Casein Micelles As Nanovehicles of Interesting Molecules for Food and Pharmaceutical Applications

Some examples

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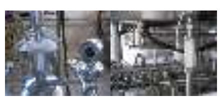
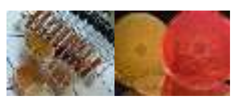
Une approche multidisciplinaire et multiéchelle renforcée par 2 Plateformes

Plateforme LAIT



Centre de Ressources Microbiologiques



STLO

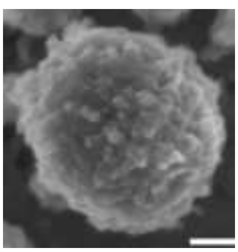
- **Mécanismes de structuration / destructuration de matrices alimentaires:**
  De la caractérisation structurale à la digestion
- **Technologies laitières et fabrication fromagère :**
  Vers des systèmes laitiers écoconçus et durables
- **Interactions microbiennes:**
  De la matrice alimentaire à l'hôte cellulaire

Please visit http://www6.rennes.inra.fr/stlo_eng

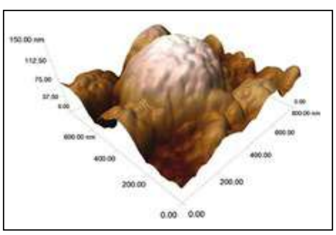
General Characteristics of Casein Micelles

- 80% of the milk proteins (25 g/l)
- 94 % of 4 types of casein molecules (α_{s1}-, α_{s2}-, β- and κ) associated with each other through 6 % of inorganic constituents (Ca, inorganic phosphate, Mg & citrate)
- Importance in dairy technologies : source of several hundred dairy products and ingredients
- Importance in nutritional properties of dairy products
  - Vectorisation of calcium
  - Essential amino-acids
  - Peptides with biological activities

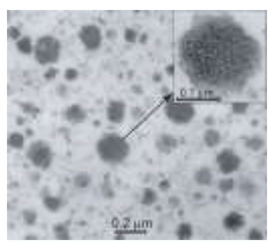
Microscopies of Casein Micelles



Scanning Electron Microscopy

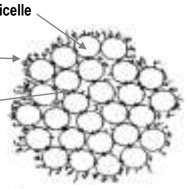


Atomic Force Microscopy



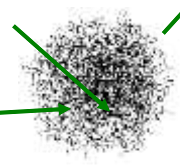
Cryo Transmission Electron Microscopy

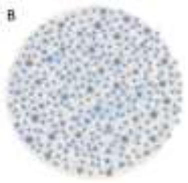
Different Structural Models of Casein Micelles

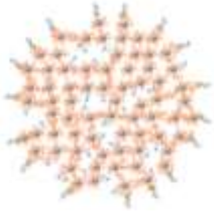


Walstra (1990)

Carl Holt's open structure casein model







Bouchoux et al. (2010).

Average Characteristics of Casein Micelles

- Diameter 120 nm : (range: 50–500 nm)
- Surface area : 8×10−10 cm^2
- Volume : 2.1×10−15 cm^3
- Density (hydrated) : 1.0632 g cm^{-3}
- Mass : 2.2×10^{-15} g
- Water content : 63 %
- Hydration : 3.7 g H_2O g^{-1} protein
- Voluminosity : 44 cm^3 g^{-1}
- Molecular mass (hydrated): 1.3×10^9 Da
- Molecular mass (dehydrated) : 5×10^8 Da
- No. of peptide chains : 5×10^3
- No. of particles per mL milk : 10^{14}–10^{16}
- Surface of micelles per mL milk : 5×10^4 cm^3
- Mean free distance : 240 nm

P.F. Fox, A. Brodkorb , *International Dairy Journal*, *18, 2008*, *677-684*

Functionalities of Caseins

- Examples of interesting functionalities :
  - Heat stability
  - Properties of rennet and/or acid gels (capacity to the gel formation, rheological properties, syneresis, …)
  - Properties of dehydration by spray drying and rehydration of powder
  - Nutritional and pharmaceutical properties
- Complexe properties depending on several parameters :
  - Type of protein,
  - Tridimensional structure and organisation/mineralization
  - Surface properties
  - Physico-chemical environment

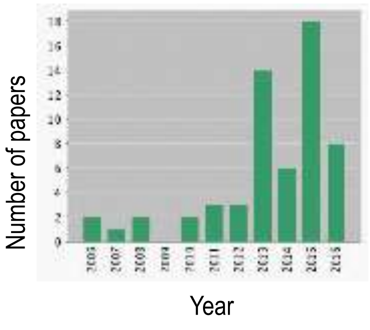
Actual Scientific Questions Concerning Casein

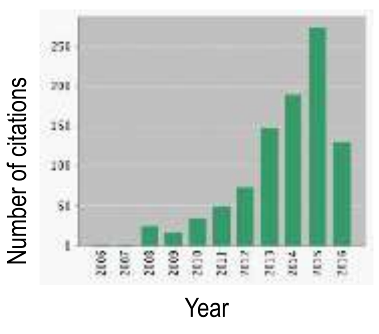
- How the internal structure of casein micelle is organized ?
- What are the role of each casein molecules in the casein micelles? (κ-casein at the surface, β-casein realtively free)

- What is the contribution of the micellar calcium phosphate in the structure and stability of the micelles
- Relationship between structure and functionalities of casein?

