



**HAL**  
open science

## **Cholecalciferol (vitamin D3) and retinyl palmitate (vitamin A) display different solubilization capacities in mixed micelle solutions: effect of interactions with mixed micelle components and of cholecalciferol self-associating properties.**

Damien P. Prévéraud, Charles Desmarchelier, David Chapron, Ali Makky, Véronique Rosilio, Patrick Borel

### ► To cite this version:

Damien P. Prévéraud, Charles Desmarchelier, David Chapron, Ali Makky, Véronique Rosilio, et al.. Cholecalciferol (vitamin D3) and retinyl palmitate (vitamin A) display different solubilization capacities in mixed micelle solutions: effect of interactions with mixed micelle components and of cholecalciferol self-associating properties.. 16. Fat Soluble Vitamins Congress - March 21-23, 2017 - Paris, Société Française des Vitamines et Biofacteurs (SFVB). FRA., Mar 2017, Paris, France. 130 p. hal-01595512

**HAL Id: hal-01595512**

**<https://hal.science/hal-01595512v1>**

Submitted on 2 Jun 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-SA 4.0 - Attribution - ShareAlike - International License



PARIS - France  
March 21-23, 2017

16th **F**at  
**S**oluble  
**V**itamins  
CONGRESS



[www.fsv-paris2017.com](http://www.fsv-paris2017.com)



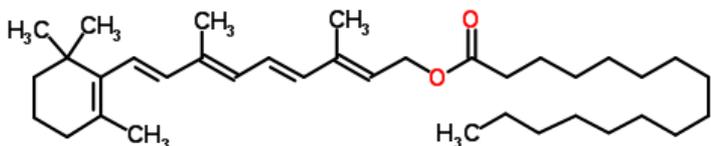
**Damien P. Prévéraud**

CHOLECALCIFEROL (VITAMIN D3) AND RETINYL PALMITATE (VITAMIN A)  
DISPLAY DIFFERENT SOLUBILIZATION CAPACITIES IN MIXED MICELLE  
SOLUTIONS:

- *EFFECT OF INTERACTIONS WITH MIXED MICELLE COMPONENTS AND OF CHOLECALCIFEROL SELF-ASSOCIATING PROPERTIES*

# Vitamins A and D in food of animal origin

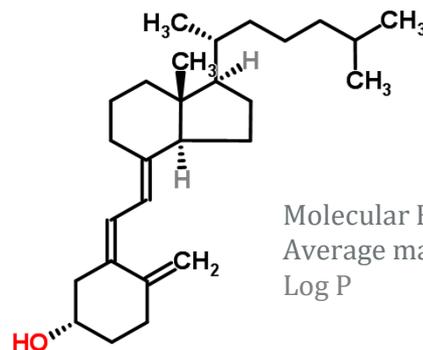
□ Vitamin A → retinyl palmitate (RP)



Molecular Formula  
Average mass  
Log P

$C_{36}H_{60}O_2$   
524.86 Da  
 $14.83 \pm 0.45$

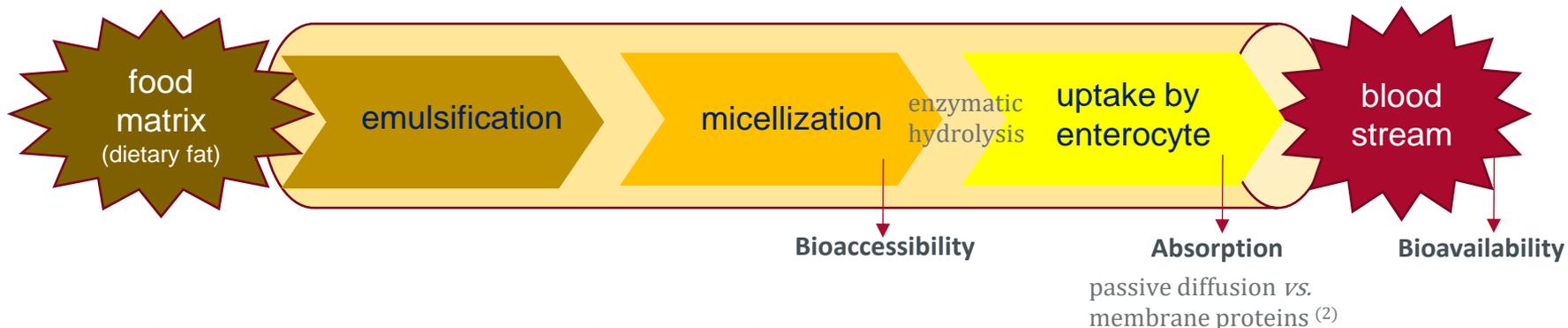
□ Vitamin D → cholecalciferol (D3)



