

Schrödinger's Capsule

a (micro)capsulate that is open and closed at the same time

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Abstract

We exploit different routes for encapsulation of food additives, such as minerals or vitamins, in a polymeric capsule. The added active ingredients should remain inside the capsule for at least a year in an aqueous environment (e.g. a dairy product), since sensory properties or functionality of the ingredients may otherwise be affected. However, after intake the active compound should readily (within 1 h) be released due to the acidic environment in the stomach. First, we propose a phenomenological model in order to study how a polymeric matrix may limit the diffusion of incorporated active molecules. The relation between the release rate of the active compound and its molecular weight is elucidated. Second, the desired capsules may be obtained by specific binding between subunits within the capsule and the active ingredient. We show two examples that rely on this mechanism: amylose-lipid complexes and mixed metal hydroxides. Amylose is able to form inclusion complexes with various types of ligands, including iodine, monoglycerides, fatty acids and alcohols, where the hydrophobic parts of the ligands are entrapped in the hydrophobic helical cavity of amylose. Mixed metal hydroxides are a versatile class of inorganic solids that consist of sheets of metal cations that are octahedrally surrounded by hydroxide molecules. In between these layers anionic species compensate for charge neutrality. In this way, various metal cations (minerals) may be incorporated with a high loading, and negatively charged actives may be placed between the layers. Upon digestion the particles dissolve and the ingredients are digested. Finally, we show that nature has already developed many intriguing capsules.

1. Company introduction

Royal FrieslandCampina is a multinational dairy company owned by the dairy cooperative Zuivelcoöperatie FrieslandCampina, which has 15,300 member dairy farms in the Netherlands, Germany and Belgium. Their products are for sale in more than 100 countries. Key regions are Europe, Asia and Africa. In 2009 sales amounted to 8.2 billion euro. The company employs 20,000 people in 24 countries and provides people around the world with all the good things milk has to offer. FrieslandCampina employs around 450 persons in R&D. Important research subjects are nutritional aspects of milk, fractionation, concentration and preservation of milk and food structuring.

2. Problem description

Milk contains many nutritional components. However, there are several cases in which the addition of extra nutritional ingredients to milk, such as minerals or vitamins, is needed e.g. to prevent nutritional deficiencies or to increase the well-being of consumers.

Research has shown that supplementation of food is often the most effective way to achieve this. Nevertheless, added ingredients often chemically interact with milk components such that functionality of the ingredients is lost and/or sensory properties of the milk product, e.g. taste, is spoiled. In order to solve this problem we are looking for (food grade) systems that 'shields' the ingredient from the milk components during processing (e.g. heat, shear) and during the shelf life of a long-life milk product, meaning that the protection should last around one year at a temperature of roughly 30°C. After consumption, in a very short time frame, the ingredient should be available at the right location in the body. This sets highly contradictory demands to the protective "capsule" that needs to be developed. Figure 1 summarizes the desired type and conditions which the capsules need to require. In addition it is required that the capsules survive sterilisation (e.g. several seconds at 120°C) and preferably survive the significant shear present during processing of the food product. Also biomolecules sensitive for lipid oxidation are interesting active ingredients to encapsulate. As long storage of foods containing these biomolecules can result in oxidative deterioration where hexanal is formed, it would therefore be favourable to encapsulate these sensitive molecules such that produced hexanal remains encapsulated to prevent sensing the off taste. In the workshop Physics with Industry 2010 we hope to find new leads for materials that possess these properties, by combining expertise from (bio) physics, physical (polymer) chemistry and colloid science.

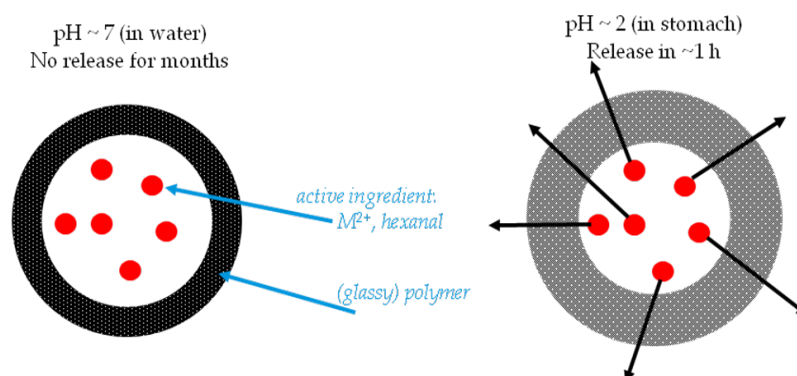


Figure 1: Desired type and conditions of encapsulation of various active ingredients

3. Problem solving strategy

- A) Control and limit diffusion of actives by polymer density/permeability. Calculate the time of diffusion across the polymer as a function of the size of active compounds to be incorporated in the matrix.
- B) Explore possible routes to encapsulate hydrophobic molecules or metal cations by mechanisms that rely on a specific binding between the polymer matrix and the active molecule.



Figure 2: Encapsulation by (a) limited permeability of actives in polymer network and (b) specific binding.

4. Results

I. Diffusion of active molecules in a polymeric capsule

A. Modelling

During the fabrication process, solubility (S) of actives in polymers plays a crucial role. There are two scenarios. For actives with high solubility, they will be dissolved in polymers. Capsules made by such substances are more like a homogeneous mixture (Figure 3a). The actives with low solubility tend to stay as cluster(s) in polymers, as a core-shell system (Figure 3b). Since it is always possible to transfer the first scenario into the second one by add an extra coating (Figure 3c), we consider a spherically symmetric percolation model showed in Figure 3d as a generic representation.



Figure 3a



Figure 3b



Figure 3c

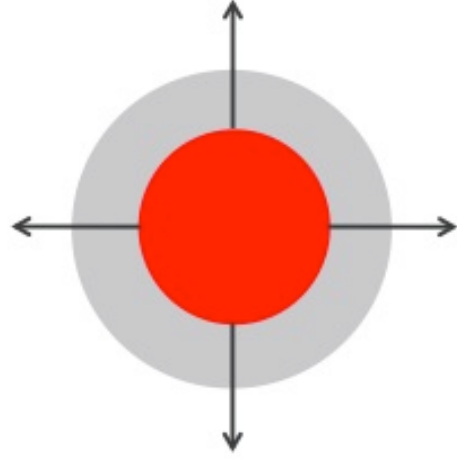


Figure 3d

B. Derivation

The permeation of active molecules through an amorphous glassy or rubbery material [1,2] is often understood in terms of a solution-diffusion process. Molecules on the high-pressure side dissolve into the polymer, diffuse down a concentration gradient, and desorb on the low pressure side. To describe this model, we consider the product of diffusion coefficient D and solubility coefficient S , which is known as permeability coefficient P_a [3]

$$P_a = D \cdot S. \quad (1)$$

This coefficient contains the information about the complicated macroscopic structure of the amorphous material and can be extracted from experiments. Physically, it corresponds to the volume of permeant passing through a unit area of polymer per unit time, with a unit pressure difference across the sample, which is [4]

$$P_a = \frac{V_{esc} \cdot h}{A \cdot t \cdot p}, \quad (2)$$

where A is the area of the active core, p is the pressure of the active, h is the thickness of polymer layer, t is the time variable, and V_{esc} is the volume of 'escaped' actives within this time. In a rough approximation, the scaling relation between the pressure and the other thermodynamic quantities can be obtained through the equation of state for an ideal gas

$$p = \frac{n}{V} RT, \quad (3)$$

where n is the amount of active in a volume V , R is the universal gas constant and T is the absolute temperature. Using the relation between the density and the molecular weight

$$\frac{n}{V} = \frac{\rho}{m}, \quad (4)$$

we ultimately find that the permeability can be expressed as

$$P_a = \frac{V_{esc} \cdot h}{A \cdot t \cdot \frac{\rho}{m} \cdot R \cdot T} \quad (5)$$

where ρ is the density of active and m its molecular weight.
 By rewriting equation (5) in order to express the mass flux, we have

$$\frac{V_{esc}}{t} = \frac{P_a \cdot A \cdot \rho \cdot R \cdot T}{h \cdot m} \quad (6)$$

From this relation we can estimate the typical release time for having a certain amount of active out of the polymer matrix.