Recent reviews on casein micelles

Growing interest on nanoparticles with milk proteins





Citation Report: **59**
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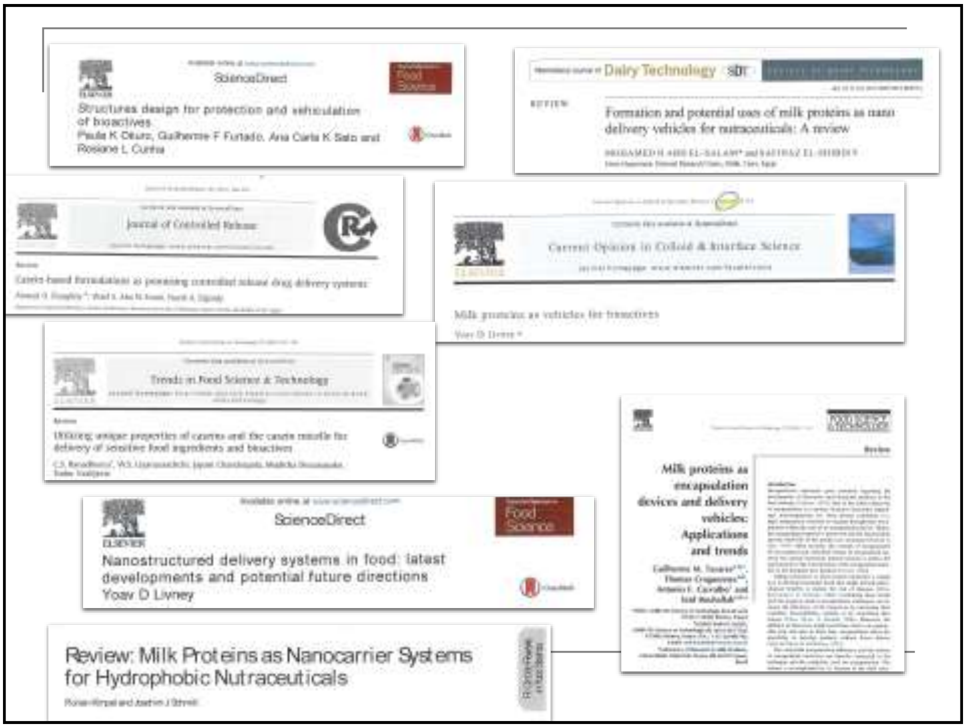
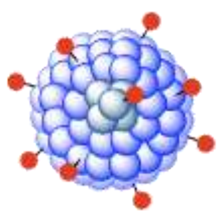
TOPIC: casein, nanoparticles, release



Objectives of this research

1. Characterize the parameters of interactions between casein and interesting molecules (association constants, stoechiometry,….)
2. Characterize the structures of the complexes
3. Characterize the stability of the complexes as a function of physico-chemical conditions
4. Characterize the biological behaviours of the complexes (protection effect, bio-utilisation, digestibility,…)



Interactions protéine-ligand

$$\text{Protéine + ligand} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{[protéine-ligand]}$$

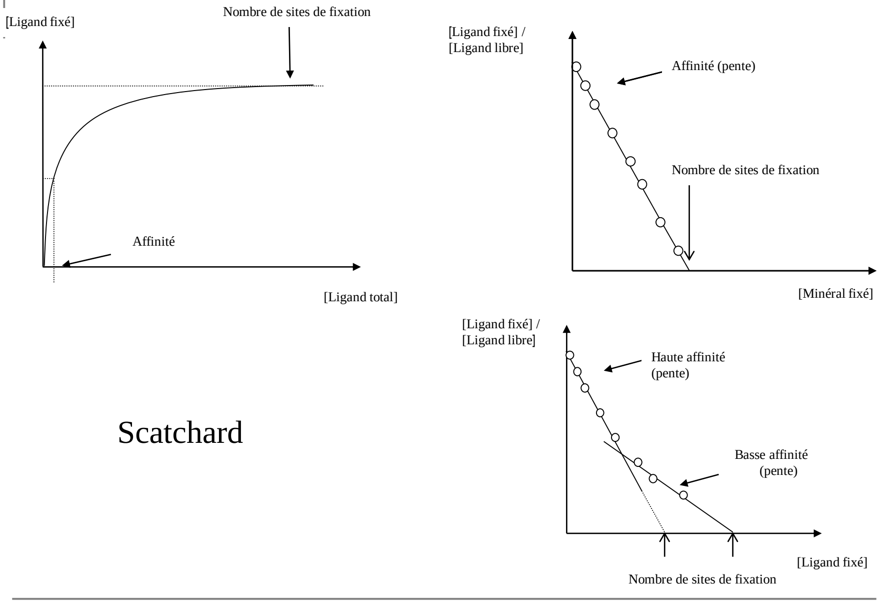
k_1 et k_2 : constantes de vitesse d'association et de dissociation

$$\frac{\text{[protéine] [ligand]}}{\text{[protéine-ligand]}} = \frac{k_1}{k_2} = Kd$$

Concentration en ligand qui permet la saturation de 50 % des sites récepteurs

Kd : constante d'équilibre de dissociation

Ka : constante d 'équilibre d'association --> 1/Kd



Scatchard

Why caseins are good candidates to be nanoparticles ?

- Source having low price and renewable
- Natural biopolymer
- Easy to prepare at industrial scale
- Natural self-assembled nanostructure
- Can exist under different aggregation states :
  - Purified casein molecules
  - Sodium caseinate (CasNa)
  - Reassembled casein micelles (rCMs)
  - Native casein micelles (CMs) also named native phosphocaseinate

How caseins can interact with interesting molecules ?

