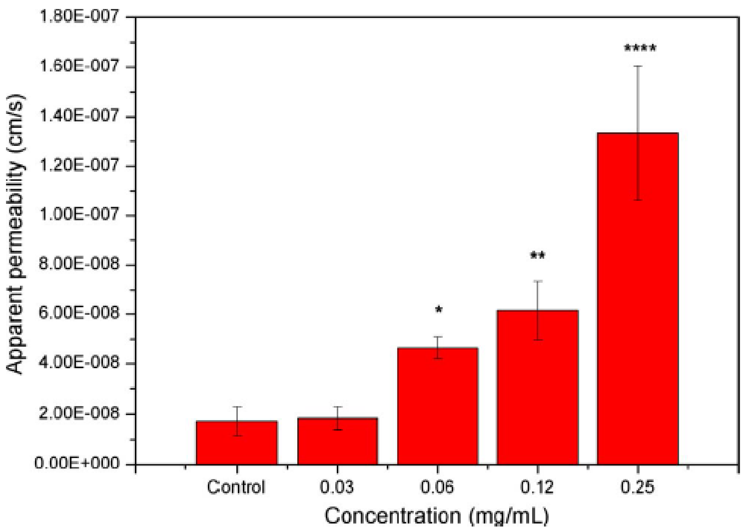
Lactose Palmitoleate (C16 1W)

Lactose Nervonate (C24 1W)

FITC-Labelled Ovalbumin 45 Kda (FOVA-45)

Lucarini S. et al., *Eur J Pharm Biopharm* **2016**, *107*, 88-96.

FITC-Dextran Permeability across Caco-2 cell monolayer



Lactose Oleate (C18 1W)

FITC-labelled Dextran 4 Kda (FD-4)

Perinelli D. R. et al., *Eur J Pharm Biopharm* **2018**, *124*, 55-62.

Antimicrobial activity against Foodborne Pathogens

Compound	MICs (µg/ml)							
	E. Coli ATCC 35150	*E. Faecalis* ATCC 29212	*L. Monocytog.* ATCC 7644	*P. Aeruginosa* ATCC 9027	*S. Aureus* ATCC 43387	*S. Enteritidis* ATCC 13076	*Y. Enterocolitica* ATCC 27729	*C. Albicans* ATCC 10231
Lacto-C10	128	512	128	128	128	128	128	128
Lacto-C12	128	128	128	128	128	128	256	512
Lacto-C14	128	128	128	128	128	128	128	128
Lacto-C16	128	128	128	128	128	64	64	64
Lacto-C16 1W	64	64	64	128	128	128	64	64
Lacto-C18 1W	128	128	256	128	128	128	128	128
Lacto-C24 1W	64	64	64	128	128	64	64	64
Gentamicin	128	64	8	16	16	4	8	-
Parabens	> 1024	> 1024	> 1024	> 1024	> 1024	> 1024	> 1024	> 1024

Lucarini S. et al., *Eur J Pharm Biopharm* **2016**, *107*, 88-96; Perinelli D. R. et al., *Eur J Pharm Biopharm* **2018**, *124*, 55-62 and Unpublished data.

Growth inhibition by time-kill experiments of Foodborne Pathogens

Food-borne pathogens:	Growth inhibition by			
	Lactose palmitoleate (μg/mL)		Lactose nervonate (μg/mL)	
	MIC	2MIC	MIC	2MIC
L. monocytogenes ATCC 7644				
3 h	2.41%	5.99%	3.55%	5.38%
6 h	5.40%	8.94%	6.49%	8.89%
24 h	9.32%	15.33%	10.64%	14.85%
E. coli O157:H7 ATCC 35150				
3 h	11.47%	14.20%	5.78%	9.51%
6 h	16.00%	20.82%	11.88%	16.50%
24 h	19.10%	**22.08%**	13.95%	**19.47%**
S. enteritidis ATCC 13076				
3 h	12.16%	15.67%	11.68%	16.81%
6 h	22.82%	28.08%	23.80%	29.66%
24 h	23.13%	**29.84%**	26.20%	**30.88%**

Percentages of growth inhibition induced by Lactose Palmitoleate and Lactose Nervonate, at their MIC and 2xMIC concentrations, toward *E. coli* O157: H7 ATCC 35150, *L. monocytogenes* ATCC 7644 and *S. enteritidis* ATCC 13076, as assessed in time-kill experiments (in bold were signed the highest percentages of bacterial growth inhibition after 24 h of incubation with each compound).

Lucarini S. et al., *Eur J Pharm Biopharm* **2016**, *107*, 88-96.

Conclusions

Lactose-based surfactants are an emerging broad group of biocompatible and biodegradable compounds with established and potential future applications in the pharmaceutical, biomedical, cosmetic and food industries.

This work:

- Enzymatic-"Green" Synthesis of aliphatic and monounsaturated fatty acid lactose esters
- CMCs decrease with the increasing of the hydrophobic chain length
- Cytotoxicity at greater concentrations of CMC (safe surfactants)
- TEER experiments and Permeability studies (FITC-OVA and -Dextran) demonstrate that our lactose-based surfactants are promising permeability enhancers
- Antimicrobial activity and time-kill studies against foodborne pathogens shown a further possible application as a safe preservatives for food, cosmetics, etc.

Acknowledgments

1506 UNIVERSITÀ DEGLI STUDI DI URBINO CARLO BO

Prof. Casettari Luca
Prof. Duranti Andrea
Dott. Campana Raffaella
Verboni Michele

UNICAM Università di Camerino 1336

Dott. Perinelli Diego R.
Prof. Palmieri Giovanni F.

UNIVERSITY OF LINCOLN

Prof. Vllasaliu Driton
Dott. Cole Hannah

The University of Nottingham

Prof. Stolnik Snjezana
Dott. Cavanagh Robert

UCL

The University of Hong Kong

Prof. Lam Jenny K. W.
Dott. Liang Wanling