C. Estimation

The typical concentration of encapsulated active load in milk is chosen as 5mg/L, while the maximum allowable leakage is 1%. We assume both active and polymer to have the same density as water, which is 1000kg/m³, and we use a value of 0.05 as weight fraction of active loading in the capsule.

In order to use equation (6), we need a relation between the permeability and the molecular weight for the same substance. Experimentally, for small molecules such as O₂ and CO₂, an exponential correlation between permeability and molecule kinetic diameter has been observed. This is shown in Figure 4. Note that here we will extend this correlation to molecules with molecular weight no larger than 1000g/mol.

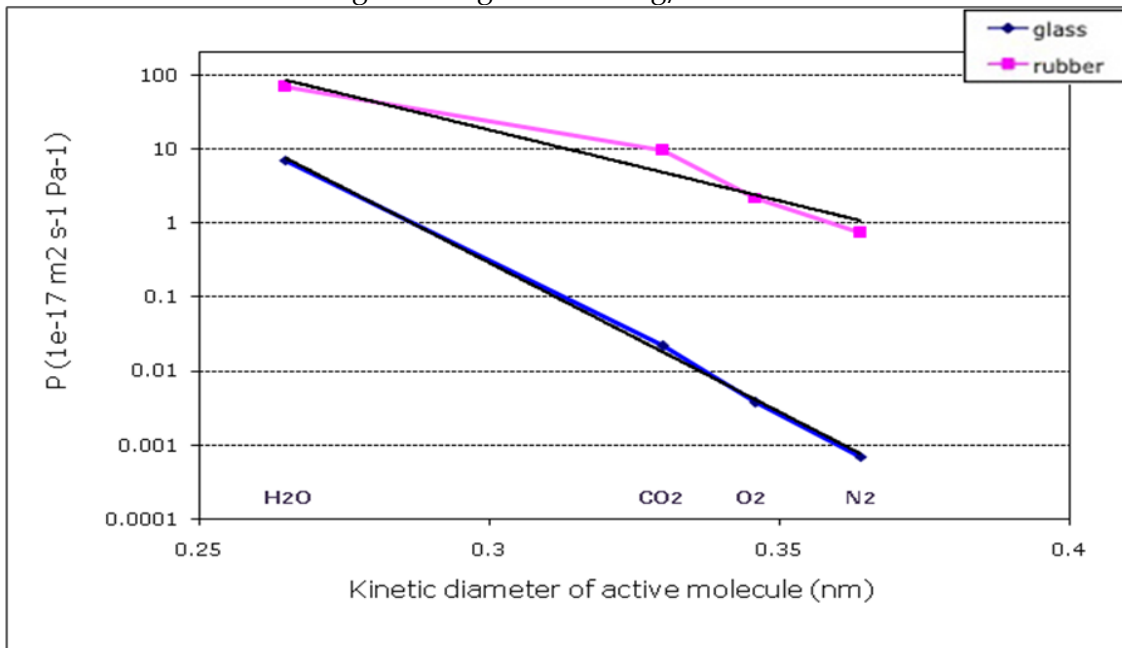


Figure 4: Permeability vs. Kinetic diameter of small molecules [4]

On the other hand, by assuming a droplet model, the molecular weight is related to the kinetic diameter through the relation

$$d = 2 \cdot \sqrt[3]{\frac{m}{N_A \cdot \rho}} \quad (7)$$

where d is the diameter of molecule and N_A is the Avogadro constant. At last, capsules are assumed to have a typical radius of 1 μ m, with an active core and 0.1 μ m polymer layer as coating.

D. Discussion

Figure 5 shows the time to release 1% active (years) as a function of the molecular weight of the active (g/mol).

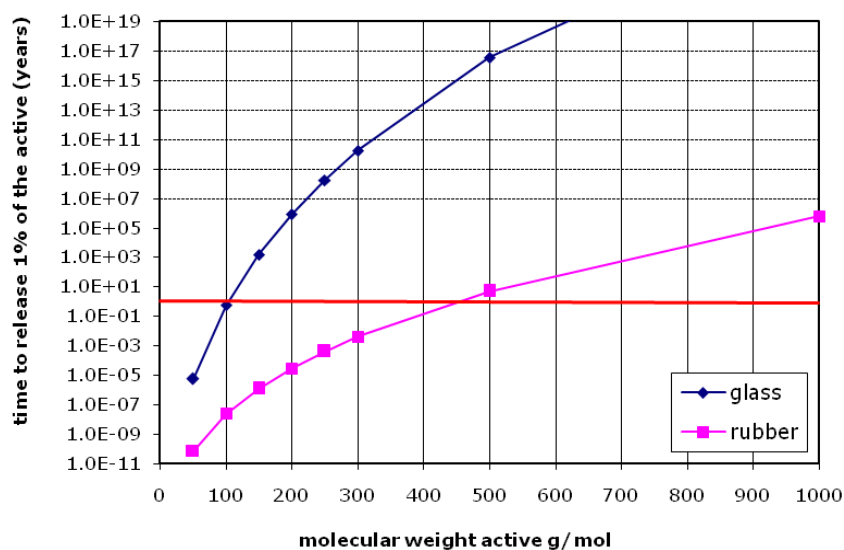


Figure 5: Time to release 1% active vs. Molecular weight of the active

It is plausible that for actives with molecular weight around 100g/mol, 1 year entrapping time is achievable by using glassy state polymers. On the other hand, such capsule is quite permeable for small molecules with molecular weight around 10-20. When the polymer changes into more loose rubbery state due to trigger mechanism (low PH at HCl, for instance), entrapping time drops significantly to 10^{-8} years, which are at seconds level. This provides an ideal mechanism for delivering nutrients into human stomach. . Even when the capsule is in the glassy state small molecules, such as mineral salts, are seen to escape very fast. This may however be solved by first complexing them with a larger molecule.

II. Encapsulation of hydrophobic actives using amylose–lipid inclusion complexes

What are amylose-lipid complexes?

Amylose is able to form inclusion complexes with various types of ligands where the hydrophobic parts of the ligands/lipids are entrapped in the hydrophobic helical cavity of amylose. This type of complex, resulting in the so called V-type X-ray pattern, normally has six glucose residues per turn to form left-handed single helix and have been found with iodine and linear ligands such as monoglycerides, fatty acids and alcohols.

The amylose complexes exist in two polymorphic forms, type I and II, characterised by the temperature of their dissociation. Type I amylose complexes melt at a temperature about 10–30° below those of type II, depending on the types of ligands and the experimental conditions. Type II complexes have a crystalline structure while type I are in the amorphous state. Inclusion complexes with active ligands particularly flavour compounds with amylose are an emerging technique for encapsulation and it is suggested that starch-flavour complexes will provide protection during processing and storage because the complexes melt at high temperature. Besides pure amylose, potato starch was used in most of studies on inclusion complex (see figure 6), since it contains little or no internal lipid which interferes with the formation of the complex.