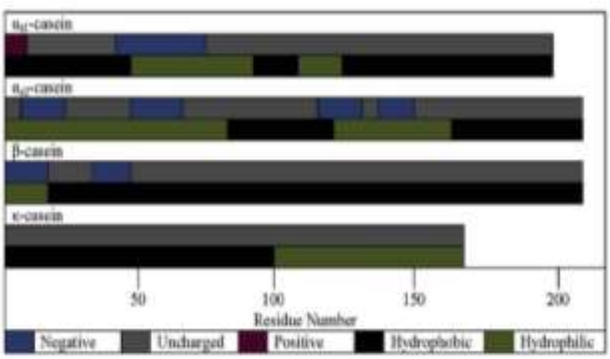
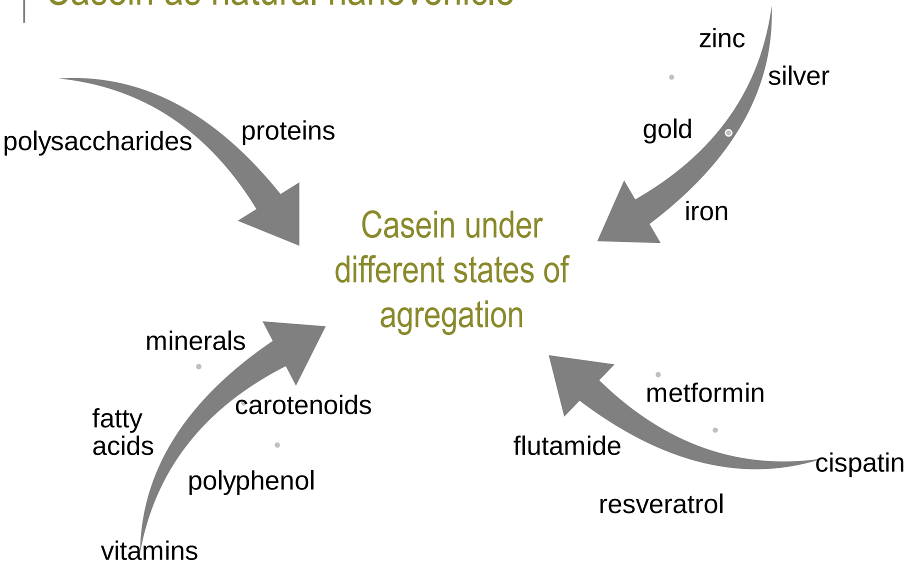


Fig. 2. Schematic diagram of the location of the mostly hydrophilic, hydrophobic, charged and uncharged regions along the casein chains at pH 6.6 (adapted and modified fro
Kessler, Menéndez-Aguirre, Hinrichs, Stubenrauch, & Weiss, 2013; Swaisgood, 2003).

Electrostatic interactions ⇨ charged molecules (ions)
Hydrophobic interactions ⇨ hydrophobic molecules

Casein as natural nanovehicle



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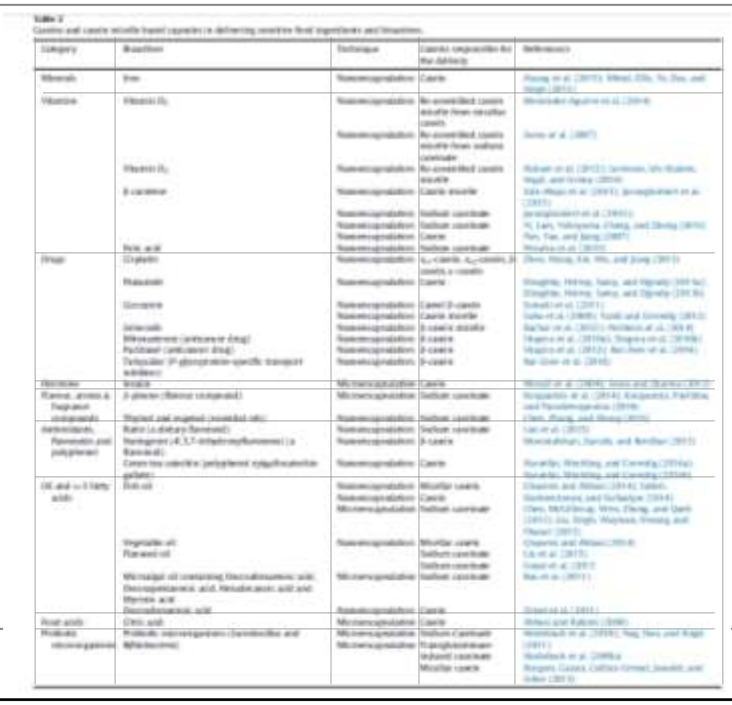
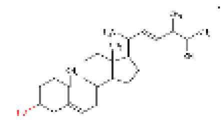


Casein micelle as a natural nano-capsular vehicle for vitamin D2



- Vitamin D2 : fat-soluble essential for calcium metabolism
- Incorporation of vitamin D2 into re-assemby casein micelles : sodium caseinate + vit D2 + citrate + phosphate + calcium / pH maintained between 6.7- 7.0
- ~ 5.5 times more concentrated within the micelles than in the serum
- No modification of the morphology and average diameter of casein micelles
- Casein micelle : partial protection against UV-light-induced degradation to vitamin D2