Molecular Formula  
Average mass  
Log P

$C_{27}H_{44}O$   
384.638 Da  
 $9.72 \pm 0.27$

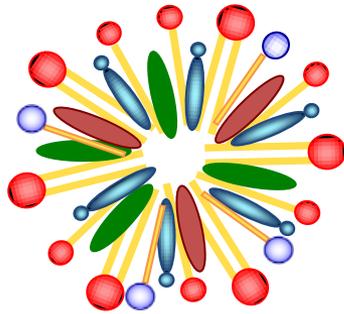
□ Digestion process in the upper gastrointestinal tract <sup>(1)</sup>:



□ Micellization is a key step for bioavailability

<sup>1</sup>Borel, P., Caillaud, D. and Cano, N. J. (2013) *Crit. Rev. Food Sci. Nutr.* 55.; <sup>2</sup>Reboul, E. and Borel, P. (2011) *Prog. Lipid Res.* 50.

# Interactions with mixed micelles components



mixed micelles

components	ORIGIN		
	diet	bile	digestion products
Bile salts		✓	
Cholesterol	✓	✓	
Phospholipids	✓	✓	
Lysophospholipids		✓	✓
Fatty acids			✓
Monoglycerides			✓

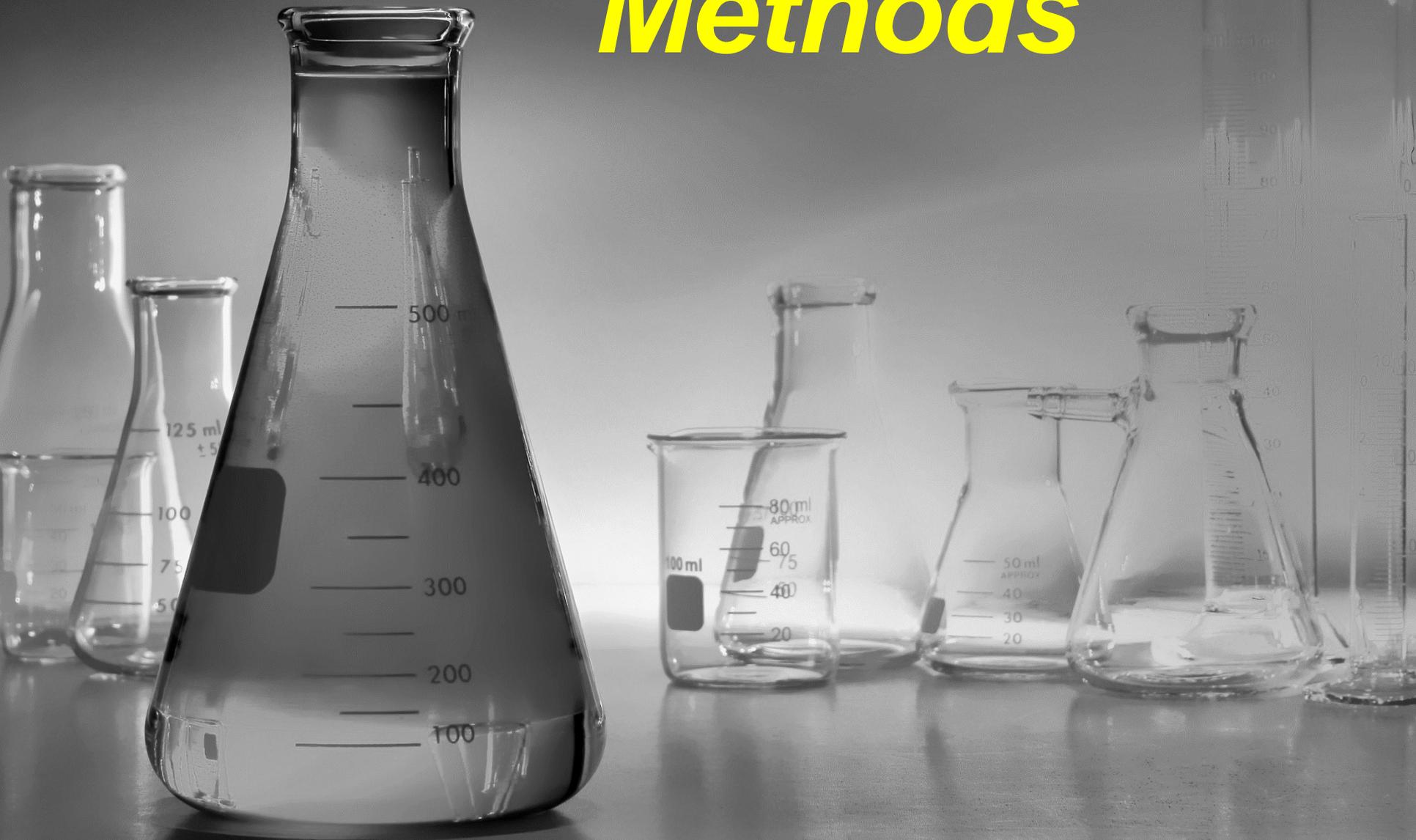
- ❑ Mixed micelle lipid composition affects the solubility of fat-soluble vitamins:
  - Substitution of lysophospholipids by phospholipids = lymphatic absorption of vitamin E  $\nearrow$  in rat (Koo and Noh, 2001 J Nutr)
  - Change in FA profile (chain length and saturation degree) = absorption of D3  $\searrow$  in perfused rat (Hollander, 1978 Gut)
  - Addition of oleic and linoleic acids = absorption of D3  $\searrow$  (Hollander, 1978 Gut)