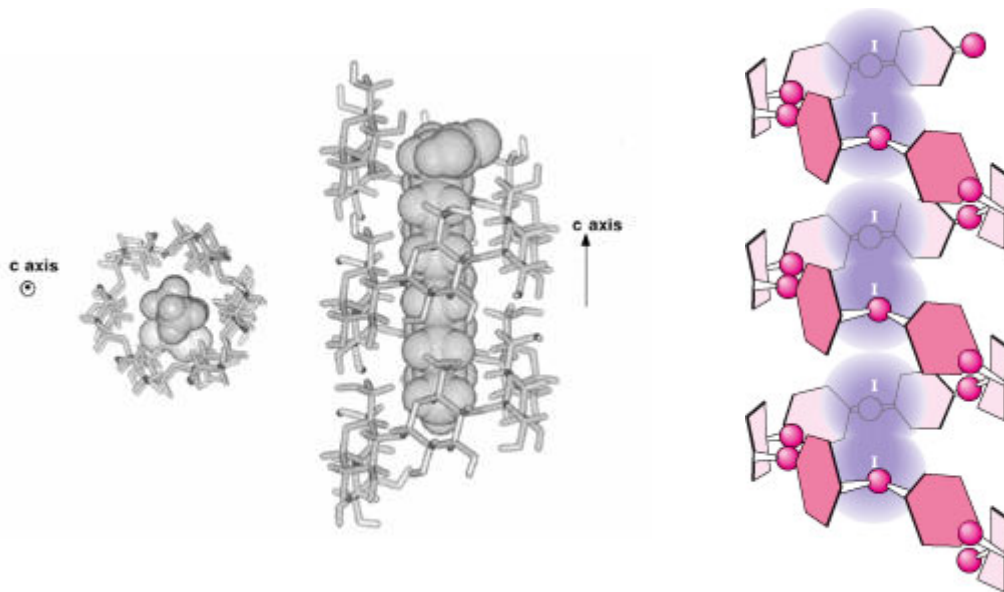


Figure 6: Inclusion Complexes between Amylose and FA or iodine

The complexes are less soluble and precipitate or form a gel. They can be stabilized upon addition of a third component (maltodextrin), which interacts, with amylose (start) and sterically can stabilize the particles (aggregates). If additional protection is needed, the encapsulate can be coated with a secondary layer using fluid bed coating.

Synthesis:

spray drying semi-gelatinized or cross-linked starches or amylose in presence of lipids, and milled afterward to the right particle size.

Factors:

active type (chain length), amylase Mw, additives, kinetics

Type of encapsulation:

specific binding (inclusion complex), followed by glass/crystal formation (upon drying or precipitation)

What can be loaded:

lipids: fatty acids, fatty alcohols, monoglycerides (MG), emulsifiers, flavors.

How much can be loaded:

1-10% (g active/g complex). If the particles do not swell significantly in water, then to keep the product rheology around 0.1-1 wt% complexes can be added: thus the active concentration will be around 0.001-0.1 wt% (1 - 100 ppm).

How stable upon storage (in milk):

binding energy

Colloidal/Physical: Stabilization of the particles/extra encapsulation - MD or other carbohydrate

How it can be released:

starch digestion, high temperature (>80C). Issue with sterilization (post addition)

Prior Art:

Amylose–Lipid Inclusion Complexes well known (bakery, flavor encapsulation/ degradation) - lot of patents and literature. There is a possibility of combining this with specific formulation, processing or both.

Safety:

Can be used in foods / all food-grade using food grade processes

Cost / Scale up:

mixing / spray drying / fluid bed coating

Case study:

We have performed self-consistent field calculations aimed to check the feasibility of scenarios of how lipid (fatty acids) can complex with amylose and then phase separate from an aqueous solution. As a full analysis is clearly not possible at this stage, we start with the Ansatz that the amylose forms locally a helical structure with an inner radius of order 1 nm and a slightly larger outer radius. We can present such a structure in a cylindrical coordinate system as a small hollow tube. The coordinate system and where the hollow tube is placed are shown in Figure 7.

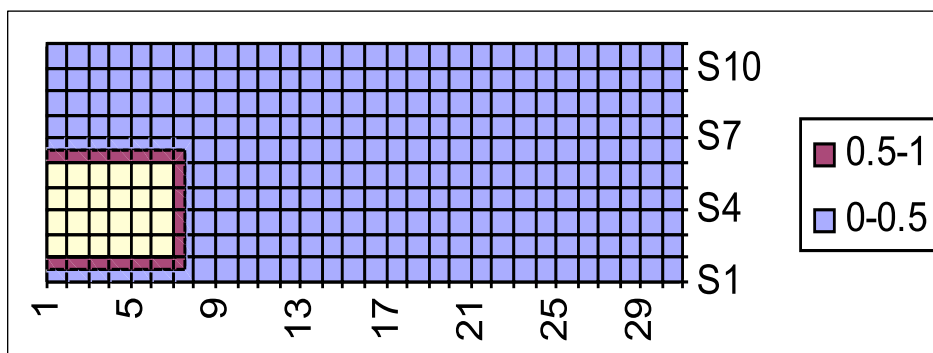


Figure 7. Two-gradient SCF calculations were performed with a long axis $z = 1, \dots, 30$ (from left to right) and a radial coordinate $r = 1, \dots, 11$. The mean field approximation is applied in a ring (z, r) , where there are $L(r) \sim 2\lfloor r \rfloor$ sites. The hollow cylinder is in the region $1 < r < 6$ and $1 < z < 8$ as indicated by the light region. At $r = 2$ the cylinder is hydrophobic, at the other sides it is hydrophilic

In this coordinate system reflecting (mirror-like) boundary conditions are implemented meaning that the tube is twice as long as indicated (i.e., 14 sites long) and is open on both sides. Recalling that the tube mimics a helical segment of an amylose fragment, the inner side of the tube is hydrophobic and the outer side as well as the front is hydrophilic (not indicated in figure 7). In this volume we assume the presence of a fatty acid with sixteen hydrocarbon segments (C) and two polar (O) groups, and the remainder of the solution is filled with water. The water phase is modelled as unimers (W).

We are solving the partition function that contains all possible and allowed conformations of the fatty acid molecules in the vicinity of the hollow amphiphilic tube. The chain conformations are treated on the freely jointed chain level. Moreover the system is taken to be incompressible, which means that the volume is completely filled by either the hollow cylinder or the water of the units of the fatty acid.

Central to the SCF approach is that we solve the mean field partition function of this problem, for which the free energy can be expressed in terms of the segment volume fractions and corresponding segment potentials. The optimization of the free energy leads to the SCF machinery, which expresses the segment potentials as a function of the volume fractions and vice versa. When the SCF equations are solved we can compute the free energy and derived from this the grand potential.

$$\Omega = F - \sum_i \mu_i n_i \quad (1)$$

Here we use Flory-Huggins interaction parameters to specify the relevant interactions in the system. First of all there is the interaction between hydrocarbon segments with water. We know from the study of the CMC as a function of the chain length that $\chi_{CW} = 1.6$. The interaction of O with W is taken to be favorable and the ad-hoc value of $\chi_{OW} = -1$ was chosen. Slightly less important is the fact that polar and apolar segments (C,O) is repulsive. Here we opted for $\chi_{CO} = 2$. In combination with the molecular architecture it is possible (using the same SCF calculations, but in a different geometry) to evaluate the critical micellisation (calculations not shown), and we have estimated the value of $\varphi^b = 4 \times 10^{-4}$ (roughly equal to molar concentration). This volume fraction specifies the chemical potential of the surfactant (fatty acid), which is relevant when we consider the amylose-fatty acid complexation.