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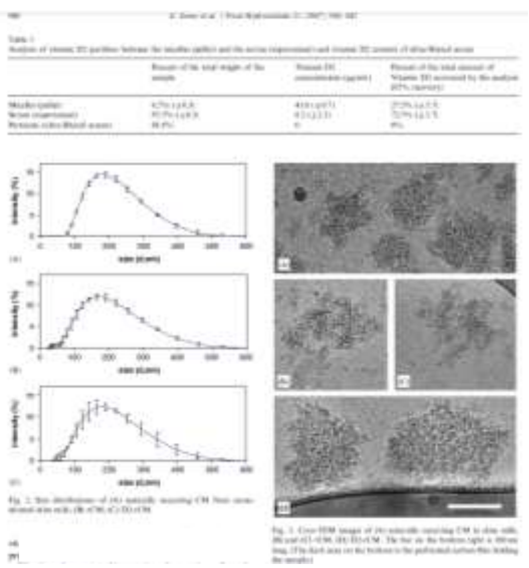
E Semo, E Kesselman, D Danino, YD Livney, Food Hydrocolloids 21 (2007) 936–942
Patent WO 2007/122613 A1 « casein micelles for nanoencapsulation of hydrophobic compounds » by Livney YD and Dalgleish D

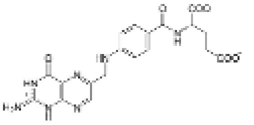
Casein and vitamin D2



This study,demonstrated that CM can be used for nano-encapsulation of hydrophobic nutraceutical substances for potential enrichment of low- or non-fat food products.

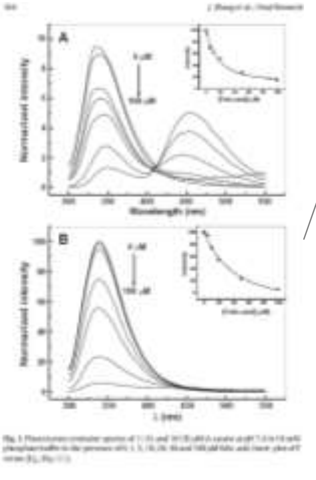
CM were shown to serve as potential nano-vehicles for added nutraceuticals such as the fat-soluble vitamin D2 chosen here as a model for hydrophobic bioactive compounds. In terms of encapsulation efficiency, 27% of the vitamin recovered from the micelle suspension was found in the reformed micelles, which contained about 5.5 times higher concentration of the vitamin compared to the surrounding serum

β-casein and acid folic



- Folate : water-soluble **vitamin B9**
- Present mainly in leafy green vegetables and legumes.
- *In vivo*, acts as a coenzyme in one carbon transfer reactions required in the biosynthesis of DNA and RNA
- Folate reduces the risk of neural tube defects and influences the likelihood of developing vascular diseases and some cancers.
- Folic acid is commonly used for nutritional fortification and for formulation of pharmaceuticals.
- Folic acid is sensitive to ultraviolet (UV) light, which causes its decomposition to inactive photoproducts

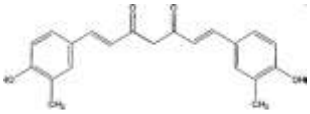
β-casein and acid folic



- β-Casein can interact with folic acid by hydrophobic contacts with a dissociation constant of ~10^{-5} M.
- Binding to β-casein appears to reduce the photodecomposition of folic acid, more so at the higher protein concentration, and inhibits folic acid photodecomposition completely at 10 μM.
- β-Casein = a carrier material suitable for folic acid delivery, and folic-acid-β-casein complex formation as a useful model of the interaction between proteins and bioactive molecules.

Curcumin-Casein micelle complexation

- Curcumin :

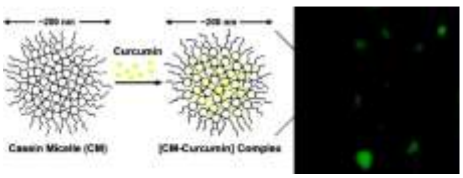


- Natural polyphenolic compound
- Lipophilic fluorescent molecules
- Low toxicity but a wide range of pharmaceutical activities including antioxidant, anti-inflammatory, antimicrobial, antiamyloid and antitumor properties

Abhishek Sahu, Naresh Kasoju, and Utpal Bora, Biomacromolecules 2008, 9, 2905–2912

Curcumin-casein micelle complexation

- Possible complexation between casein micelles and curcumin with formation of spherical particles
- Hydrophobic interactions with binding constant of 1.48 x 10^4 M^{-1} and the complexes are stable



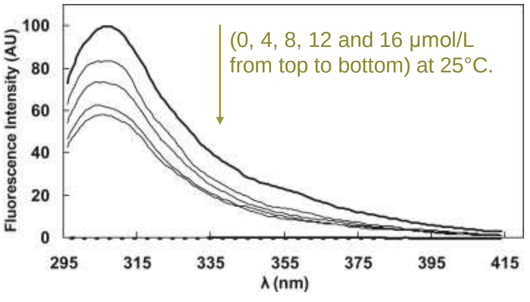
- Cytotoxicity and cellular uptake of complex and free curcumin on in vitro cultured HeLa cells were similar

↳ casein micelles = drug nanocarrier

Abhishek Sahu, Naresh Kasoju, and Utpal Bora, Biomacromolecules 2008, 9, 2905–2912

Intrinsic fluorescence of camel B-CN (5 µmol/L) in the presence of different [curcumin]

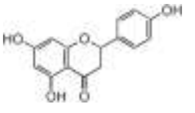
Excitation 280 nm



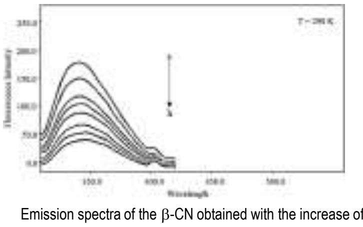
- **Fluorescence intensity of a protein is mainly due to Trp residues**. Camel B-CN contains 5 Tyr and 10 Phe residues, which are mainly located in the hydrophobic part of its primary structure, which is devoid of Trp (comparing to bovine B-CN which contains 1 Trp, 4 Tyr and 8 Phe residues).
- Interaction between curcumin and B-CN at different temperatures resulted in a **quenching of the intrinsic fluorescence at 280 nm**. Quenching of the protein fluorescence upon interaction with curcumin does not induce any shift in the spectra neither to blue nor to red.