## Objectives:

- (i) compare the relative solubility of D3 and RP in the aqueous phase rich in mixed micelles
- (ii) study the interactions between these vitamins and the mixed micelle components



# *Methods*



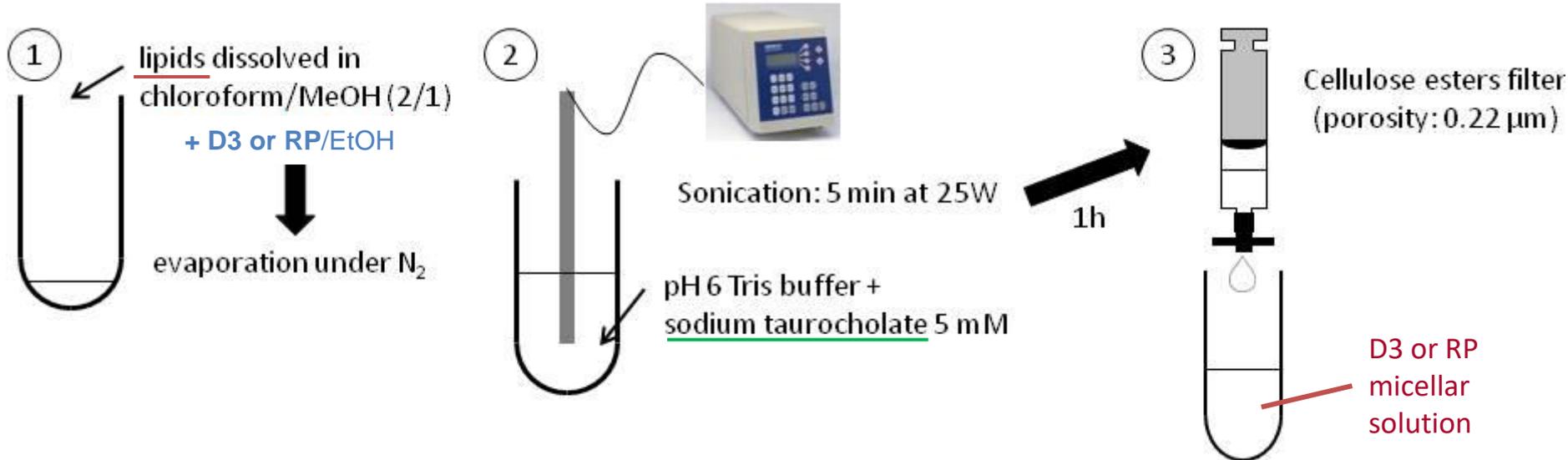
# Incorporation efficiency of RP and D3 in mixed micelles

LDP:NaTC  
1.1:5

	Concentration (mM)
Monoolein	0.30
Oleic acid	0.50
Phosphatidylcholine (PC)	0.04
Cholesterol	0.10
LysoPC	0.16
<b>Taurocholate (NaTC)</b>	<b>5.00</b>
Cholecalciferol	0.001 - 1
Retinyl palmitate	0.001 - 5

Lipid Digestion Products (LDP) 1.1 mM

Desmarchelier *et al.* (2013) MNFR

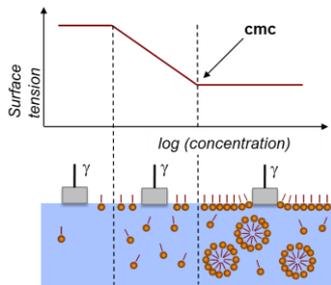


☐ Vitamin analysis by HPLC



# interactions between RP and D3, and the mixed micelle components

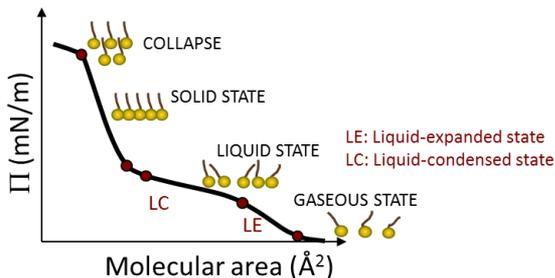
- ☐ Surface tension measurement:



Tensiometer K10 (Krüss)

→ Critical Micellar Concentration (CMC)

- ☐ Surface pressure measurement:



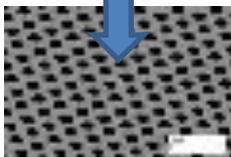
Langmuir trough

→ Compression isotherms  
→ Surface compressional modulus

$$K = -A \frac{d\Pi}{dA}$$

- ☐ Cryo-TEM (Transmission Electron Microscopy) analysis:

D3 micellar suspension



Copper grid (TedPella)

liquid ethane bath



JEOL 2200FS + CCD camera

→ Electron micrographs

$$x=L$$

$$\sum_{i=1}^x (\alpha_i + b)^2$$

$$Z = \frac{2P - ax - by}{c}$$

# Results

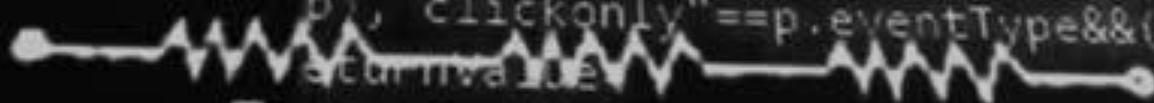
$$T_R = q(V_A - V_B)$$

$$U = \int \vec{E} \cdot d\vec{v}$$

$$V = \int \frac{e^{-\lambda x}}{x^2} dx$$
$$\sqrt{\sum_{i=1}^n (x_i - \bar{x})^2}$$