When the fatty acids are introduced in the system (having excess water) specified in Figure 7, the head groups of the fatty acid adsorb onto the hydrophilic faces of the hollow cylinder (i.e. to the outside). Upon the increase of the fatty acid concentration, the adsorption increases and near the CMC value a condensation of the layer takes place and then a dense monolayer appears. This monolayer bridges with its mirror image sitting on a neighboring cylinders in the radial direction. Figure 8a shows the equal density contour plot for the head groups of the fatty acids and in Figure 8b we present the volume fraction distribution of the hydrophobic segments.

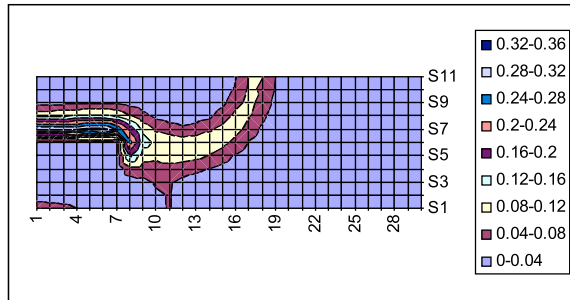


Figure: 8a

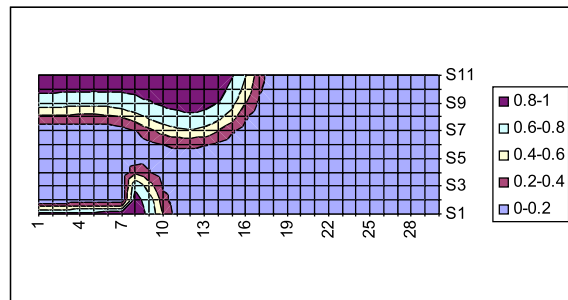


Figure: 8b

Figure 8. The equilibrium distribution of (a) the head groups of the fatty acid (b) the hydrocarbon segments of the fatty acid, near the hollow cylinder shown in figure 7. Details are given in the text. Interaction parameters with the cylinder are the following: Outside surface: $\chi_{SoO} = -6$ (very weak H-bond), $\chi_{SoC} = 0$ and $\chi_{SoW} = -1$ (water prefers the outside over C). Inside surface $\chi_{SiC} = -1.5$ (weakly attractive), $\chi_{SiW} = 2$ (water does not like inside), $\chi_{SiO} = 0$ (head groups are indifferent).

We mention once again that the chemical potential of the fatty acid is consistent with the CMC in the solution (highest possible concentration of the fatty acid in solution). To understand the distributions shown in figure 8 we should recall that the boundary conditions are reflecting. This means that the fatty acids form some type of bilayer in between two neighboring cylindrical objects. Consistent with this, the tail density is high near the S11 ($r = 11$) boundary layers. The head groups, which have the highest density near the hydrophilic face of the cylinder (they are taken to adsorb preferentially at this face, e.g. through hydrogen bonding) and they form a cap around the hydrophobic region, simply to prevent too many contacts of the hydrophobic segments with water. Interestingly, the aggregated bulges out into the water phase and the end-cap is somewhat wider than the

'body'. This fact is known, e.g. from the shape of cylindrical micelles, for which the end caps are wider than the central body.

Even though the inner volume of the hollow cylinder is very small, we can see from figure 8 that a fatty acid molecule has wormed itself inside the cavity. Of course this is assisted by the hydrophobic nature of the inner side of the cylinder.

The fact that the fatty acid inserts itself inside the cylinder and that the affinity apparently overcomes the conformational entropy loss for doing so, we conclude that there is a positive driving force for it. In other words there is a free energy gain for inserting the fatty acid into the cavity. In the same token, we conclude that the insertion of a fatty acid helps the formation of the helical shape of the amylose chain.

It must be understood that these results are very preliminary. To really predict the optimal structure (unit cell), we have to evaluate the equilibrium distance between the hollow cylinders, which in the above results was fixed as a constraint. We also should optimize how long the cylinder can be in order to have the optimal match for the fatty acid (for amylose this is assumed to be a value that can be adopted). Again, in the present calculations the length of the cylinder is imposed.

The preliminary results discussed above prove that it is feasible to bind amylose molecules together with the help of fatty acids using very reasonable interaction parameters. This may help us to understand why the amylose-lipid complexes are insoluble in water and why these complexes phase separate. Hence, the amylose-lipid complexes can be the internal phase of a capsule.

Returning to the capsule problem, we remind the reader that one can use these complexes to formulate a range of biologically active molecules. More specifically hexanal can be solubilised in the fatty-acid rich regions and receive an H-bond from the amylose molecules. Capsules made of the amylose-lipid complexes are easily digested and this gives a natural release mechanism for the active compound. The formulation of the amylose-lipid capsules should be possible using the casein molecules readily available in milk.

III. Encapsulation of metal cations using mixed metal hydroxides

What are mixed metal hydroxides?

Clay is a versatile material used by mankind and nature and mainly consists of a stiff, sticky fine-grained earth. Clay particles, especially mixed metal hydroxides (MMH) (also called layered double hydroxide (LDH)), can be divided into cationic clays and anionic clays. The anionic clays are found in sporadic amounts in nature and in lattice structure they consist of positively charged layers with charge balancing anions in the interlayers (figure 9a). The cationic clays are the opposite of anionic clays. MMH are a class of ionic lamellar solids with positively charged layers with two kinds of metallic cations and exchangeable hydrated gallery anions.

Of the types of mixed metal hydroxide the hydrotalcite is interesting for its use and properties in various fields in science, industry, and pharmaceuticals and other. The MMH platelets thank their interest due to the property that they are an easy-to-synthesize class of colloidal, disk shaped materials that they also can be adapted chemically to contain different metal ions and intercalated anions (see figure 9b). This class of inorganic compounds can easily be synthesized through various synthesis routes [5].

The general formula of a MMH is $[M^{2+}_{1-x}M^{3+}_x(OH)_2]^{b+}[A^{n-}]_{b/n} \cdot m H_2O$, where M^{2+} and M^{3+} represent the mixed metal cations (such as Mg^{2+} , Fe^{2+} and other), $(OH)_2$ making it a double hydroxide, b represents the charge of the layer, anion A^{n-} and $m H_2O$ situated in the interlayer area.

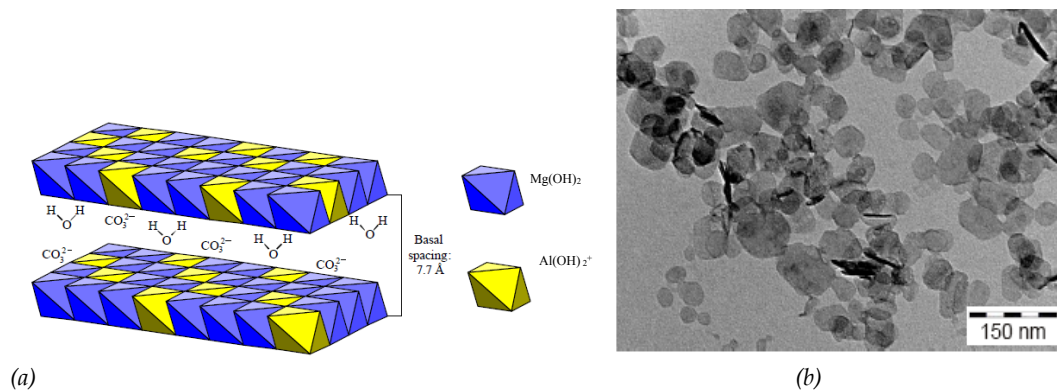


Figure 9: (a). MMH host lattice of hydrotalcite; (b). Transmission electron micrograph picture of hydrotalcite particles [14].