LWT - Food Science and Technology, Volume 44, Issue 10, 2011, 2166–2172
Beta casein-micelle as a nano vehicle for solubility enhancement of curcumin; food industry application
Mansoore Esmaili, S. Mahmood Ghaffari, Zeinab Moosavi-Movahedi, Malihe Sadat Atri, Ahmad Sharifizadeh, Mohammad Farhadi, Reza Yousefi, Jean-Marc Chobert, Thomas Haertlé, Ali Akbar Moosavi-Movahedi

Naringenin with β-casein



- Naringenin : flavonoids polyphenol present in fruits and vegetables
- Studies of interaction by fluorescence



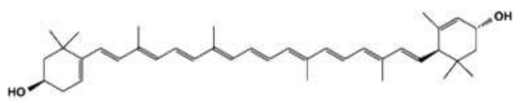
Emission spectra of the β-CN obtained with the increase of the naringenin concentration (T ¼ 298 K; lex ¼ 295 nm). [b-CN] ¼ 10.0 mM; a ¼ 0 to h ¼ 14 mL at increments of 2.0 mL.

Binding constant (K_b), and binding site numbers (n) of the NGEN-β-CN complex at 298, 303 and 308 K

pH	T (K)	K_b (× 10^3 M^{-1})	n	R^2
7.0	298	3.219	1.135	0.9984
	303	2.875	1.159	0.9994
	308	2.555	1.181	0.9991

Amir-Abbas Moeiniafshari, Ali Zarrabi, Abdol-Khalegh Bordbar, Exploring the interaction of naringenin with bovine beta-casein nanoparticles using spectroscopy Food Hydrocolloids (2015) 51, 1-6

Interactions between lutein and milk proteins

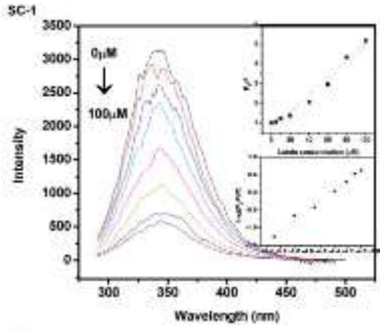


- Lutein : belonging to the family of the xanthophyll group of **carotenoids**
- Leafy greens like spinach, collard greens, kale, corn, persimmons, and broccoli are its main sources
- As a **natural antioxidant**, lutein was found to have a protective effect against oxidative damage of egg yolk lecithin liposomal membranes induced by exposure to UV radiation and incubation

Characterization of milk proteins–lutein complexes and the impact on lutein chemical stability

Food Chemistry, Volume 200, 2016, 91–97 Jiang Yi, Yuting Fan, Wallace Yokoyama, Yuzhu Zhang, Liqing Zhao

Interactions between lutein and milk proteins



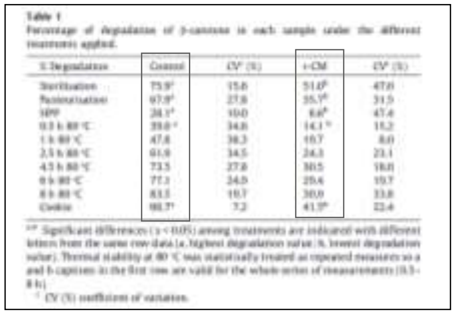
Fluorescence emission spectra 0.5 mg/mL SC,, in the presence of various concentrations of lutein (0, 5, 10, 20, 40, 60, 80, and 100 μM) in 10 mM pH 7.4 PB at 25 °C.

SC were found to interact with lutein by **hydrophobic interactions** with binding constants of 2.8 × 10^5 and 5.0 × 10^5 M with stock lutein alcoholic solution probably, and 2.4 × 10^5 and 3.5 × 10^5 with stock lutein PB, respectively.
No significant effects were observed for both proteins' secondary structure after interacting with lutein

Milk proteins–nutrient complexes might be useful in stabilizing bioactive compounds and the development of functional foods especially low-fat food.

Food Chemistry

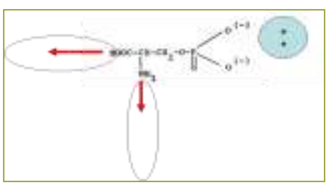
Thermal protection of β-carotene in re-assembled casein micelles during different processing technologies applied in food industry



Binding of Minerals by Casein

Caseins	Charge at pH 6	Mineral binding
α_{s1}-Casein	-42.6	Fe, Zn, Ca,….
α_{s2}-Casein	-31.2	Zn, Ca,…
β-Casein	-30.5	Fe, Zn, Ca, Mg, Mn, Cu
κ-Casein	-6.9	Ca,….