$$\omega_1 = \frac{2}{L} \sin^2 \frac{\pi x}{L}$$

Red = function !=



$$I_1 = I_2 = I_3$$

$$R_1$$

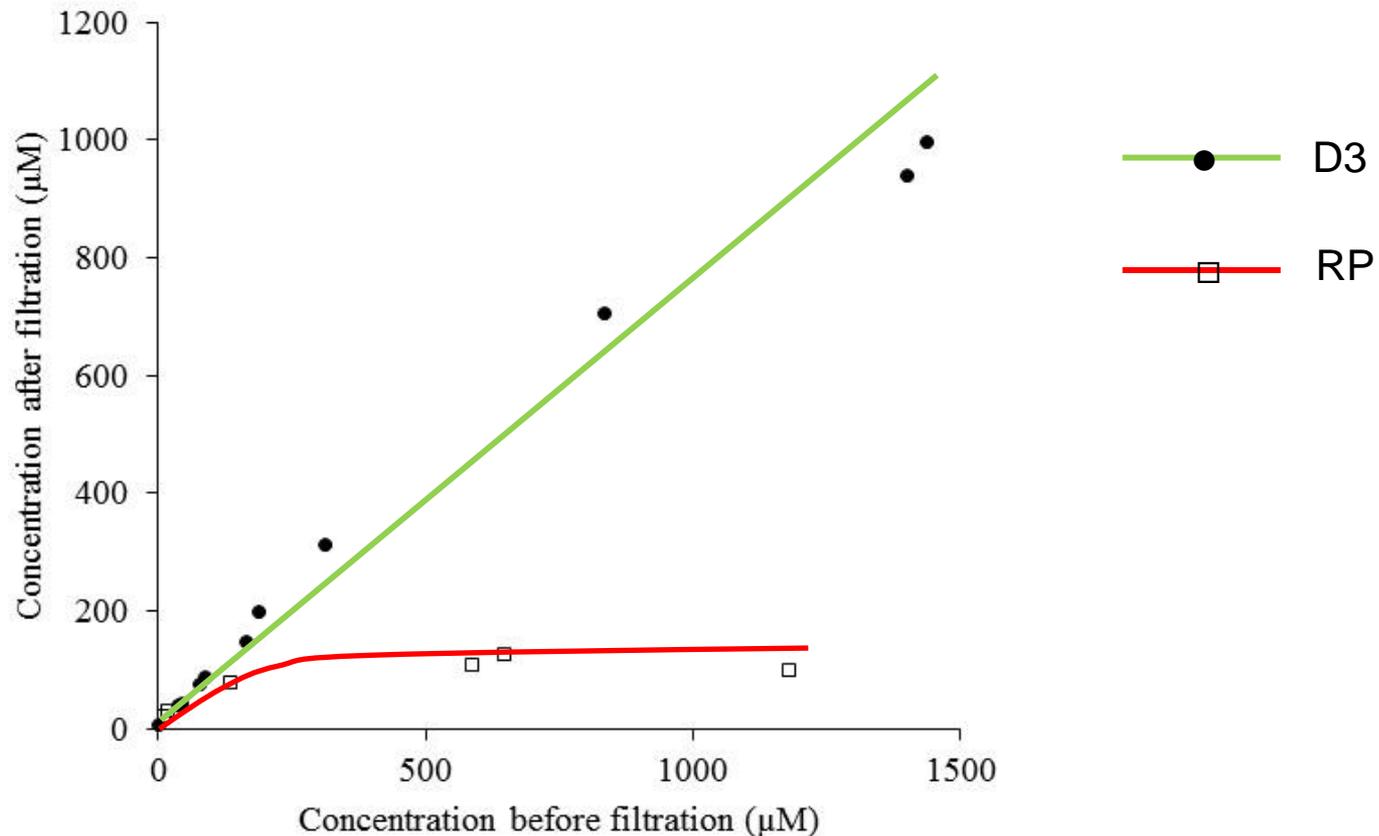
$$R_2$$

$$S = \text{function}(a, d, b, c, e, f)$$

$$\text{function}(b) \{ \text{var } c=a, e=d, f=!1; \text{"mouseenter"} == c ? c = mo$$

$$U = U_1 + U_2$$

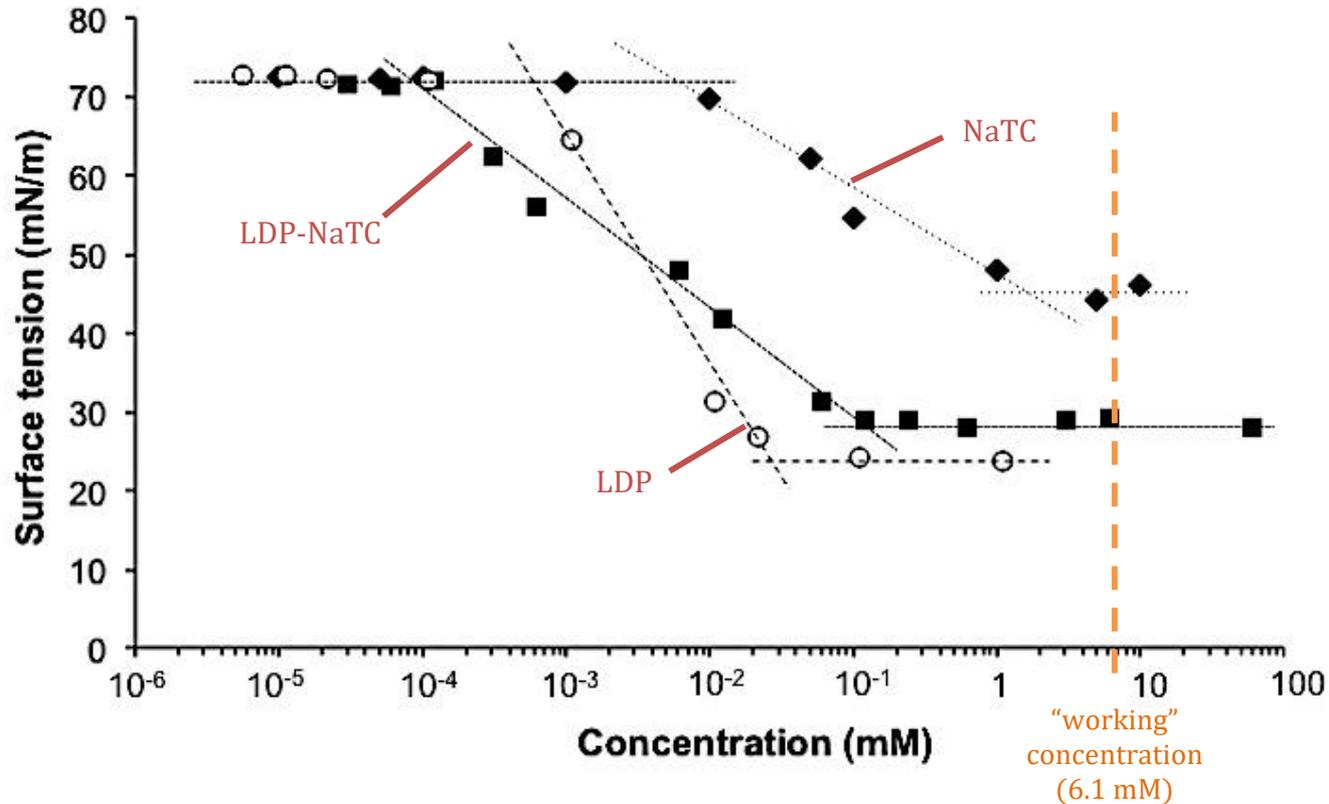
# Solubilization of RP and D3 in mixed micelle solutions



- D3 solubilization was linear ( $R^2=0.98$ , regression slope=0.71) and higher than that of RP, which reached a plateau with a maximum concentration around 125 µM