The ordering of hydroxide layers is similar to that of brucite, $[\text{Mg}(\text{OH})_6]^{4-}$ where each Mg^{2+} cation is octahedrally surrounded by 6 OH⁻ anions and the different octahedral $[\text{Mg}(\text{OH})_6]^{4-}$ groups share edges to form infinite sheets. In table 2 (Appendix 2) is shown a small overview of the types of mixed metal hydroxides known and the type of metals that can be combined inside the MMH structure.

Of the MMH family the mineral hydrotalcite (general formula $\text{Mg}_6\text{Al}_2\text{CO}_3(\text{OH})_{16} \cdot 4 \text{H}_2\text{O}$) is commonly used and the $\text{Mg}^{2+}/\text{Al}^{3+}$ isomorphous substitution in the octahedral sites of hydroxide sheet results in a net positive charge. The hydrotalcite sheet with their net positive charge is balanced by interlayer anionic species. To give an impression of the amount of anion that can be stored in a hydrotalcite, models have calculated that up to 20 lattice layers there are more than 2000 anions present per layer [4]. Most synthetic anionic clays contain a small amount of carbonate in the anionic interlayers. This is due to the presence of CO_2 in the atmosphere. Carbonate is the most stable anion in a MMH because carbonate binds tightly in the interlayers and it is therefore difficult to remove from the MMH structure [7].

Synthesis: A commonly used route to synthesize MMH colloidal particles is co-precipitation under basic conditions of salts with different $\text{M}^{2+}:\text{M}^{3+}$ ratios, after which the solution is aged at elevated temperatures. In this way particles can be synthesized with typical sizes between 20 and 400 nm and a polydispersity of 20-40%.

Type of encapsulation / what can be loaded: It is possible to encapsulate a range of metallic cations (see table 2 in Appendix 2) by incorporation into the metal hydroxide layers. Charge balancing anionic species can be placed in the interlayers and can be of various type, which include inorganic [7], organic [8], drug molecules and even DNA [9]. Figure 10 shows how MMH can host free fatty acids (which are food grade), like stearate inside its interlayer [10].

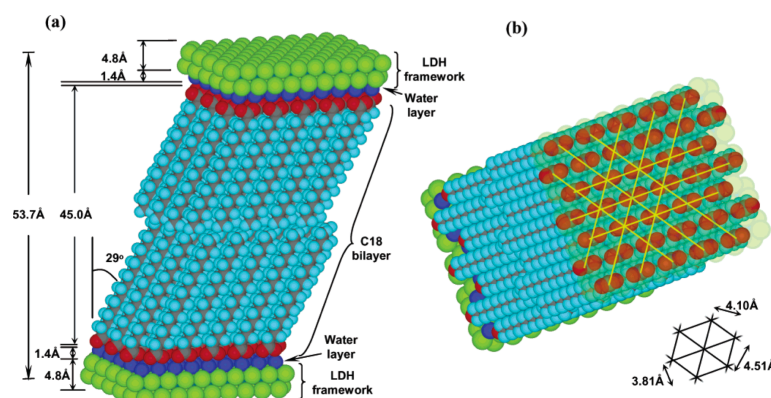


Figure 10: Stearate bilayers in (Mg/Al) LDH framework: (a) tilted stearate bilayer and water molecules in LDH; (b) the regular packing of stearate molecules.

How much can be loaded: Metal cations can be incorporated in the metal hydroxide layers at a high loading. Hydrotalcite ($\text{Mg}_6\text{Al}_2\text{CO}_3(\text{OH})_{16} \cdot 4 \text{H}_2\text{O}$) contains 24 wt% Mg and 9 wt% Al, whereas pyroaurite ($\text{Mg}_6\text{Fe}_2\text{CO}_3(\text{OH})_{16} \cdot 4 \text{H}_2\text{O}$) contains 17 wt% Fe. For anionic interlayer dopants typically one molecule per 8 metal atoms can be included. It should be possible to load dairy products with 0,1 wt% MMH, without changing the rheological properties. At significantly higher concentrations the rheological properties will change (see Figure 11b) and gel structures or liquid crystal phases may eventually form [12]. Within the typical range of mineral loadings in foods and beverages ,the rheology is unaffected. Furthermore, the concentration of free metal cation is expected to be well below the taste threshold for metal ions, since the minerals are strongly attached to the LDH framework.

How stable upon storage: from experience we know that colloidal dispersions of clay particles can be stable for years.

How it can be released: The release of metal ions starts at pH 3 and at pH 1 the MMH is completely dissolved [11]. Figure 11a shows that at low pH the percentage of hydrotalcite dissolution steeply increases. Based on experimental experience the MMH is insoluble in water at pH 7.

Prior Art: Hydrotalcites have been used for medical purposes and therefore we expect that they can be used in foods, but we are unaware of a food grade label.

Safety: Their generally non-toxic nature allows MMH to be used in the formulation of materials with applications in storage, delivery and controlled release of pharmaceutical and other compounds. A known example of MMH is its effective medical application against heartburn. The hydroxides from the MMH will bind to the protons in your stomach. Water will be formed and that will contribute to buffer your stomach to pH 3-4.

Cost / Scale up: In literature many examples are available to scale up the production of MMH. One example is to use a colloid mill to fabricate MMH of several tonnes per year [13]. The costs of the chemicals used for the synthesis are low.

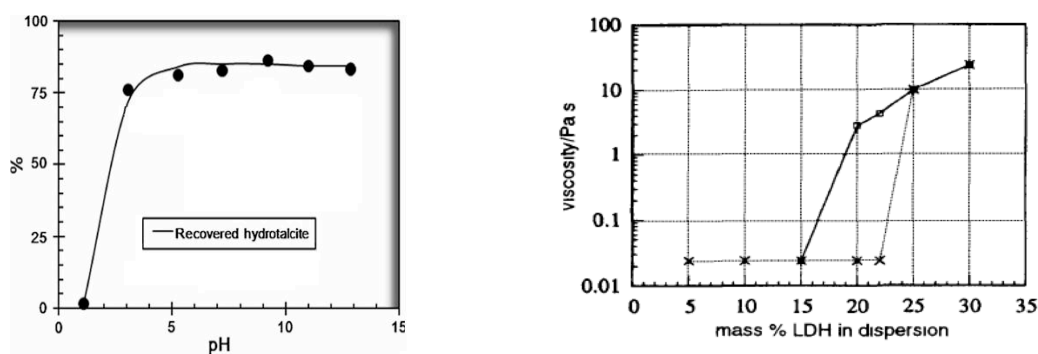


Figure 11: (a) Dissolution of hydrotalcite as a function of pH [11]; (b) Viscosity of hydrotalcite as a function of w%: x, as prepared; □, after shaking [12].