Negative charge due to phosphoseryls residues



Interactions majeures des cations sur phosphosérines

- **Caséine β**

  -Glu - Ser(P)[15] - Leu - Ser(P)[17] - Ser(P)[18] - Ser(P)[19] - Glu - ... - Gln - Ser(P)[35] - Glu -

- **Caséine α_{s1}**

  -Gly - Ser(P)[46] - Glu - Ser(P)[48] - Thr - ... - Glu - Ser(P)[64] - Ile - Ser(P)[66] - Ser(P)[67] - Ser(P)[68] - Glu - ... - Asn - Ser(P)[114] - Ala -

- **Caséine α_{s2}**

  -Val - Ser(P)[8] - Ser(P)[9] - Ser(P)[10] - Glu - Glu - ... - Gly - Ser(P)[56] - Ser(P)[57] - Ser(P)[58] - Glu - Glu - Ser(P)[61] - Ala -

  - Ile - Ser(P)[16] - Gln - Glu - ... - Leu - Ser(P)[129] - Thr - Ser(P)[131] - ... - Glu - Ser(P)[143] - Thr

- **Caséine κ**

  Glu - Ala - Ser(P)[149] - Pro - Glu -

Contribution des résidus carboxyliques

Facteurs influençant la liaison entre les caséines et les cations

Caséine

- Type (α_{s1}-, α_{s2}-, β ou κ)
- Etat de phosphorylation
- Charge

Cation

- Charge (+ 1, + 2 ou + 3)
- Taille
- Hydratation
- Type de liaison (électrostatique ou coordination)

↕

Liaison caséines-cations

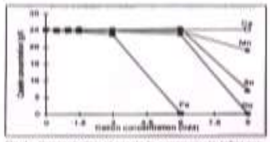
↕

Environnement physico-chimique

- pH
- Force ionique
- Présence d'ions interférants (phosphate, citrate, hydrocolloïde, etc)
- Température

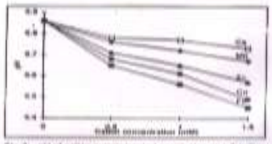
Interaction cations - caseins

Precipitation



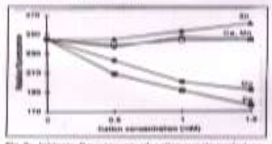
- Addition of 9 mmol.l^{-1} of cation to caseinate ↳ casein precipitation with the decreasing order :
  - $Fe^{3+} > Cu_{2+} > Zn^{2+} > Ca^{2+}$
  - ↳ charge neutralisation

pH



- Decrease in pH

Fluorescence



- Changes in the structure determined by fluorescence

Stability of complexes cations – caseins as a function of [NaCl]

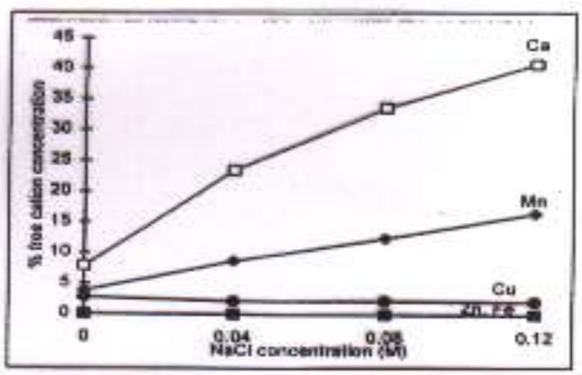
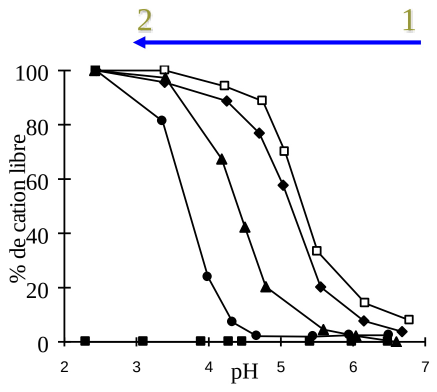


Fig. 6: Effect of NaCl concentration of cation-supplemented caseinates on the % of free cation concentration. 100% correspond to 1.5 mM-cation

Effets of pH on caseins-cations interactions



Calcium (❺)
Manganese (◆)
Zinc (⬩)
Copper (●)
Iron (III) (■)

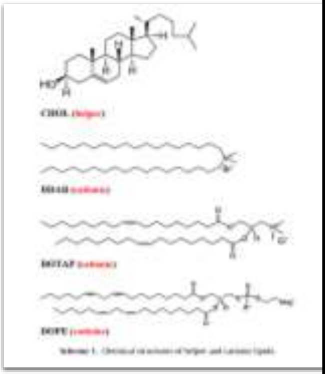
➘ of the binding (except iron) when pH ➘ (decrease in ionisation)
Differences in the mode of binding : electrostatic, coordinative (Fe)

Casein and lipids

International Journal of Biological Macromolecules

Association of lipids with milk α- and β-caseins

F. Bourassa, L. Bekale, H.A. Tajmir-Riahi



Casein and lipids

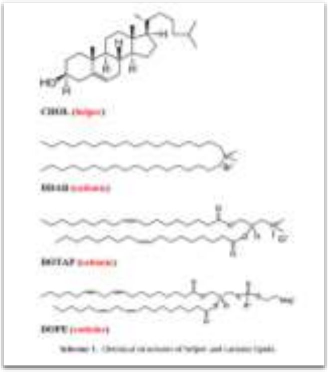
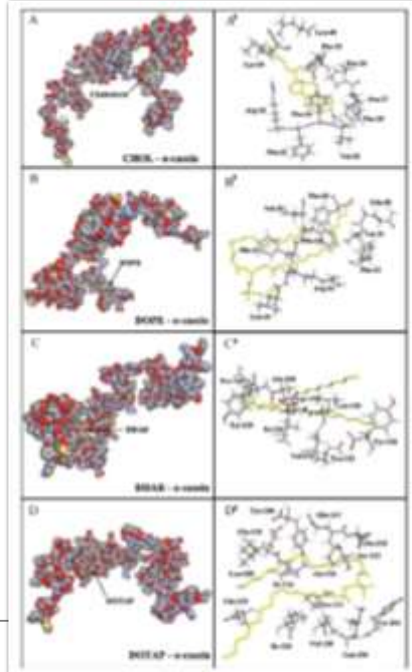


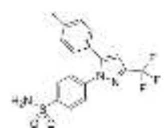
Table 1
Binding parameters for lipid–casein complexes.