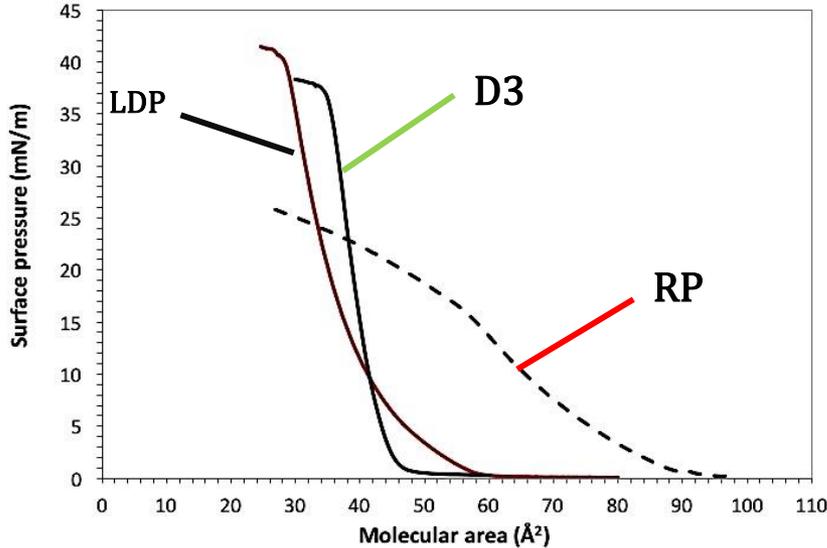


# Interfacial behaviour of micelle components



- ❑ LDP showed better surface parameters (CMC and surface tension) than NaTC
- ❑ LDP aimed to improved NaTC surface tension and CMC by inserting well in NaTC domain
- ➔ D3 and RP solubilization depend on their capacity to penetrate the LDP and NaTC domains

# Interfacial behaviour of the 2 vitamins and lipids



Monolayer	$A_c$ (Å <sup>2</sup> )	$\pi_c$ (mN/m)	$K_{max}$ (mN/m)
PC	54.0	40.0	112.7
LysoPC	32.0	36.0	44.8
Monoolein	27.0	41.0	94.7
Oleic acid	26.0	37.3	96.5
Cholesterol	38.0	44.4	939.8
LDP mixture	29.0	41.0	115.4
D <sub>3</sub>	35.2	38.0	187.4
RP	56.0	16.2	42.8

$A_c$ : collapse molecular area,  $\pi_c$ : collapse surface pressure.  
 $K_{max}$ : maximum compressibility modulus.

- Based on  $K_{max}$ , the lipid monolayers can be classified into **poorly organized** ( $K < 100$  mN/m, for lyso-PC, monoolein, and oleic acid), **liquid condensed** ( $100 < K < 250$  mN/m, for POPC and the LDP mixture) and **highly rigid monolayers** ( $K > 250$  mN/m, for cholesterol)
- D3 and LDP exhibit similar interfacial behavior, whereas RP show very different behavior compared to the micelle lipids

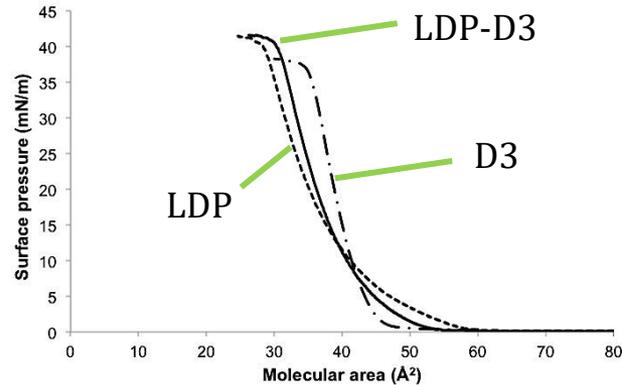


# Interaction of the 2 vitamins with NaTC and LDP

Sample	$C_{\text{micelle}}$ (mM)	$C_{\text{vitamin}}$ (mM)	Surface tension (mN/m)
NaTC micelles	5	0	$44.9 \pm 0.1$
NaTC micelles + D3	5	0.1	$36.8 \pm 0.5$
NaTC micelles + RP	5	0.1	$37.9 \pm 0.5$

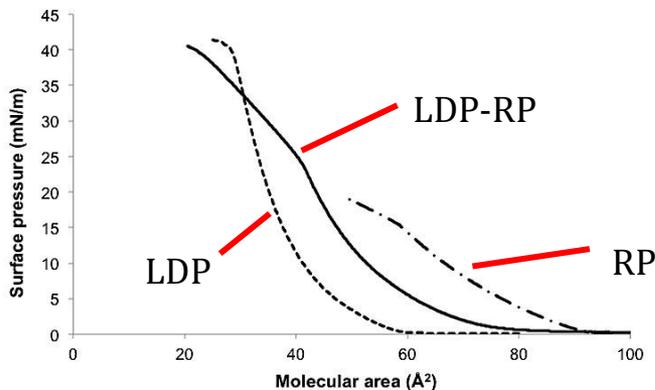
□ NaTC micelle barely affect by D3 or RP.