IV. Encapsulation inspired by nature

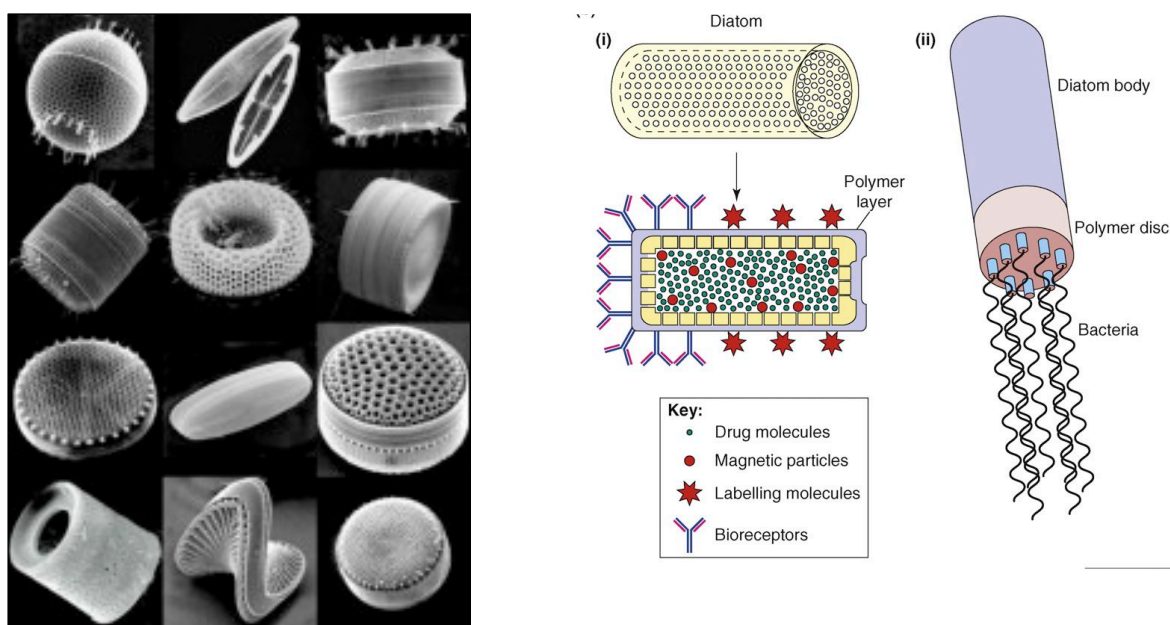
Development of new technologies is often based on nature, which is formally known as biomimicry. It involves the study of nature's design and applying the knowledge to solve human problems, e.g. the lotus effect (superhydrophobicity). Further investigation on our goal of encapsulation of metal ions and small bioactive molecules guided us also back to nature.



Figure 12: Encapsulation in nature: various kind of pollens (left[22]), sea urchin (middle) [20] and HIV-virus (right) [21].

Encapsulation is encountered in nature in a wide range of intriguing and highly efficient systems (see figure 12). This strategy is for instance applied in skin, pearls, fruit, sea and eggshells, and also every cell is encapsulated by a membrane. Different environments make needs of different mechanical properties and therefore nature has developed multiple mechanisms to create encapsulation with different stabilities. We were especially interested in crystal encapsulation as found in eggshells and seashells.

The use of biogenic minerals by living organisms creates properties that differ from the inorganic counterparts- exactly what we are looking for. Mineralized tissue has been found in a wide range: egg and sea shells using calcium carbonate or eukaryotic animals called diatoms that have highly structured silica shells. These are structured to such a degree that even MEMS-technology is not able to reproduce them as seen in figure 13. The "emptied" capsules have already been used in e.g. toothpaste. Further investigations are focused now on the use as a drug delivery system even guided by chemo taxis [20].



delivery [19].

Figure 13: Various forms of diatoms and a possible function in drug

To use nature ideas the mechanism has to be understood. Investigations in egg and seashells showed that there is most likely a four-step mechanism that creates the crystal structure. In the beginning a microenvironment has to be created, that implies crystal production at a certain localisation but prevents it of unwanted expansion. In general it is a hydrophobic ordered structure that creates a scaffold for a polyanionic protein that is needed for Ca^{2+} -binding. The need of their specific properties is not just in binding calcium ions due to high number of aspartic and glutamate amino acids but also in their secondary structures. Both steps are necessary to activate nucleation and additional proteins e.g. OC-17 (see figure 14) influence structure and orientation of the crystal layer but also determine the arrest of the crystal layers [17,18].

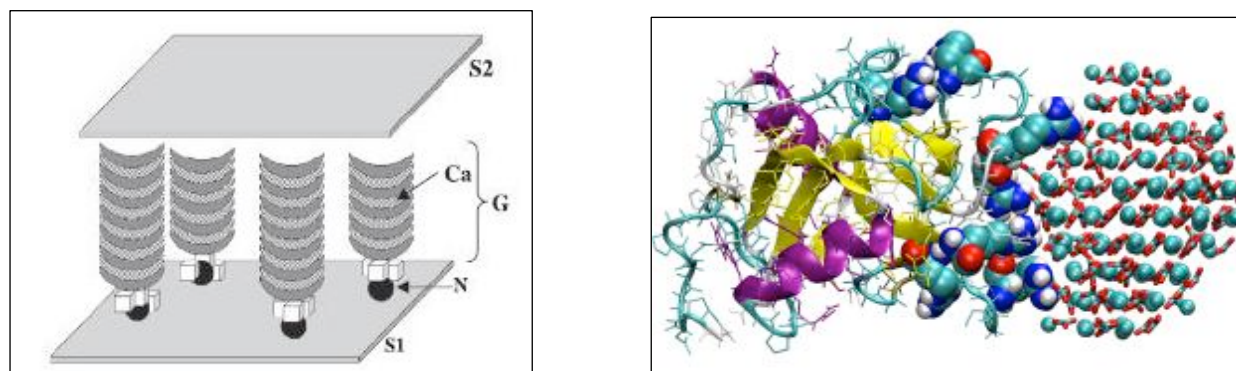


Figure 14: The four-step mechanism of the crystal process [17] and the structure of OC-17, a specific protein that works as a catalyser [19].

The knowledge of the precise mechanism in combination with the use of genetic tools could give us the possibility of encapsulation of a metal ion of interest. Besides encapsulation of molecules, a second requirement of the desired system is fast release upon the pH trigger in the stomach. It is well known that eggshells dissolve in acidic environment and release calcium ions, which is very likely to be a more generic mechanism of similar structures. Understanding nature's mechanism and its tools gives rises to ideas for technology developments not just for encapsulation but also for all scientific environments.

Conclusions and recommendations

In order to entrap active ingredients in capsules by a dense polymer matrix, a spherically symmetric percolation model yields reasonable results. The large difference (~ten magnitudes) on diffusion time between of 'big' active molecule and of 'small' trigger molecule, as well as between through glassy polymer barrier and through rubbery polymer barrier, provides a possible mechanism to store actives for years in milk while release them in seconds in human stomach.

Nevertheless, there are two main approximations in the calculation, which may limit the validity of this model:

1. The permeability of active ingredients with 100-1000g/mol molecular weight is obtained by fitting small gas molecules' data
2. The pressure of actives within the polymer matrix is derived by applying the state equation of an ideal gas

The overall effect of these two approximations is an over-estimation of diffusion time. In a future model more accurate approaches should be used.