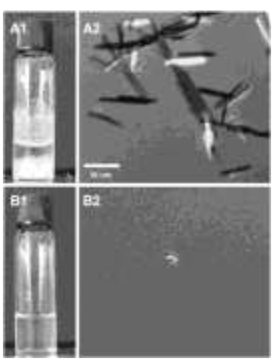
Complexes	Casein	K-fluorescence (10^4 M^{-1})	K_A [illegible]	n
CHOL–casein	α	1.0 ± 0.1	1.3 ± 0.3	0.7 ± 0.1
	β	1.0 ± 0.3	1.9 ± 0.3	1.1 ± 0.2
DOPE–casein	α	0.5 ± 0.07	0.12 ± 0.03	0.6 ± 0.04
	β	0.15 ± 0.06	0.18 ± 0.04	0.7 ± 0.06
DDAB–casein	α	2.0 ± 0.6	6 ± 0.4	0.7 ± 0.1
	β	1.7 ± 0.3	3.3 ± 0.5	0.9 ± 0.2
DOTAP–casein	α	1.5 ± 0.80	2.9 ± 0.5	1.1 ± 0.2
	β	2.1 ± 0.30	5.8 ± 0.7	1.0 ± 0.2



β-casein and celecoxib

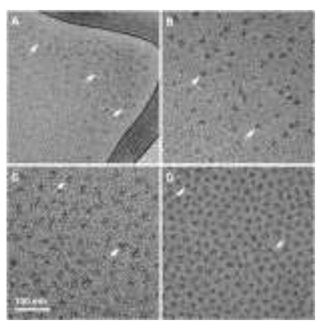
<u>Celecoxib</u> : **nonsteroidal anti-inflammatory drug** for the treatment of **rheumatoid arthritis and osteoarthritis**.





(A) celecoxib in buffer (pH 6.8). Solubility of drug is low, and it arranges into large crystals.

(B) β-casein and celecoxib in buffer, at 20 mg/mL protein and protein:drug mole ratio



Cryo-TEM images show an increase in micellar diameter upon increasing drug loading.
(A) 5 mg/mL β-casein, 1:1 protein:drug mole ratio,
(B) 5 mg/mL β-casein, 1:8 protein:drug mole ratio,
(C) 20 mg/mL β-casein only
(D) 20 mg/mL β-casein, 1:15 protein:drug mole ratio

↳ Celecoxib is solubilized by β-casein.

↳ β-casein : nanocarrier of celecoxib

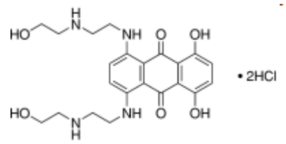
Development and characterization of a novel drug nanocarrier for oral delivery, based on self-assembled β-casein micelles

Journal of Controlled Release, Volume 160, Issue 2, 2012, 164–171

β-casein nanovehicles for oral delivery of chemotherapeutic drugs

- β-casein : one of the four mains casein in bovine milk - Amphiphilic structure with possible self association ↳ stable micelle-like structure in aqueous solution

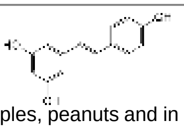
- **Mitoxantrone** = hydrophobic anticancer drug revealed by Trp fluorescence and absorbance



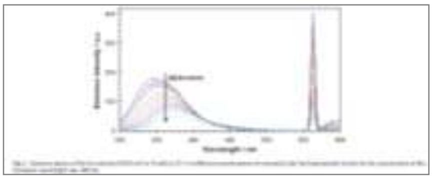
- Formation of a stable complex [β-casein-Mitoxantrone] with an optimal loading molar ratio : 3.3 Mitoxantrone/ 1 β-casein with an association constant of 2.45 x 10^{-5} M^{-1}

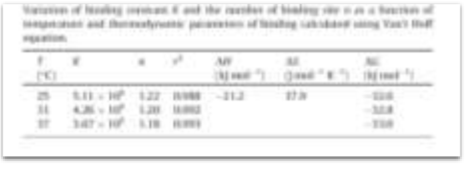
A Shapira, YG Assaraf, YD Livney in Nanomedicine: Nanotechnology, Biology, and Medicine 6 (2010) 119–126

Casein and resveratrol

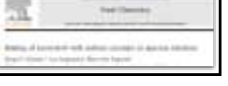


- **Resveratrol** : polyphenol found in the skin of grapes, apples, peanuts and in some traditional herbs.
- Consumption of plants and plant products that are rich in polyphenols has been related with protective effects against cardiovascular disease and certain forms of cancer
- Study of interaction between resveratrol and sodium caseinate by fluorescence



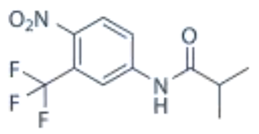


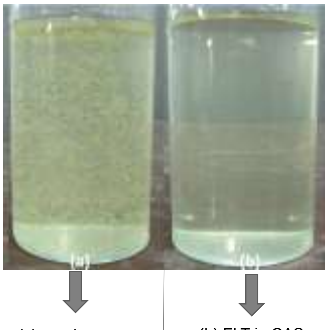
- The interaction between resveratrol (Res) and sodium caseinate (Na-Cas) has been studied by measuring fluorescence quenching of the protein by resveratrol. Quenching constants were determined using Stern–Volmer equation, which suggests that both dynamic and static quenching occur between Na-Cas and Res.
- Binding constants for the complexation between Na-Cas and Res were determined at different temperatures.
- The large binding constants (3.7–5.1 10^5 M-1) suggest that **Res has strong affinity for Na-Cas.**
- This affinity decreases as the temperature is raised from 25 to 37°C.
- The binding involves both **hydrogen bonding and hydrophobic interaction**, as suggested by negative enthalpy change and positive entropy change for the binding reaction.
- The present study indicates that Na-Cas may be used as a carrier of Res, a bioactive polyphenol which is insoluble in both water and oils.