→ D3 and RP insert into NaTC domain (same effect on its organization)



□ For LDP/D3, surface pressure was controlled by LDP

□ For LDP/RP, surface pressure was controlled by RP



□ D3 conferred a better rigidity of LDP monolayers than RP:

→ Micelles are more stable

→ Better capacity to solubilize at low concentration

# Interaction of the 2 vitamins with mixed micelle

→ Is that also true for higher concentration ?

Sample	$C_{\text{micelle}}$ (mM)	$C_{\text{vitamin}}$ (mM)	Surface tension (mN/m)
Mixed micelles	6.1	0	29.0 ± 0.2
Mixed micelles + D3	6.1	0.1	31.6 ± 0.1
Mixed micelles + D3	6.1	1	30.5 ± 0.5
Mixed micelles + RP	6.1	0.1	29.5 ± 0.2
Mixed micelles + RP	6.1	1	31.8 ± 0.5

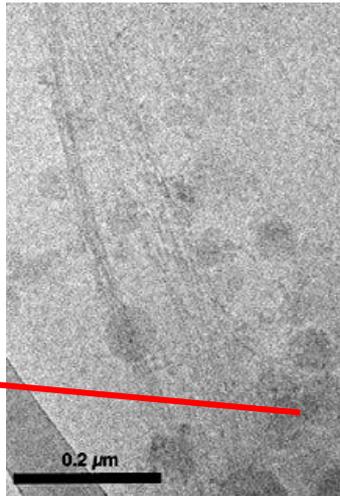
□ Low impact of vitamin concentration in mixed micelles

# cholecalciferol self-associating properties

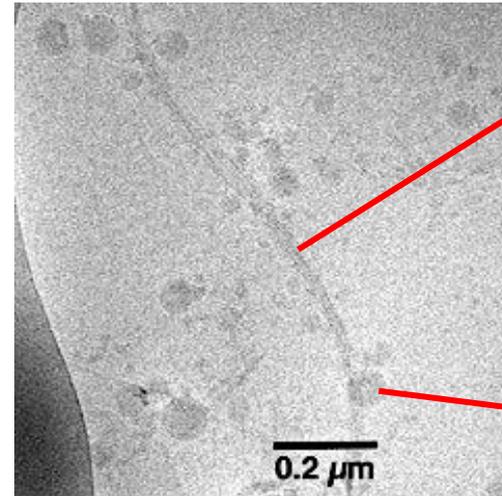
- Since (i) D3 showed an interfacial behavior similar to that of the lipid mixture, and since (ii) it could be solubilized at very high concentrations in an aqueous phase rich in mixed micelles, then its self-assembling properties were more specifically investigated:

Cryo-TEM analysis:

aggregates



Fibers  
(nanotube)



micelles

- D3 can self-associate to form micelles at concentrations  $\geq 0.1$  mM
- D3 forms various organized systems at higher concentrations (1 mM):  
Fibers (nanotubes), aggregates

***Conclusion***



# Summary of Findings - Conclusion

- ❑ D3 displays a higher solubility in mixed micelles than RP... WHY ?
- ❑ Solubility of vitamins in mixed micelles is function of their interfacial behavior and affinity with micelle components:
- ❑ Even if  $[LDP] < [NaTC]$  in micelle, solubility of RP and D3 is dependent of their capacity to insert into both NaTC and LDP domains:
  - ❑ D3 has similar behavior than LDP, whereas RP is very different
  - ❑ Both RP and D3 can insert into NaTC
- ❑ the different abilities of the two vitamins to solubilize into mixed micelles is based on their interactions with micelle components,
- ❑ but it is also due to the fact that, conversely to RP, D3 can self-associate in structures that are readily soluble in an aqueous phase.
- ❑ Whether these D3 self-assemblies are available for absorption by the intestinal cell needs further studies.



# Cholecalciferol (vitamin D3) and Retinyl Palmitate (vitamin A) display different solubilization capacities in mixed micelle solutions: effect of interactions with mixed micelle components and of cholecalciferol self-associating properties

Damien P. Prévéraud<sup>1\*</sup>, Charles Desmarchelier<sup>2</sup>, David Chapron<sup>3</sup>, Ali Makky<sup>3</sup>, Véronique Rosilio<sup>3</sup>, Patrick Borel<sup>2</sup>

<sup>1</sup>Adisseo France SAS, Centre of Expertise and Research in Nutrition, Commentry, France; <sup>2</sup>UMR 1260 INRA/1062 INSERM/Aix-Marseille University, "Nutrition, Obesity and Risk of Thrombosis", Marseille, France; <sup>3</sup>University Paris-Sud, CNRS UMR 8612, Institut Galien Paris Sud, Châtenay-Malabry, France; \*[damien.preveraud@adisseo.com](mailto:damien.preveraud@adisseo.com)