From the approach to bind the active ingredients in capsules through binding mechanisms, mixed metal hydroxides represent a promising candidate for encapsulation of metal ions and biomolecules. This versatile type of inorganic encapsulates/containers can easily be tuned to your desired parameters and properties. The conclusions about MMH as encapsulate are summarized as followed:

- Easy synthesis and tailoring properties/size
- Encapsulation metal ions and biomolecules possible
- High loading
- Release mechanism at low pH
- Sterilization possible
- Long-term stability
- Used in medicine
- Cost are low / simple process (scale-up)

Experiments should be performed to determine the performance of interesting MMH in the actual product and processes.

The development of new encapsulates may be improved by using self-consistent field calculations, as shown in the amylose-lipid example, and by looking at nature's way of encapsulating. Nevertheless, basic research has to be done to figure out the binding strength, maximum loading capacity, heat treatment, high shear forces, long-term stability, etc.

References

- [1] P.G. de Gennes, *Scaling Concepts in Polymer Physics*, Cornell University Press, 1979
- [2] J.D. Ferry, *Viscoelastic Properties of Polymers*, John Wiley and Sons, Inc., 1980
- [3] *Diffusion in polymers'*, Partho Neogi, Marcel Dekker Inc, 1996
- [4] *Diffusion and Permeability in polymers'*, Powerpoint slides, <http://users.encs.concordia.ca/~woodadam/GCH6101/Diffusion%20and%20permeability%20in%20polymers.pdf>
- [5] J. T. Klopogge, R. L. Frost and L. Hickney, "Klei, Glas en Keramiek Wetenschap", 21, 1999, 7
- [6] Z. Ni; G Pan; L. Wang; W. Yu; C. Fang; D. Li, "Structure and Properties of Hydrotalcite Using Electrostatic Potential Energy Model", *Chinese Journal of Chemical Physics*, 19, 3, 27 June 2006
- [7] C. Vonk, "Synthesis of hydrotalcite", internal report Van 't Hoff laboratory, March-September 2006
- [8] S.P. Newman and W. Jones, *New. J. Chem.*, 1998, 105
- [9] Z.P. Xu, G.S. Stevenson, C. Lu, G.Q. Lu, P.F. Bartlett and P.P. Gray, *J. Am. Chem. Soc.*, 128, 2006, 36
- [10] T. Itoh, N. Ohta, Tetsuya Shichi, Tatsuto Yui, and K. Takagi, *Langmuir*, 19, 2003, 9120
- [11] S. J. Santosa et al., *Applied Surface science*, 254, (2008), 7612-7617
- [12] L. Albiston, K. R. Franklin, E. Lee, J. Bas and A. F. Smeulders, *Rheology and microstructure of aqueous layered double hydroxide dispersions*, *J. Mater. Chem.*, 1996, 6(5), 871-877
- [13] D. G. Evans and X. Duan, "Preparation of layered double hydroxides and their applications as additives in polymer, as precursors to magnetic materials and in biology and medicine", *The Royal Society of Chemistry, Chem. Commun.*, 2006, 485-496
- [14] M.C.D. Mourad, E.J. Devid, M.M. van Schooneveld, C. Vonk and H.N.W. Lekkerkerker, *Formation of nematic liquid crystals of sterically stabilized layered double hydroxide platelets*, *J. Phys. Chem. B*, 112, 2008, 10142
- [15] "Biomimetic processes through the study of mineralized shells", *Arias & Fernandez*, 2003
- [16] "Mimicking the Function of Eggshell Matrix Proteins: The Role of Multiplets of Charged Amino Acid Residues and Self-Assembly of Peptides in Biomineralization", *Ajikumar et al*, 2005
- [17] "Research on eggshell structure and quality: An historical overview", *Hunton*, 2005
- [18] "Synthetic Seashells: Biomimetic Mineral Nucleation at a Langmuir Monolayer", *DiMasiet al*, 2001
- [19] "The Glass Menagerie: diatoms for novel applications in nanotechnology", *Gordon, et al*, 2008
- [20] "Sea Urchin Yields Key Secret of Biomineralization" *University of Wisconsin-Madison, Science Daily*, 2008
- [21] "HIV Virus", www.Live science.com
- [22] *Dartmouth Electron Microscope Facility*, remf.dartmouth.edu/imagesindex.html
- [23] A. Poortinga, *Presentation about encapsulations from Royal FrieslandCampina* for

workshop Physics with Industry 2010

Samenvatting

Het verrijken van levensmiddelen met vitamines en mineralen is gewenst om de gezondheid en het welbehagen van mensen te verhogen. Echter, deze componenten kunnen hun functie verliezen tijdens opslag of processing. Daarnaast kunnen deze componenten leiden tot ongewenste veranderingen in het product (smaak, kleur, houdbaarheid, etc). Encapsulatie, het beschermen van actieve ingrediënten m.b.v. een coating of drager, kan deze problemen voorkomen.

Dit rapport beschrijft verschillende routes en mogelijkheden om mineralen of biomoleculen te encapsuleren. We hebben hierbij aangenomen dat de actieve ingrediënten in de capsules minstens een jaar in een waterig milieu intact blijven, maar in zuur milieu (de maag) dienen ze binnen een uur vrij te komen vanuit de capsules.

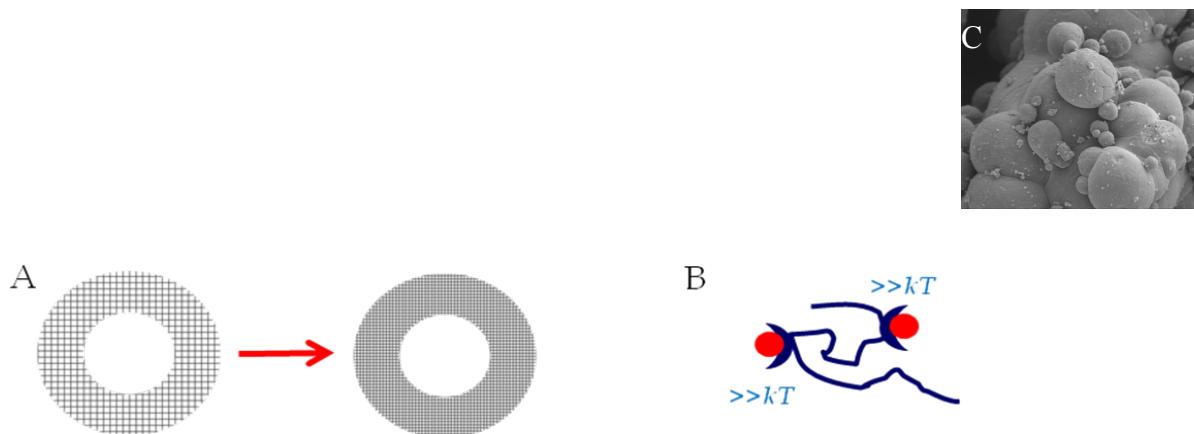
De eerste route (figuur 1a en 1c) omtrent capsulieren richt zich op polymere capsules waarbij de diffusie van een biomolecuul afhangt van het molgewicht en de permeabiliteit van de capsule. De tweede route beschrijft alternatieve manieren die mogelijk gebruikt kunnen worden om zowel metaalionen als biomoleculen te capsulieren. Deze route focust zich om de actieve ingrediënten te capsulieren via binding mechanismes (figuur 1b).

Amylose is het eerste voorbeeld dat besproken wordt waarbij het in staat is om via diverse liganden complexe bindingen te vormen met biomoleculen zoals vetzuren.

Ons tweede voorbeeld van capsulatie is gebaseerd op inspiratie vanuit de natuur. Door de natuur zijn behalve isotropische (bolvormige) capsules al exotische, anisotropische (plaatvormige, staafvormige) capsules ontwikkeld met ideale capsulatiekenmerken.