Casein and flutamide

- Flutamide (FLT) is an antiandrogenic agent presently used for monotherapy of androgen-dependent prostate cancer
- Low solubility → low bioavailability



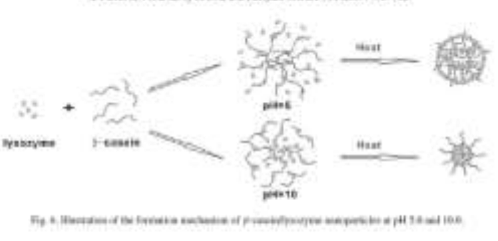


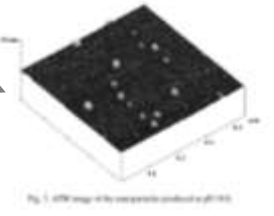
(a) FLT in water; solubility of drug is poor, and it precipitates into large crystals,

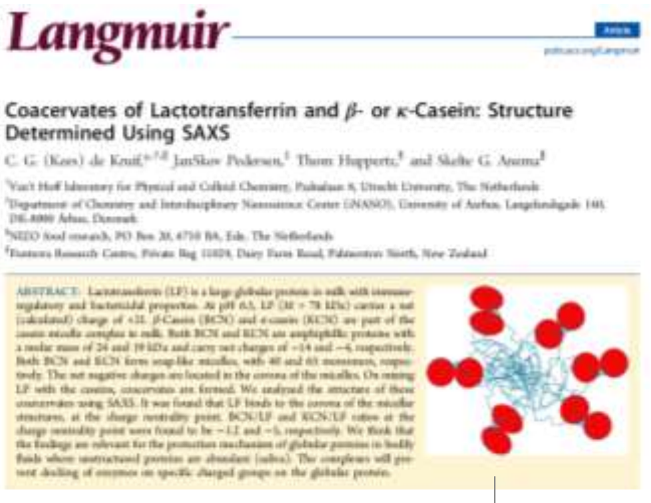
(b) FLT in CAS micelles; this solution is clear, and no drug crystals are visible.

Ahmed O. Elzoghby, Maged W. Helmy, Wael M. Samy, Nazik A. Elgindy, **Spray-dried casein-based micelles as a vehicle for solubilization and controlled delivery of flutamide: Formulation, characterization, and in vivo pharmacokinetics** European Journal of Pharmaceutics and Biopharmaceutics, 84, 2013, 487–496

Self-assembly of β-casein and lyzozyme





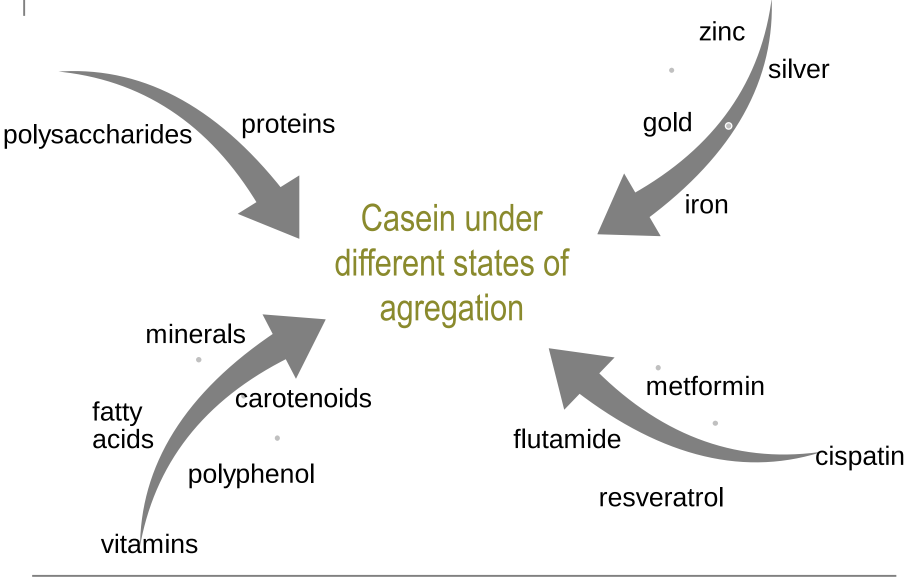

Langmuir

Coacervates of Lactotransferrin and β- or κ-Casein: Structure Determined Using SAXS

C. G. (Kees) de Kruif, Jan Skov Pedersen, Thom Huppertz, and Skelte G. Anema

Cartoon of the binding of lactotransferrin to BCN micelles. Lactotransferrin is depicted as a dumbbell according to protein structure in the Swiss Protein Data Bank.28

Casein as natural nanovehicle



Conclusion

Due to increase in the knowledge in dairy science and dairy technology

⇩

Possibility to have nanoparticules with different functions

Techno-functionality

➯ physico-chemical (pH, temperature, pressure,…) and enzymatical (proteases, TGAse, desamidase, …) modifications

⇩

Thermal stability, acid and rennet gels properties, emulsifying properties, *etc*

Health

➯ addition of external compounds :

- Ca,
- curcumin,
- vitamins,
- anticancer drug,
- *etc*

- **Merci pour votre attention**
- **Merci aux comités scientifiques et d'organisation**
- **Merci au Professeur Mohammed Ali Ayadi**