## Scope

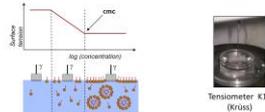
- Vitamin A (as **retinyl palmitate**, RP) and vitamin D (as **cholecalciferol**, D3) : the 2 main fat-soluble vitamins found in food of animal origin. The solubilization of these vitamins in the human upper gastrointestinal tract during digestion is assumed to follow that of dietary lipids<sup>(1)</sup>; this includes **emulsification, solubilization in mixed micelles, diffusion across the unstirred water layer and absorption by the intestinal enterocyte** (2).
- Solubilization in mixed micelles (**micellarization**) : a key step for their **bioavailability**.
- Mixed micelles are mainly made of a mixture of **bile lipids** (bile salts, cholesterol, phospholipids, lysophospholipids) and **dietary lipids** (phospholipids, lysophospholipids, cholesterol, fatty acids and monoglycerides).
- Mixed micelle lipid composition has been shown to significantly affect **vitamin solubility**<sup>(3)</sup> and the possibility of **self-association** in the aqueous environment of the intestinal tract lumen<sup>(6-7)</sup>.
- This study was designed to compare *in vitro* the relative solubility of D3 and RP in the presence of mixed micelles and to study by surface tension and surface pressure measurements the interactions between these vitamins and the mixed micelle components.

## Methods

- Synthesis of mixed micelles and measurement of D<sub>3</sub> and RE incorporation efficiency by HPLC



- Surface tension measurement and determination of the critical micelle concentration (cmc)



- Analysis of the molecular interactions between micelle components and vitamins by surface pressure measurements (Langmuir trough)



- Cryo-TEM (Transmission Electron Microscopy) analysis



## Conclusion

The results obtained show that D3 displays a higher solubility in mixed micelles than RP. This difference is mainly due to the different abilities of the two vitamins to solubilize into mixed micelles based on their interactions with micelle components, but it is also due to the fact that, conversely to RP, D3 can self-associate in structures that are readily soluble in an aqueous phase. Whether these D3 self-assemblies are available for absorption by the intestinal cell needs further studies.

Come and see our poster !  
Oslo room (Level -1)

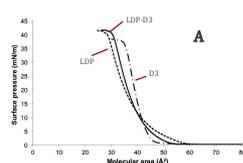
Figure 3. Interaction of D3 and RP with NaTC

Composition of solution	Micellar solution concentration (mM)	Vitamin concentration (mM)	Surface tension (mN/m)
NaTC micelles	5	0	44.9 ± 0.1
NaTC micelles + D3	5	0.1	34.3 ± 0.3
NaTC micelles + RP	5	0.1	37.9 ± 0.3

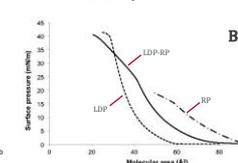
→ Both D3 and RP insert into NaTC domains

Figure 4. Interaction of D3 (A) and RP (B) with LDP

→ In LDP-D3, the surface pressure and molecular area at collapse are controlled by LDP



→ In LDP-RP, the interfacial behavior is controlled by RP



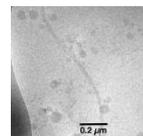
D3 solubilization was linear (R<sup>2</sup>=0.98, regression slope=0.71) and higher than that of RP, which reached a plateau with a maximum concentration around 125 μM

Figure 5. Self-assembling of D3 at concentrations higher than 1 μM

Component	Area (nm <sup>2</sup> )	K <sub>max</sub> (mN/m)
LDL	40.0	113.0
LDL+D3	30.0	44.8
LDL+RP	37.0	90.7
LDL+D3+RP	30.0	96.7
LDL+D3+RP	30.0	104.0
D3	30.2	107.0
RP	30.0	42.8

- Poorly organized → **lyso-PC, monoolein, oleic acid**
- Liquid condensed → **PC, LDP mixture**
- Highly rigid monolayers → **cholesterol**

→ D3 showed an interfacial behavior similar to that of lipid mixture



→ Co-existing molecular assemblies in 1 mM pure D3 hydrated system

# Thank you for your attention !

Damien P. Prévéraud



Centre of Expertise and Research  
in Nutrition, Commentry (France)

Charles Desmarchelier,  
Patrick Borel



UMR 1260 INRA/1062  
INSERM/Aix-Marseille University,  
"Nutrition, Obesity and Risk of  
Thrombosis", Marseille (France)

David Chapron, Ali Makky,  
Véronique Rosilio



CNRS UMR 8612, Institut Galien  
Paris Sud, University Paris-Sud,  
Châtenay-Malabry (France)