Een bekend voorbeeld van anisotropische deeltjes is klei en in het bijzonder mixed metal hydroxides (MMH) (ook wel bekend als layered double hydroxides (LDH)).

Deze plaatvormige deeltjes zijn eenvoudig te synthetiseren en bezitten het vermogen om hun eigenschappen aan te passen naar keuze. Dit en vele andere voordelen maken MMH een interessante optie voor verscheidene toepassingen onder meer in de voedingsmiddelenindustrie.



Figuur 1: Encapsulatie door (a) gelimiteerde diffusie van actieve ingrediënten in een polymeermatrix en (b) specifieke binding aan de matrix. (c) Een scanning electron microscoop foto van een biopolymeercapsule, zoals gebruikt in voedingsmiddelen [23].

Appendix 1

Mineral deficiencies

Humans need various micronutrients (for example minerals) for health, survival and well-being. Minerals are micronutrients that are required to regulate processes within the body. Minerals are present in different forms within the body, e.g. in enzymes, hormones, skeletal bones, skeletal tissues, teeth and fluids¹. Calcium and phosphorus are the two most common minerals found in the body. Other minerals that can be found in the body are iron, zinc, sodium, potassium, magnesium, sulfur, copper, chloride.

Mineral deficiency occurs when the concentration of (some of the) minerals essential to human health is low in the body. A low mineral concentration can be defined as that concentration leading to impairment in a function dependent on the mineral¹.

Of the sixteen essential minerals, 11 of them are required in such small amounts and/or are so abundant in food and drinking water that their deficiencies are uncommon². The remaining five are present in minor quantities in most foods, so an unbalanced diet can easily result in deficiencies. These minerals are iodine, iron (Fe), zinc (Zn), calcium (Ca) and selenium (Se). For example, iron deficiency leads to anemia (and may lead to other symptoms too); iodine deficiency leads to goiter (swelling in the thyroid gland leading to swelling of the neck).

According to certain sources on internet, even in developed countries many deficiencies are present. Table 1 shows some examples for the United States.

Table 1: Percentage mineral deficiencies in the United States

Deficiency	U.S. Population
Magnesium	75%
Iron	58%
Copper	81%
Manganese	50%
Chromium	50%
Zinc	67%

To avoid deficiencies, food can be fortified. Fortification is a means to increase the amount of micronutrients to a higher level than that found in normal, unprocessed food. In some cases, fortification can be achieved by simply adding these minerals³ and micronutrients⁶. However, in many cases it does not work, because of difficulties in achieving a satisfactory load without affecting colour, taste and/or odour. Iron, for example, may react with fatty acids in the fortified food, forming free radicals that induce oxidation.

These micronutrients can therefore be coated with a protective shell (either by covering the micronutrient directly with a coating or by coating a complex containing the micronutrient where the complex may also consist of the coating material) to keep the desired colour, taste and/or odour. The coating of active substances for protective purposes (e.g. micronutrients) is called encapsulation. Encapsulation may also (1) permit controlled release of the nutrients in time, (2) enhance the stability of ingredients to extreme conditions during processing, (3) improve flow properties and (4) reduce dusting when nutrients are added to dry mixes.

Note that after encapsulation, the encapsulated micronutrient still needs to have a high bioavailability. Bioavailability is defined as the fraction of an administered dose of

unchanged drug that reaches the systemic circulation. So, after encapsulation the micronutrient should not only become available in the human body, but also be taken up. Several different procedures exist for encapsulation (see, for example, Pegg and Shahidi⁴ or Gouin⁵). However, encapsulates that are commercially available do not fulfill the requirements (no release in aqueous product at neutral release and rapid release in the gut, high shear and temperature stability and small encapsulate size) or they are too expensive.

References mineral deficiencies

1. <http://kulvis.com/mineral-deficiency-health-and-wellness-problems/>
2. Gomez-Galera S, Rojas E, Sudhakar D, Zhu C, Pelacho AM, Capell T, Christou P. 2010. Critical evaluation of strategies for mineral fortification of staple food crops. *Transgenic Res* 19:165-180.
3. <http://www.lohmann-chemikalien.de/index.php/mineral-fortification-beverages.html>
4. Pegg, R. B. Shahidi, F. 2004. Encapsulation, Stabilization, and Controlled Release of Food Ingredients and Bioactives. *Food science and technology*, 167, 509-570.
5. Gouin S. 2004. Microencapsulation: industrial appraisal of existing technologies and trends. *Trends in Food Science & Technology* 15:330-347.
6. http://www.dsm.com/en_US/html/dnp/hnh_vitamins.htm.

Appendix 2

Table 2: Example of various types of mixed metal hydroxide minerals/colloids

Type of mixed metal hydroxide	Structure formula
Hydrotalcite	$\begin{array}{c} \text{Mg} \\ 6 \\ \text{Al} \\ 2 \\ [(\text{OH}) \\ 16 \\ \text{CO} \\ 3 \\] \cdot 4\text{H} \\ 2 \\ \text{O} \end{array}$
Pyroaurite	$\begin{array}{c} \text{Mg} \\ 6 \\ \text{Fe} \quad 3+ \\ 2 \\ [(\text{OH}) \\ 16 \\ \text{CO} \\ 3 \\] \cdot 4\text{H} \\ 2 \\ \text{O} \end{array}$
Stichtite	$\begin{array}{c} \text{Mg} \\ 6 \\ \text{Cr} \\ 2 \\ [(\text{OH}) \\ 16 \\ \text{CO} \\ 3 \\] \cdot 4\text{H} \\ 2 \\ \text{O} \end{array}$

Desautelsite	Mg 6 Mn 3+ 2 [(OH) 16 CO 3] · 4H 2 O
Takovite	Ni 6 Al 2 [(OH) 16 CO 3] · 4H 2 O
Reevesite	Ni 6 Fe 3+ 2 [(OH) 16 CO 3] · 4H 2 O
Sergeevite	Ca 2 Mg 11 (CO 3) 13 · 10H 2 O

Brugnatellite	Mg 6 Fe 3+ [(OH) 13 CO 3] · 4H 2 O
Coalingite	Mg 10 Fe 3+ 2 [(OH) 24 CO 3] · 2H 2 O
Ankerite	Ca(Fe 2+ ,Mg,Mn 2+)(CO 3) 2
Scarbroite	Al 5 (OH) 13 CO 3 · 5H 2 O

Indigirite	$\begin{array}{c} \text{Mg} \\ 2 \\ \text{Al} \\ 2 \\ [(\text{CO} \\ 3 \\) \\ 4 \\ (\text{OH}) \\ 2 \\] \cdot 15\text{H} \\ 2 \\ \text{O} \end{array}$
Alumohydrocalcite	$\begin{array}{c} \text{CaAl} \\ 2 \\ (\text{CO} \\ 3 \\) \\ 2 \\ (\text{OH}) \\ 4 \\ 3\text{H} \\ 2 \\ \text{O} \end{array}$
Para-alumohydrocalcite	$\begin{array}{c} \text{CaAl} \\ 2 \\ (\text{CO} \\ 3 \\) \\ 2 \\ (\text{OH}) \\ 4 \\ 6\text{H} \\ 2 \\ \text{O} \end{array}$

Tunisite	NaCa 2 Al 4 (CO 3) 4 (OH) 8 Cl
Wermlandite	Ca 2 Mg 14 (Fe,Al) 4 CO 3 (OH) 42 29H 2 O